The Year in Helicobacter 2020

Guest Editors: Francis Mégraud & Peter Malfertheiner
The Year in Helicobacter

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Review: Epidemiology of Helicobacter pylori

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Abstract
This review summarizes the recent knowledge on the epidemiology of Helicobacter pylori and the potential modes of transmission. In addition to English language publications, the authors have included original full-text publications from Russia and Latin America published in the original languages. High H pylori prevalence has been reported in Russia, Jordan, Iran, China, and Latin American countries as well as in Arctic populations in Canada. Indigenous inhabitants in the Arctic were found to be infected substantially more frequently than non-indigenous inhabitants. In Amsterdam, the Netherlands, the ethnic minority groups were at a significantly higher risk of being H pylori seropositive compared to the Dutch population. For the first time, data on the prevalence from Armenia have been published indicating 41.5% H pylori prevalence. Convincing evidence on the decline of H pylori prevalence in Southeast Hungary and Taiwan was published. A study from Chile suggested high infection rates in newborns during the first month after birth. Two meta-analyses covered the potential correlation between H pylori and periodontal diseases, therefore addressing the potential oro-oral transmission rates. Periodontal disease was found to be more prevalent in H pylori-infected subjects. Other studies addressed the potential role of drinking water and food products as well as socioeconomic factors in transmitting the infection. Several studies in Asia addressed annual reinfection rates of H pylori, ranging from 1.5% in China to 3.1% in Korea. Finally, a review was published on the current evidence and future perspective of analysing H pylori in ancient human remains by a metagenomic approach.

KEYWORDS
acquisition, ancient remains, epidemiology, H pylori, prevalence, reinfection, risk factors, transmission

1 HELICOBACTER PYLORI PREVALENCE WORLDWIDE

Several studies on H pylori prevalence were published during the last year. The key results of selected studies and reviews are briefly summarised below.

1.1 Europe

The HELIUS study (Healthy Life in an Urban Setting) having enrolled a random sample of the largest ethnic groups in Amsterdam, The Netherlands (2011-2015), explored the prevalence of H pylori and CagA seropositivity among 4683 adults. H pylori seroprevalence was highest in the Ghanaian (84%), followed by Moroccan (81%), Turkish (66%), African Surinamese (51%), South-Asian Surinamese (48%), and Dutch (17%) participants. All ethnic minority groups had a significantly higher risk of being H pylori seropositive compared to the Dutch group. All groups, except the Moroccans, had a significantly higher proportion of individuals with CagA + H pylori strains compared to the Dutch participants.1

The epidemiological study in Southeast Hungary that investigated H pylori infection in 1001 healthy blood donors by H pylori IgG serology reported a prevalence of 32%.2 A higher H pylori
prevalence was found among males (34.9% vs 29.2%, \( P = .052 \)) and in rural areas (36.2% vs 27.9%, \( P = .005 \)). Additionally, agricultural/industrial workers were more likely to be positive than office workers (38.3% vs 30.1%, \( P = .0095 \)). Authors concluded that the prevalence of \( H \) \textit{pylori} infection decreased in recent decades in Southeast Hungary (eg previous serological studies in blood donors conducted in 1993 and 1999 reported 63.3% and 62.3% seroprevalence, respectively); however, it remains high in middle-aged rural populations.\(^2\)

Results from a few recently published studies in Russian language on the prevalence of \( H \) \textit{pylori} in Russia have also been included. The prevalence of \( H \) \textit{pylori} among medical employees was assessed in two cities in Russia – Moscow and Kazan (in Southwest of Russia, capital of the Republic of Tatarstan) using the \(^{13}\)C-urea breath test (UBT) HELICARB (Russia) and locally validated methodology.\(^3,4\) The overall \( H \) \textit{pylori} prevalence was 53% (52.6% men, 57.6% women) among 286 subjects. Higher prevalence was observed with increasing age: 44.1% were found to be infected in the age group 18-24 years, and 66.6% in the group above 60 years. The prevalence of the infection in Moscow was substantially lower (49.8%) than in Kazan (67%).

A serology-based study that conducted on healthy volunteers in Ryazan in central Russia (2017-2018) revealed IgG positivity (using IMMULITE 2000, Germany) in 65.6% of the adults and 20.8% of the children not treated previously for \( H \) \textit{pylori}.\(^5\)

The first study on the prevalence of \( H \) \textit{pylori} in Armenia was published in 2019. \( H \) \textit{pylori} seropositivity was revealed in 41.5% (39.6% men, 42.0% women) of symptomatic health check-up attendees based on an ELISA test (Enzygnost anti-\( H \) \textit{pylori} II IgG, Siemens, Munich, Germany). The lowest infection rate (13.6%) was reported in the youngest age group - 18-25 years, and the highest rate (83.3%) in the age group over 65 years.\(^6\)

### 1.2 | Asia

A prospective, cross-sectional study HIOC (\textit{Helicobacter pylori} Infection in Oilfield Community) involving employees and their family members was conducted in the Jidong community of Hebei province, China.\(^7\) Among the 4796 consecutive study subjects tested by UBT, \( H \) \textit{pylori} infection was present in 52.3%. The infection was found more frequently in married subjects than singles (\( P = .02 \)), those with a lower education level (\( P = .01 \)), using barreled vs. piped water (\( P = .04 \)), and more frequently consuming garlic (\( P = .03 \)) as well as in alcohol consumers when compared to non-drinkers (\( P = .02 \)). Furthermore, responders that were familiar with the route of \( H \) \textit{pylori} transmission and related diseases had a lower prevalence of the infection than those who were not aware (\( P = .01, P = .03 \), respectively).\(^7\)

A large study originating from a third-tier (fast-growing small and medium-sized cities) city, Wenzhou in China, was published, reporting data on 53 260 study subjects having undergone medical check-ups (2013-2017). Based on the UBT results, 48.4% prevalence was reported.\(^8\) The authors claim that this rate which is lower than in Linqu city and the rural region in Shandong Province as well as Shanghai city reflects the level of urbanisation, sanitation, and access to clean water as well as the socioeconomic status. No convincing increase in the prevalence with age was observed, yet a decline in the infection was observed in individuals born between 1980 and 2004.\(^9\)

A small study from Taiwan reported 21.2% \( H \) \textit{pylori} prevalence in asymptomatic adults and 37.9% prevalence in patients with dyspepsia. Older age and presence of dyspeptic symptoms were independently associated with a higher prevalence of \( H \) \textit{pylori} infection. The authors claimed a decrease in the prevalence that was 54.4% in 1992 in Taiwan.\(^9\)

### 1.3 | Middle East

Two studies reported high seroprevalence in the general population of the Middle East region.

A cross-sectional study was conducted in Jordan (2015-2016) by enrolling asymptomatic individuals, relatives of health-seeking individuals in randomly selected healthcare centres covering every governorate of the country. Altogether 88.6% of 460 study subjects were positive with an ELISA test (Enzygnost\textsuperscript® anti-\( H \) \textit{pylori} II IgG, Siemens, Munich, Germany). The factors associated with \( H \) \textit{pylori} positivity were: increased age (the highest prevalence was in age \( \geq \) 50 years (93.4%)), consumption of raw milk compared to those who did not consume milk, and location of residence. Drinking water sources, consumption of undercooked meat, and consumption of traditional wild herbs were not associated with \( H \) \textit{pylori} infection.\(^10\)

A study addressing the correlation between \( H \) \textit{pylori} and cardiovascular diseases was conducted in Karaj, Iran. A 68.0% seroprevalence was found among 97 adult subjects.\(^11\)

### 1.4 | Latin America and the Caribbean

The systematic review and meta-analysis by Curado et al included studies published between 1987 and 2012: 22 articles from 14 counties in Latin America and the Caribbean area were in the final analysis with a total population of 24 178 individuals. The overall prevalence of \( H \) \textit{pylori} infection was 57.6%; in children and adolescents the prevalence was 48.3%, and 69.2% in adults. It should be noted that no difference was observed in relation to gender.\(^12\)

A retrospective study, based on reports of gastric endoscopic biopsies performed in a private laboratory affiliated with the Brazilian Health System was performed by Rodrigues et al. The prevalence of \( H \) \textit{pylori} was 31.7% (out of 4604 participants). Furthermore, the prevalence was significantly higher in patients consulting the public health service (42%) compared to patients consulting the private health service (25.6%), (\( P < .01 \)).\(^13\)

The study led by Castabeda et al\(^14\) suggested a relatively high prevalence of \( H \) \textit{pylori} as well as virulent strains in the Peruvian...
population. The prevalence of *H pylori* was 62.9% in the entire study population and 60.8% in the gastric cancer subset. CagA was detected in 79.9% of *H pylori*-infected patients in the whole series; vacAs1 and vacAm1 alleles were identified in 41.6% and 60.7% of the patients respectively. In addition, detection of Epstein Barr virus (EBV) with quantitative real-time polymerase chain reaction (qPCR) was performed on DNA using the Primerdesign EBV kit (Genesig Advanced, Southampton, UK) with a region of BRF1 as the target. The prevalence of EBV was 14.1% in the entire study population, and it was higher in gastric cancer cases; a co-infection of *H pylori* and EBV was found in 7.8%, and it was also higher in the case of gastric cancer.14

A population-based study in Cuba indicated that among 1,274 three-year-old children, a 5% prevalence of *H pylori* infection, and positivity was associated with sleeping together. Furthermore, the protective factors were found to be drinking water from delivery trucks and living in a nuclear family unit.15

1.5 | Arctic communities in North America

The prevalence of *H pylori* infection in Western Canadian Arctic communities was investigated by Fagan-Garcia et al16 A total of 878 participants were recruited between 2008 and 2013, of whom 62% were *H pylori*-positive (by UBT, histology and/or culture). The largest variation in prevalence concerned ethnicity, with non-indigenous participants having a much lower prevalence (22%) than indigenous participants (66%). Furthermore, the prevalence of atrophic gastritis was 43% among *H pylori*-positive individuals, and the prevalence of intestinal metaplasia was 17%.16

2 | HELICOBACTER PYLORI ACQUISITION

The acquisition and transmission of *H pylori* infection remain debatable. Direct person-to-person transmission (oral-oral, gastro-oral, faecal-oral, breastfeeding and iatrogenic pathways) appears to be the main route.17 Other routes of transmission were investigated including waterborne, zoonotic, milk ingestion-based and raw vegetable-based, each of which requiring a contaminated intermediate environmental reservoir.

2.1 | Person-to-person transmission

To study the dynamics of colonisation/infection by *H pylori* during the first six months of life, Chilean authors evaluated faecal specimens from 67 mothers prior to giving birth, and from their children at seven days, one month and six months of age by using a stool antigen test. 71.6% of the pregnant mothers were positive for *H pylori*. During the first month of life, prevalence and incidence of the infection were 23.9% and 13%, respectively. These results suggest that there is a high risk of *H pylori* infection during the first month of life.18

A growing interest for *H pylori* infection in the oral cavity and its role in the oral-oral route of transmission was noted. A meta-analysis was performed on 11 studies including 1993 participants (1319 with chronic periodontitis (CP) and 674 controls) who had oral and gastric *H pylori* tested by PCR and rapid urease test. When compared to the *H pylori*-negative population, *H pylori*-positive patients had a significantly increased risk of CP (OR = 3.42, 95% CI = 2.71-4.31).13 Another meta-analysis of 13 studies involving 6800 patients, also suggested that the risk of periodontal diseases in the patients with oral *H pylori* positivity was 2.31 (95% CI, 1.00-2.68) times higher than those with *H pylori* negativity.20 These findings suggest that periodontal pocketing and inflammation may favour *H pylori* colonisation.

2.2 | Waterborne transmission

To address the potential role of aquatic environments and sewage sludge (SS) in the transmission of *H pylori* infection, a study was designed to detect the presence of *H pylori* in different aquatic environments and in SS using PCR-based methods. A total of 88 samples were collected from various aquatic environments as well as anaerobically digested SS in the city of Isfahan, central part of Iran. Using *H pylori* 16S rRNA gene as target, *H pylori* was detected in 36% (14/39) of wastewater samples and 8% (2/25) of water samples, while amplification of the ureA gene yielded in only two positive samples, which indicates a lack of specificity of the first target. None of the SS samples were positive for *H pylori*. The authors suggest that *H pylori* can be detected in drinking well water samples without the detection of faecal coliform bacteria which are commonly used as an indicator of water quality.21

A cross-sectional study among the tribal (Mizo origin) population in Northeast India was performed to evaluate the peculiar habit of the use of tobacco smoke-infused aqueous solution called “tuibur” on *H pylori* infection. *H pylori* infection was searched in 475 endoscopic samples by the rapid urease test and PCR amplification of the ureC gene. The use of “tuibur” was associated with an increased OR of *H pylori* infection (OR: 3.32; 95% CI, 1.95-5.83). The habit of “tuibur” consumption may be a contributing factor to the high prevalence of *H pylori* infection and gastritis among the Mizo population.22

A high positivity rate of *H pylori* contamination in drinking water has been implicated as one of the causes of the high gastric cancer rates observed in some countries. A Peruvian study aimed to detect the presence of *H pylori* in the tap water in the homes of 82 recently diagnosed gastric cancer patients in Lima city. Water samples were analysed for hspA and ureA genes by qPCR *H pylori* was detected in 69.5% of gastric tissues and in 12.2% of analysed tap water. No correlation was identified between gastric infection and water contamination (70% vs 69.4%, P = .971).23

Several studies addressed the prevalence of *H pylori* in seafood. The presence of vacA by qPCR was found in Spanish commercial seafood samples; it was positive in 12 of 100 samples, with 67% (8/12)
identified in mussels, 25% (3/12) in clams, and only 8% (1/12) in cockles. Another study showed that *H pylori* was able to survive in artificially contaminated mussels for six days, two days in a cultivable form and four days in a non-cultivable form.

### 2.3 Raw vegetable-based transmission

It has been suggested that *H pylori* can also be acquired from vegetables. Free-living amoebae are protozoans found in vegetables. They are transmission vehicles for amoeba-resistant bacteria, among which *H pylori* was included. In a study of 20 lettuce samples, 26 amoebic infection of *H pylori* (by means of PMA-qPCR in 55% of the samples and viable intra-amoebic *H pylori* cells in 25% of the samples using the DVC-FISH technique.

### 2.4 Milk ingestion-based transmission

Household animal’s milk has been assumed to be a probable source of *H pylori* infection in humans. The first study in Algeria regarding the zoonotic aspect of *H pylori* was performed. Cows’ milk and faecal samples were used to detect and identify *H pylori* using a bacteriology culture method. Out of 200 sera and 200 milk samples, 12% and 4% were positive, respectively, for the *H pylori* IgG antibody. The glmM gene was also detected in the milk of 13% of the cows and was confirmed in all cows presenting *H pylori* IgG in the milk.

### 3 REINFECTION

Several studies were published on the reinfection rates of *H pylori*. In a large cohort from South Korea (10 468 eradicated subjects), reinfection of *H pylori* was calculated to occur at the rate of 3.06% per person-year. In a multi-centre prospective, observational study in China involving 5193 subjects, the annual reinfection rate was 1.5%. Reinfection was associated with the following risk factors: minority groups (HR = 4.7, 95% CI, 1.6-13.9), a low level of education (HR = 1.7, 95% CI, 1.1-2.6), a family history of gastric cancer (HR = 9.9, 95% CI, 6.6-14.7), and the residence located in Western China (HR = 5.5, 95% CI, 2.6-11.5) following by Central China (HR = 4.9, 95% CI, 3.8-11) all P < .05.

### 4 FURTHER STUDY PLANS

Two study protocols for systemic reviews were published: for a systematic review of *H pylori* prevalence in Southwest China to address longitudinal changes during recent periods, and for a meta-analysis on epidemiologic studies between Tibetan and Han populations by considering the high prevalence of infection in Tibet.

Finally, Maixner et al reviewed available data on ancient human remains to demonstrate the presence of *H pylori*. While the best evidence so far is available for the European Copper Age mummy known as Iceman, other studies on the analysis of stomach tissue or stool samples in mumified materials from Chile, Mexico, Alaska, and South Korea are being discussed. Based on these achievements, the investigators are currently collecting and analysing further ancient contents and coprolites by metagenomic diagnostic approach and genome reconstruction.

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### CONFLICT OF INTEREST

The authors have declared no disclosures of interests related to this work.

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Review: Diagnosis of *Helicobacter pylori* infection

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Abstract

New imaging techniques are still the topic of many evaluations for both the diagnosis of *Helicobacter pylori* gastritis and the detection of early gastric cancer. Concerning invasive tests, there were studies on the reuse of the rapid urease test material for other tests, and a novel fluorescent method to be used for histology but with limited sensitivity. Progress occurred essentially in the molecular methods area, especially next-generation sequencing which is applied to detect both *H pylori* and the mutations associated with antibiotic resistance. For non-invasive tests, a few studies have been published on the validity of breath collection bags, the shortening of the testing time, the performance of different analysers or the added value of citric acid in the protocol. The accuracy of serological immunochromatographic tests is also improving. Multiplex serology detecting antibodies to certain proteins allows confirmation of a current infection. Dried blood spots can be used to collect and store blood without a loss of accuracy. Finally, the serum antibody titer can be useful in predicting the risk of gastric cancer. Several stool antigen tests were evaluated with good results, and a novel test using immunomagnetic beads coated with monoclonal antibodies is potentially interesting. PCR detection in stools can also be effective but needs an efficient DNA extraction method. The use of easyMAG\textsuperscript{®} (bioMérieux) combined with Amplidiag\textsuperscript{®} *H pylori* + Clarir (Mobidiag) appears to be powerful.

Keywords

linked colour imaging, new generation sequencing, rapid urease test, serology, stool antigen test, urea breath test

1 | ENDOSCOPIC DIAGNOSIS

New endoscopic techniques, especially linked colour imaging (LCI) and blue laser imaging (BLI), were extensively evaluated this year either for the diagnosis of *Helicobacter pylori* gastritis or the detection of early gastric cancer (EGC).

In the first case, the studies followed a similar protocol. Endoscopy was performed with the new imaging technique as well as white light imaging (WLI) on a group of patients for whom the *H pylori* status was established by various methods. Several images were obtained from different parts of the stomach and submitted blindly to a group of endoscopists (at least 4) for evaluation. The first aim was to determine the sensitivity and specificity of the new method vs WLI. LCI always performed better than WLI with sensitivity and specificity for LCI ranging from 83.8% to 85.4% and 79.5% to 99.5%, respectively (Table 1).

The difficulty stemmed from the fact that there are different stages of *H pylori* gastritis, from active inflammation to atrophy and intestinal metaplasia, which exhibit very different aspects. Nevertheless in their study, Wang et al concluded that identification of *H pylori* gastritis by LCI was especially reliable in the corpus.\(^1\) In another study by Jiang et al, using a score of 3.5 as the cut-off value, sensitivity and specificity were 83.8% and 99.5%, respectively, for the diagnosis of *H pylori* gastritis among 358 patients.\(^2\) Ono et al\(^3\)
found that LCI significantly improved the accuracy of active gastritis diagnosis in patients with a past *H. pylori* infection. Takeda et al also compared BLI-bright in their evaluation, following the Kyoto classification of gastritis on 261 patients. All endoscopists reported improved visibility with LCI, but BLI-bright was the best for detecting intestinal metaplasia.\(^6\) Zhu et al\(^5\) confirmed that the LCI mode gave the best rates for *H. pylori* infection and BLI-bright with magnification for atrophy and intestinal metaplasia.

The microvascular architecture pattern of the gastric mucosa was used with success to predict the *H. pylori* status or atrophy in a study carried out in Ecuador.\(^6\)

Studies using artificial intelligence (AI) were also carried out last year. The presence of *H. pylori* infection determined by LCI was “learned” through machine learning. A validation protocol was then performed. Images from 105 consecutive patients were submitted in comparison to the diagnosis made by experienced endoscopists leading to a sensitivity and specificity of 90.4% and 85.7% respectively, with no significant difference regarding the diagnosis of the endoscopists.\(^7\) A convolutional neural network was also evaluated on 452 patients in a validation cohort, resulting in a sensitivity and specificity of 81.4% and 90.1%, respectively.\(^8\)

With regard to detection of EGC after *H. pylori* eradication, LCI significantly improved the mean visibility scores and coloured differences compared to WLI, leading to significantly lower miss rates compared to WLI (30.7% vs 64.0%).\(^9\) In a similar study, Majima et al noted map-like redness and the absence of regular arrangements of collecting venules as a feature of EGC.\(^10\) The difference in colour contrast has been used as an objective way of quantifying colours with the so-called red, green and blue (RGB) pixel brightness combined with LCI. A receiver operating characteristic (ROC) curve was calculated for differentiating cancer from non-cancer. They obtained an area under the curve of 0.767 with a sensitivity of 60.5% and a specificity of 92.1%.

Given the progress in endoscopy both for the diagnosis of *H. pylori* infection and the evaluation of gastric cancer (GC) risk, the endoscopic Kyoto classification published in 2013 has been reevaluated.\(^12\)

The Italian Society of Digestive Endoscopy also applied valid quality indicators to endoscopy wards in the country. They noted failures on some points, especially concerning *H. pylori* sampling which had not been done in 30% of the centres.\(^12\)

**2 | INVASIVE TESTS**

### 2.1 Rapid urease test

A review on the continuing use of the rapid urease test (RUT) highlighted the advantages of this cheap, rapid, and specific assay, but reminded the readers of its limited sensitivity, with potential false-negative results with standard commercial assays if the bacterial load is less than $10^4$ in the biopsy. Furthermore, false-positive results may occur with some urease positive bacteria, e.g. *Staphylococcus capitis ureolyticus*.\(^13\) A study from Germany compared various commercial RUTs and found them to be equally sensitive and specific for *H. pylori* diagnosis and surprisingly more sensitive than histology after previous exposure to PPI and/or antibiotics.\(^14\) In addition, the gastric biopsy used for the RUT can be reused for other tests like *H. pylori* PCR. Given that a small proportion of GCs are caused by Epstein Barr virus (EBV), the RUT material was reused for a qPCR targeting the EBV oriP gene in a Japanese study. Out of 10 cases, *H. pylori* was detected in nine and EBV in four.\(^15\)

### 2.2 Histology

Detection of *H. pylori* by histology is still one of the most commonly used diagnostic methods by regular staining or immunostaining. A novel method using γ-glutamyl transpeptidase (GGT) activatable fluorescent probe was proposed this year. The γ-glutamyl hydroxy methyl rhodamine green probe reacts with GGT and immediately produces fluorescence. It was used ex-vivo on gastric biopsies to quantify the GGT activity of *H. pylori*. Its main advantage is the rapidity of the result (15 min) while the sensitivity is still limited (75%-82%).\(^16\)

Another study focused on pathology and also showed the role of *H. pylori* on Brunner’s gland hyperplasia and hamartomas in the duodenum.\(^17\)

### 2.3 Molecular methods

#### 2.3.1 PCR

PCR including the assay targeting the 16S rRNA gene confirmed to be a far more sensitive method for *H. pylori* detection in gastric biopsies compared to other methods.\(^18\)

**TABLE 1** Performances of endoscopic methods to detect *Helicobacter pylori* gastritis

<table>
<thead>
<tr>
<th>Author</th>
<th>Technique</th>
<th>No. patients/No. Hp+</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al(^1)</td>
<td>LCI</td>
<td>103/27</td>
<td>85.4</td>
<td>79.7</td>
<td>Histology + RUT</td>
</tr>
<tr>
<td>Jian et al(^2)</td>
<td>LCI</td>
<td>358/127</td>
<td>83.8</td>
<td>99.5</td>
<td>Histology + RUT</td>
</tr>
<tr>
<td>Ono et al(^3)</td>
<td>LCI</td>
<td>127/</td>
<td>84.4</td>
<td>88.9</td>
<td>UBT + Serology</td>
</tr>
<tr>
<td>Yasuda et al(^7)</td>
<td>LCI + AI</td>
<td>105/42</td>
<td>90.4</td>
<td>85.7</td>
<td>2 methods among histology, serology, SAT, UBT</td>
</tr>
<tr>
<td>Zheng et al(^8)</td>
<td>WLI + CNN</td>
<td>452/104</td>
<td>91.6</td>
<td>98.6</td>
<td>IHC ± UBT</td>
</tr>
</tbody>
</table>

Abbreviations: AI, artificial intelligence; CNN, convolutional neural network; IHC, immunohistochemistry; LCI, linked colour imaging; RUT, rapid urease test; SAT, stool antigen test; UBT, urease breath test; WLI, white light imaging.
Evolving antibiotic resistance continues to be a critical problem in the management of *H. pylori* and before culture, real-time (RT) PCR is being used increasingly given its high sensitivity and specificity, its turnaround time of a few hours and its convenient transport conditions. Several commercial RT-PCRs are available, they have the added value of detecting macrolide resistance conferred by three mutations (A2142G, A2143G and A2142C) on the 23S rRNA gene. Only one commercial assay, a line probe assay (Genotype HelicoDR assay, Hain Lifescience, Germany), detects common point mutations in both the 23S rRNA gene and the *gyrA* gene (N87K, D91G, D91N, D91Y) to determine clarithromycin and levofloxacin resistance, respectively.\textsuperscript{13}

### 2.3.2 Next-generation sequencing

The decrease in sequencing costs now allows the addition of next-generation sequencing (NGS) to the armamentarium to detect *H. pylori* and its antibiotic resistance. Using NGS, detection of *H. pylori* mutations that are known to confer resistance to clarithromycin, levofloxacin, and tetracycline was undertaken directly from formalin-fixed paraffin-embedded (FFPE) gastric biopsy specimens on an Ion Torrent (Thermo Fischer) platform in the USA: 133 *H. pylori* positive gastric biopsy specimens were identified histologically and subsequently analysed by NGS to detect mutations in *gyrA*, 23S rRNA, and 16S rRNA genes. The method successfully detected *H. pylori* in 126 of 133 cases (95% sensitivity). Mutations conferring resistance were present in 92 cases (73%), including 63 cases with one mutation (50%) and 29 cases with mutations in several genes (23%). In the 58 cases where treatment history was available, there was a good correlation between therapy failure and the number of mutated genes: no failure in cases with no mutation (0/15), 19% (5/27) failure in cases with one gene mutation, and 69% (11/16) failure in cases with more than one mutated gene. Common 23S rRNA gene mutations (A2142G or A2143G) were present in 88% (14/16) of failed cases as opposed to only 10% (4/42) of eradicated cases (*P* < .001). Although this is a small study, it shows the feasibility of NGS to detect multidrug resistance in culture negative biopsies. It can be used on clinical specimens collected during standard of care.\textsuperscript{19}

A study in Switzerland showed a high congruence of >99% between phenotypic antibiotic susceptibility results for clarithromycin, levofloxacin, and rifampicin and single nucleotide polymorphisms (SNPs) identified in the 23S rRNA, *gyrA*, and *rpoB* genes using whole genome sequencing (WGS). However, it was not possible to infer a resistance phenotype for metronidazole based on the occurrence of distinct SNPs in *frxA* and *rdxA* genes.\textsuperscript{20} The same good correlation was also found in Cambodia.\textsuperscript{21} Other studies, using WGS analysis of *H. pylori* strains isolated from gastric biopsies, described new mutations or identification of multiple mutations conferring resistance to rifampicin, metronidazole and amoxicillin.\textsuperscript{22-24}

Other studies using NGS platforms were also carried out to study the virulence genes of *H. pylori*\textsuperscript{25,26} as well as to decipher the gastric and duodenal microbiota.\textsuperscript{27}

### 3 NON-INVASIVE TESTS

#### 3.1 Urea breath test

Few articles have been published during this past year concerning urea breath tests (UBT). One study evaluated the validity of breath collection bags in detecting *H. pylori* on more than 250 patients using the BreathID Hp Lab System (Exalenz Bioscience Ltd, Israel).\textsuperscript{28} This system contains an application to control the process and can measure up to 10 sets of bags consecutively and automatically within approximately 25 minutes. In comparison to histology and RUT, the authors concluded that this system is highly accurate and has several advantages compared to the previous BreathID Hp device, eg the possibility to analyse more samples simultaneously and the good stability of the bags facilitating the transport.

Another article aimed to compare a short vs a standard sampling time during UBT, the BREATHQUALITY UBT (AB Analitica, Padua, Italy).\textsuperscript{29} The authors obtained comparable accuracy by shortening the testing time to 15 minutes.

A prospective study was carried out to compare two devices: the IRIS-Doc2 (Wagner Analysein-Technik, Germany, now Mayoly Spindler Group, France) and the BreathID Hp Lab System used as the reference.\textsuperscript{30} Five hundred eighteen patients were enrolled and each filled two bags prior to the test and two bags after ingestion of the test solution with the same protocol. The correlation between the two devices was excellent confirming that a single protocol can give similar results with different devices.

A study evaluated the effect of citric acid on the accuracy of UBT. In this prospective study, 1207 *H. pylori* positive patients received UBT after a successful eradication treatment. They were then divided into two groups: one received a citric acid meal whereas the other one did not. The UBT values after use of citric acid were significantly higher compared to the control group (*P* < .001). In addition, 122 patients were also evaluated by endoscopic biopsy methods. Compared to invasive tests, there was no significant difference in the UBT performance between the two groups, even if gastric atrophy and intestinal metaplasia decreased UBT accuracy.\textsuperscript{31}

Finally, a meta-analysis by Abd Rahim et al, reported the different \textsuperscript{13}C-UBT accuracy studies conducted in Asia.\textsuperscript{32} In total, 15 protocols were referenced and analysed by the authors. Sensitivity and specificity of the studies were excellent, even if heterogeneity was observed in the different evaluations. However, the review shows that it is possible to reduce heterogeneity by adjusting for the dose and breath sample collection time.

#### 3.2 Serology

A study performed in Japan aimed to evaluate the diagnostic accuracy of four commercially available kits, 2 ELISA kits and 2 latex immunoassay kits for the detection of *H. pylori* infection. Kits were all manufactured in Tokyo, Japan, coming from Eiken Chemical Co, Ltd, or Denka Seiken Co, Ltd. The diagnostic accuracy was determined
using samples from 213 dyspeptic patients, and UBT was the reference. The authors concluded that, in terms of accuracy, the latex immunossays are as reliable as ELISAs but they have some advantages in terms of time and cost.  

A study performed primarily on African-Americans in the Southeastern United States aimed to detect active infection by seropositivity to H pylori proteins. They measured antibody responses to 13 H pylori proteins using multiplex serology in serum samples of a training set (n = 78) and validation set (n = 49) collected concurrently from patients undergoing UBT. To determine sensitivity, a cutoff was applied to achieve 90% specificity. They finally concluded that seropositivity to ≥ 2 of H pylori proteins VacA, GroEL, HcpC, and HP1564 indicates active H pylori infection at high specificity and sensitivity and may allow an approximation of the prevalence of active H pylori infection in large cohorts.  

Another study evaluated the utility of dried blood spots (DBS) in detecting H pylori infection in comparison to serological assay. A total of 264 paired DBS samples prepared from intravenous blood (IVB) were stored at 4°C, then at −20°C after a period of 24, 48 and 72 hours. Specific IgG antibodies detected by ELISA in DBS were compared with those from IVB. No significant difference between these 2 pre-analytical processes was observed confirming that DBS collected even in field conditions can be used for detecting H pylori infection.  

Finally, a study was performed to clarify the role of anti-H pylori antibodies in the evolution to GC by combining them with the Kyoto classification endoscopic score. A multivariate analysis carried out on 874 cases revealed that nodularity, atrophy, and age between 40-59 years were correlated with a high serum antibody titer in H pylori-infected patients. In summary, serum antibody titer changes with age, reflects gastric mucosal inflammation, and is useful in predicting the risk of GC.  

3.3 | Stool antigen tests

Opekun et al evaluated the clinical performance of the automated Liaison® Meridian H pylori stool antigen test (SAT). The test was used on 277 patients for whom an endoscopy was performed. Compared to a composite reference test including histology, culture and a RUT, the Liaison® Meridian H pylori SAT gave a sensitivity of 95.5% and a specificity of 97.6% confirming its usefulness in detecting H pylori.  

An ELISA, the RIDASCREEN® (R-Biopharm, Darmstadt, Germany) and an immunochromatography test (ICT), the RIDAQUICK® (R-Biopharm), were evaluated on 266 stool samples from patients in comparison to a chemiluminescence assay, the Liaison® H pylori SA. Results were in concordance, showing that the 2 kits can be used for H pylori detection.  

A Taiwanese study aimed to determine the accuracy of a new ICT, the Vstrip® H pylori stool antigen rapid test (Vstrip®HpSA) (Vstrip, Taiwan), in the detection and surveillance of H pylori prevalence in Taiwan. A total of 347 subjects were included in the study, 152 asymptomatic volunteers and 195 symptomatic patients. Stool samples were collected and tested for H pylori with three different commercialised kits: Vstrip®HpSA, ImmunoCard STAT® Campy (Meridian), another ICT, and an ELISA, Premier Platinum HpSA® PLUS (Meridian). UBT was considered as the gold standard. Performances of the 2 ICTs were similar. They are very convenient and showed an excellent agreement with the ELISA.  

Finally, a novel SAT has been developed to detect H pylori using immunomagnetic beads coated with monoclonal antibodies capturing the bacterium coupled with a polyclonal antibody-conjugating quantum dot probe. The final detection was achieved using a fluorescence spectrometer. This test provides a novel potential method for non-invasive detection of the H pylori faecal antigens but further testing in clinical studies is needed.  

3.4 | Molecular tests

Concerning molecular tests in stool samples, a study compared an optimised high throughput, semi-automated workflow to a reference manual workflow previously described to detect H pylori and its resistance to clarithromycin on unpreserved stools and gastric biopsies. For this study, material from 96 symptomatic patients was collected and DNA extracted in parallel with both the Magna Pure 96 (Roche) and the QIAamp Fast Stool kit (Qiagen). Results obtained from stools and gastric biopsies showed that the semi-automated workflow can be used to detect and genotype H pylori. Cut-off temperatures were different between stools and gastric biopsies.  

A prospective multicentre study including 1200 patients was performed to evaluate the Amplidiag® H pylori + ClariR assay which can detect H pylori and its resistance to clarithromycin in stools. The results of the assay performed on DNA extracted from stools using an automatic extraction (EasyMAG® bioMérieux) were compared with the results obtained with culture/E-test and a quadruplex real-time PCR performed on two gastric biopsies to detect the H pylori glmM gene and mutations in the 23S rRNA genes conferring clarithromycin resistance. In this cohort, only 160 patients were positive. The sensitivity and specificity of the Amplidiag® H pylori + ClariR assay were respectively 96.3% and 98.7%.  

Seligova et al aimed to find a reliable nested PCR for the detection of H pylori in stools, gastric biopsies and saliva. After having tested the different published nested PCRs, the authors designed a new one to avoid the lack of specificity of the primers previously used. Thus, they used primers targeting the variable regions of the 16S rRNA gene. Eighty-one patients were enrolled, and the material was collected simultaneously. The sensitivity of their nested PCR in gastric biopsies was equivalent to that of UBT, but it was much lower in stools. Concerning, saliva, the nested PCR was able to detect H pylori but correlation with its presence in the stomach was not relevant.  

3.5 | Other tests

Auampan et al reported their experience using a rapid urine test to detect H pylori antibodies. Gastric biopsies from 94 dyspeptic patients
were collected and *H pylori* detected by RUT, culture and histopathology. Urine samples collected at the same time were tested with the RAPIRUN® *H pylori* antibody test (Otsuka Pharmaceutical., Ltd.). The rapid urine test was indeed less efficient than the other tests with a specificity of 90.8% and a sensitivity of 86.2%.44

A frequent problem in the evaluation of a new test is the lack of reference. The use of latent class analysis to evaluate different *H pylori* diagnostic tests was proposed for such cases. This model uses a combination of observed and estimated results. The authors showed that the use of latent class analysis is possible to evaluate *H pylori* tests based on histology, SAT and serology.45

**CONFLICT OF INTEREST**
The authors declare no conflict of interest for this article.

**REFERENCES**


eradication therapy in a region with a high prevalence of atrophic gastritis. 


1 INTRODUCTION

1.1 Helicobacter pylori shape and surface

The helical cell shape of H pylori facilitates efficient colonisation of the human stomach. This shape is generated and maintained by the action of a combination of peptidoglycan (PG) modifying enzymes and non-enzymatic proteins that act as scaffolds. With three recent publications,1-3 the group of N.R Salama made significant progress in the characterisation of the corresponding actors and underlying mechanisms. Using a genome-wide screen, they identified Csd7, an integral inner membrane protein, as an essential determinant of H pylori helical shape that is important for efficient mouse colonisation.1 Through protein-protein interaction with two other cell shape
determinants, the Csd1 periplasmic PG endopeptidase, the Csd2 protein and interaction with the filament forming bactofilin CcmA, Csd7 was found to stabilise Csd1. In H. pylori, cell shape is controlled by a highly orchestrated program recruiting many partners that form a "helical shapesome." A combination of 3D confocal microscopy and volumetric image analysis was applied to characterise the colonisation of murine gastric glands by a H. pylori Δcsm6 straight rod mutant. This mutant was found to be attenuated shortly after infection but it then persists during chronic colonisation although eliciting less inflammation. Helical shape maintenance implies the control of several cell parameters such as diameter, length and helical pitch. By applying quantitative 3D microscopy analysis of short pulses with metabolic probes, Taylor et al. revealed that cell growth is enhanced at both sidewall curvature extremes and they observed increased levels of the actin-like filament MreB and CcmA at negative and positive Gaussian curvature sites of the bacterium, respectively. These two proteins thus promote PG synthesis at specific sites and this patterning is one mechanism maintaining the helical shape.

The H. pylori lipopolysaccharide (LPS) surface structures play an important role in the interaction with the host and in immune escape. Li et al. established the first complete LPS biosynthesis pathway using H. pylori strain G27 as a model. Analysing this pathway in a large panel of H. pylori strains from different ethnic origins, they found that the genes involved in LPS heptan incorporation are lacking in East-Asian strains. Given the high gastric cancer (GC) rate in East-Asia, this observation opens interesting questions on a potential role of LPS heptan in H. pylori pathogenesis. H. pylori phospholipid composition is also unusual in that it contains a large proportion of cyclopropane fatty acids (CFA). Jiang et al. identified the gene encoding CFA synthase, hp0416. This function is dispensable for H. pylori in vitro growth but a Δhp0416 mutant presents increased acid sensitivity, disruption of membrane permeability and reduced capacity to survive in murine macrophages and to colonise the mouse stomach. Finally, a major actor in the biogenesis pathway of H. pylori cell-envelope polysaccharides was identified by Gasiorek et al. HupA, an enzyme combining undecaprenyl pyrophosphatase with phosphatidylglycerol phosphate phosphatase plays a dual function in synthesis of cell-wall polysaccharides and phospholipids. HupA is essential for murine stomach colonisation and is involved in cationic anti-microbial peptide (CAMP) resistance. These results underline the importance of maintaining PG and LPS composition while facing the hostile gastric niche.

1.2 | Natural transformation, mobile genetic elements and within-host genomic evolution

H. pylori displays exceptionally high genetic diversity and variability that is generated by inefficient DNA repair mechanisms, competence for natural transformation (NT) and high levels of recombination. NT allows bacteria to import DNA from the environment and internalise it into the cytoplasm where it can be integrated into the genome. Damke et al. showed that the ComH protein is a receptor for the import of transforming DNA during NT into the periplasm. ComH binds DNA at its C-terminal domain and delivers it to the ComEC inner membrane channel, a complex able to translocate DNA into the cytoplasm. Mobile elements like integrative and conjugative elements (ICEs) represent an important source of genetic diversity among bacteria. Weiss et al. characterised the excision/integration and horizontal transfer characteristics of H. pylori ICEHptfs4. They reported that transfer of this ICE is independent of its conjugation genes and that homologous recombination is more efficient than site-specific integration into the recipient chromosome. Understanding H. pylori population dynamics inside the gastric environment during infection is a question of major interest. The genomes of H. pylori strains from multiple gastric biopsies taken from different stomach regions were analysed by Ailloud et al. They observed that within-host evolution of H. pylori is niche-specific and probably associated with the physiological differences existing between the antral and oxyntic gastric mucosa. Evidence of natural selection of niche-specific genotypes was revealed for functions such as chemotaxis, regulatory functions and outer membrane proteins that probably contribute to specific adaptation to the antral and oxyntic mucosa. Finally, they found that antibiotic exposure can induce population bottlenecks; this is undoubtedly an important parameter that shapes the "within-host" population structure of H. pylori in patients.

1.3 | Regulation of gene expression and stress response

Response generated by inefficient DNA repair mechanisms, competence for natural transformation (NT) and high levels activation of heat-shock proteins ensuring cell protection. Promotor sequence methylation is an understudied level of gene regulation in bacteria. Narayanan et al. showed that a type III DNA methyltransferase, M.HpyAXI, whose expression is upregulated at low pH, forms an active tetramer able to bind DNA and regulate essential genes, only during acid stress. The M.HpyAXI is thus an enzyme licensed to act only under acidic conditions like those encountered by H. pylori in the gastric niche. In H. pylori, HrcA is a major repressor known to regulate the expression of genes encoding crucial chaperones. Using whole genome RNA-sequencing, Roncarati et al. found that 49 genes were deregulated in an hrcA mutant. The HrcA regulon includes genes coding for chaperones and stress-related proteins as well as other genes encoding functions important for H. pylori survival and virulence.

1.4 | Gastric colonisation and microbota

Colonisation of the gastric epithelium by H. pylori relies on its capacity to swim into the mucus and its preference for injury sites of the stomach, a tropism that relies on chemotaxis. Using a murine gastric organoids (gastroids) model in which single cell damage could be introduced by photodamage and subsequent repair monitored,
Aihara et al demonstrated that *H pylori* rapidly accumulates at the damage site in an actin polymerization-dependent mechanism and locally limits repair.\textsuperscript{12} The actual chemoattractant sensed by *H pylori* is unknown but the TlpB chemoreceptor was shown to be required for the orientation of the bacteria to the repair site.\textsuperscript{13}

TlpD, one of the four *H pylori* chemoreceptors, is a non-canonical cytosolic transducer-like protein important for stomach antrum colonisation. Perkins et al showed that bleach (hypochlorous acid, HOCl) is a powerful chemoattractant for TlpD. They established a reconstituted full chemotaxis signalling complex in vitro assay in which HOCl was sensed at micromolar concentrations by TlpD, much lower concentrations than other reactive oxygen species (ROS). The predicted physiological source of HOCl is neutrophils and chemoattraction to this molecule may constitute a strategy of *H pylori* to persist in the neutrophil-infiltrated gastric glands in order to acquire important nutrients.\textsuperscript{14}

Chemotaxis can also influence global bacterial behaviour such as biofilms. An *H pylori* self-produced quorum sensing molecule, autoinducer-2 (AI-2), was previously found to act as a chemorepellent and to reduce biofilm formation. Agent-based models (ABMs) are useful tools for exploring the influence of simple interactions between cells on the properties of bacterial communities. Using ABM, Sweeney et al provide new insights into how local chemotactic behaviour can shape *H pylori* biofilms.\textsuperscript{15}

How *H pylori* persists in the human stomach was investigated by Fung et al who propose a model involving the establishment of *H pylori* in a microenvironment deep in the gastric glands.\textsuperscript{16} The infection of adult or neonate mice with PMSS1 isogenic *H pylori* strains, differentially fluorophore-tagged, coupled to quantitative 3D confocal microscopy and passive CLARITY technique (PACT) analysis, showed bacteria clonal population islands inside glands. Only few bacteria initiated colonisation, leading to glands resistant to subsequently added bacteria. The bacterial density was age- and T-cell response-dependent. As supported by computer simulation, gland colonisation expanded locally as patches by infecting adjacent glands. The authors proposed that glandic glands constitute a micro-niche for *H pylori*, allowing a continuous reинфекtion of the superficial mucosa.\textsuperscript{16} *H pylori* colonisation also impacts the gastric microbial community. Mongolian gerbils infected with *H pylori* strain 7.13 and isogenic ∆cagA mutant presented CagA-dependent alterations of the gastric mucosal microbiota diversity which evolves with the severity of gastric lesions.\textsuperscript{17}

1.5 | Virulence factors

1.5.1 | The cag type 4 secretion system (cagT4SS)

Injection of the *H pylori* CagA oncoprotein into gastric epithelial cells relies on the cagT4SS system and causes important cellular dysregulation that, in some cases, lead to the development of GC. The high-resolution structure of this system, one of the largest bacterial secretion system assemblies, has recently been solved.\textsuperscript{18}

Activation of the NF-κB signalling pathway leading to massive interleukin 8 (IL8) production represents a major inflammatory response of human epithelial cells to infection by *H pylori* strains with a functional T4SS. Several publications recently reported that this activation relies on the ALPK1-TIFA signalling axis and that the *H pylori*-produced signalling molecule (PAMP) for this axis is heptose 1,7-bisphosphate (HBP), an LPS intermediate. However, Pfannkuch et al identified ADP-glycero-beta-D-manno-heptose (ADP heptose), a derivative of HBP, as the predominant activator of the ALPK1-TIFA signalling axis.\textsuperscript{19} The mechanism of translocation or uptake of ADP heptose into host cells and the extent of the cagT4SS contribution remains to be established.

Passage of *H pylori* through the mouse model is known to cause the loss of T4SS function, which is frequently associated with recombination in the cagY gene that encodes a T4SS core complex protein. Hansen et al analysed a collection of mouse-passaged strains and found that, among those that had lost T4SS functionality, 51% presented recombination in cagY and, for the others, they identified inactivation of 13 additional essential cagT4SS genes, mainly cag5, cag10 and cagA.\textsuperscript{20}

Toll-like receptors (TLRs) are innate immune receptors. *H pylori* has recently been shown to activate the expression of TLR5. However, the archetypal activator of TLR5 is flagellin, a protein that, in *H pylori*, lacks the conserved and essential D1 interaction domain. Here, the authors discovered that one of the cagT4SS components, the protein CagL present at the tip of the secretion pilus, can act as a TLR5 activator. CagL possesses a D1-like motif and is necessary for NF-κB activation in a TLR5-dependent manner. Using Tlr5-knockout mice, they showed that TLR5 is important for efficient control of *H pylori* infection possibly by modulating the immune response.\textsuperscript{21}

It is now well established that the *H pylori* cagT4SS exploits the specific interaction between the bacterial HopQ adhesin and the CEACAM cellular adhesion molecules for translocation of CagA into human gastric epithelial cells. In the study of Behrens et al, the role of this interaction on immune cells was studied.\textsuperscript{22} Using human and CEACAM-humanised (hCEACAM) mouse PMNs, they found that the *H pylori* HopQ-dependent interaction strongly enhanced CagA translocation and phosphorylation. PMNs but not macrophages or dendritic cells from hCEACAM mice significantly upregulated the proinflammatory chemokine MIP-1-alpha. In a model of chronic mouse infection, the hCEACAM1 and –6 receptors were found to be downregulated on neutrophils. These data reveal an important role of *H pylori*-CEACAM interaction for CagA translocation into neutrophils, a probable strategy of the pathogen to regulate the host immune response during the progression of gastric pathology.\textsuperscript{22}

1.5.2 | The VacA vacuolating cytotoxin

VacA is a major virulence factor of *H pylori*. This secreted toxin inserts into the host cell membranes to form chloride-sensitive channels and impairs host endolysosomal trafficking, causing an accumulation of dysfunctional lysosomes and autophagosomes. The role of intracellular
**Host-pathogen interaction**

### 1.6.1 Host response and CagA oncogenic properties

Understanding the mechanisms by which the CagA oncoprotein promotes *H. pylori* carcinogenic activity is a very important question. Using a human gastric organoids model, Buti et al found that the apoptosis-stimulating protein of p53 2 (ASPP2), contributed to the survival of CagA-positive *H. pylori* bacteria in gastric organoids. CagA–ASPP2 protein interaction promoted remodelling of the partitioning-defective (PAR) polarity complex and led to cell polarity loss.

A major consequence of CagA injection is cell transformation by promoting an epithelial-to-mesenchymal transition (EMT)-like phenotype thereby disrupting cell junctions and enhancing motility and invasiveness of the infected cells. Upon contact with gastric epithelial cells (GECs), *H. pylori* activates signalling pathways such as Hippo, an important player in the promotion of EMT. The tumour suppressors LATS1/2 and their substrate YAP1 are components of the Hippo pathway which, once activated, restricts tissue overgrowth. As reported by Molina-Castro *et al., H pylori* upregulates LATS2 and YAP1. LATS2 protects GECs against *H pylori*-mediated aberrant differentiation, characterised by the induction of EMT and the development of intestinal metaplasia. Interestingly, the lysosomal associated protein transmembrane 4B (LAPTM4B), previously proposed as prognostic factor in GC, is induced in *H pylori*-infected GECs and participates in EMT.

Palrasu *et al.* addressed the question of how the gastric cells resolve the strong oxidative and genotoxic stress caused by *H pylori*. Using human gastric tissues and infected animals, they identified the proapoptotic factor Siva1, as a new actor in the host response. *H pylori* enhances Siva1 ubiquitination and proteasomal degradation, through induction of the XIAP E3 ubiquitin ligase via the PI3K/Akt pathway activation. This process is p53-independent and relies on an intact Cag T4SS.

The upstream stimulating factor 1 (USF1) is a basic-Helix-Loop-Helix (bHLH) transcription factor, known to stabilise p53 in response to genotoxic stress. Costa *et al.* reported a protective role of USF1 against *H pylori*-induced carcinogenesis. In *Usf1*−/− mice, they showed that the lack of USF1 accelerated *H pylori*-induced dysplasia, concomitantly with p53 depletion. In vitro, in GECs, *H pylori* inhibits nuclear levels of USF1 and p53 and induces USF1 foci close to cell membranes. This mechanism which impairs p53 and USF1 nuclear function promotes genetic instabilities. Another bHLH transcription factor, BHLHE40, is upregulated during *H pylori* infection by activation of a CagA-dependent ERK signaling pathway. Through p-STAT3 interaction, BHLHE40 promotes CXCL12 production and contributes to gastric inflammation. These novel findings contribute to the understanding of how *H pylori* increases survival of human cells with damaged DNA.

Finally, Wrobleski *et al.* investigated how *H pylori* causes aberrant stem cell activation, a landmark of the carcinogenesis process. The leucine-rich repeats and Ig-like domains 1 (Lrig1) is known to mark a distinct population of progenitor cells. Using a model of ex vivo Lrig1 progenitor cells and following specific Lrig1 lineage expansion in a mouse model, they found that *H pylori* infection increased Lrig1-expressing cells in a cag-dependent manner. In human samples, Lrig1 expression was increased in pre-malignant lesions. They concluded that chronic *H pylori* infection stimulates Lrig1-expressing progenitor cells, which may contribute to promote gastric carcinogenesis.

### 1.6.2 Epigenetic regulation and microRNA response to *H pylori* infection

Genome-wide DNA methylation analysis of human gastric tissue revealed *H pylori*-induced CpG methylation that is inflammation-triggered. In line with this, chronic inflammation in *H pylori*-infected mice was found to be associated with the inhibition of the telomerase reverse transcriptase (TERT) gene expression, as a consequence of DNA hypermethylation of its promoter. DNA methylation microarray analysis in the context of *H pylori*-triggered chronic inflammation allowed Yamashita *et al.* to show that targets of aberrant DNA methylation differ between “old” and “young” human gastric mucosae. MicroRNA are small non-coding RNAs that mediate post-transcriptional regulation. Interestingly, microRNA genes were also found to be susceptible to aberrant methylation. Chen *et al.* reported CagA-dependent DNA methylation in the promoter of the TRPM3 gene that encodes miR-204. During evolution of gastritis...
lesions to preneoplasia, the progressive increase of TRPM3 DNA methylation results in a gradual decrease in miR-204 levels. One miR-204 target is the Bir2C gene that is overexpressed in GC patients with a poor prognosis. By targeting Bir2C, miR-204 inhibits TNFα-mediated activation of NF-KB signalling pathways, as shown in cancer GECs. With a xenograft model, they showed that overexpression of miR-204 inhibited tumour growth.35 Also using xenograft model and GECs cultures, Ou et al demonstrated the oncogenic properties of another microRNA, miR-155. In H pylori-infected cells, miR-155 which is induced in a CagA-dependent manner, suppresses the Kruppel-like transcription factor 4 (KLF4) expression, leading to promotion of EMT and tumorigenesis.36 miR-155 was also found to be upregulated during gastric MALT lymphoma development both in patients and mice, together with miR-150, miR-196a and miR-138 that could act synergistically in common signalling pathways to promote lymphomagenesis.37 Furthermore, specific miRNAs may contribute to H pylori persistence and carcinogenesis. As shown by Codolo et al, expression of the class II major histocompatibility complex transactivator (CIITA) is inhibited through upregulation of let-7f-5p, let-7i-5p, miR-146b-5p and miR-185-5p in H pylori-infected macrophages as well as in gastritis, preneoplasia and GC patients.38 These recent studies highlight that epigenetic modifications through DNA methylation and microRNA-mediated regulation are key events in the H pylori-induced tumorigenesis process.

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CONFLICT OF INTERESTS

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Review - Helicobacter, inflammation, immunology and vaccines

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Abstract
Understanding the mechanisms involved in induction and regulation of the immune and inflammatory response to Helicobacter pylori is extremely important in determining disease outcomes. H pylori expresses a plethora of factors that influence the host response. Vaccines against H pylori are desperately needed for the prevention of gastric carcinogenesis, especially with the increasing trends in antimicrobial resistance. This review summarizes some important findings, published between 1 April 2019 and 31 March 2020, in the areas of H pylori-mediated inflammation, immunity and vaccines.

KEYWORDS
adaptive immunity, cytokines, Helicobacter pylori, inflammation, innate immunity, vaccines

1 | INFLAMMATION

1.1 | Epithelial interactions

Uotani et al developed a human gastric epithelial organoid monolayer culture model to investigate host-Helicobacter pylori interactions. Most research uses human gastric adenocarcinoma-derived cell lines, which do not accurately model normal gastric epithelial cells. Gastric mucosal spheroids were differentiated as monolayers. Their polarised cell morphology resembled that of the human gastric mucosa, and H pylori infection resulted in transient changes in tight junction integrity. The infection induced Interleukin-8 (IL-8) expression but, in contrast to commonly used cell lines, equivalent levels were induced by a cag pathogenicity island (cagPAI) knockout mutant and the wild-type (WT) strain. When cagPAI-negative clinical isolates were applied to the gastroid monolayers, they also induced high-level IL-8 secretion; no response was observed by cell lines. The authors suggested that this culture model models the human gastric mucosa more closely than cell lines.1

Several host factors were identified as playing an important role in H pylori-mediated gastritis. The pro-forms of IL-1β and IL-18 require activation via inflammasomes and cleavage by proteases. Semper et al investigated the role of the cag type IV secretion system (T4SS) in activating the NLRC4 inflammasome during H pylori infection, and its impact on gastritis. They showed that the cagT4SS was essential for NLRC4 inflammasome activation and IL-18 production from human and murine gastric epithelial cells. Unlike WT control mice, Nlrc4−/− mice did not produce gastric IL-18 when infected with H pylori, and gastritis was milder. IL-18 inhibited expression of β-defensin 1 via an NF-κB-dependent mechanism, resulting in higher H pylori colonisation. The authors concluded that activation of the NLRC4 inflammasome and IL-18 contribute to H pylori persistence and inflammation.2

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The transcription factor STAT3 plays a major role in inflammation, and phosphorylation of STAT3 at serine 727 (pS727) is required for maximal expression of STAT3-regulated genes. The role of serine phosphorylated STAT3 (pS-STAT3) was examined by Balic et al. Constitutive pS-STAT3 staining was observed in gastric biopsies from patients, regardless of H pylori status. Gastric tissue from Helicobacter felis-infected mice lacking this pS site, contained markedly fewer immune cells compared with WT mice. This indicated that pS-STAT3 plays an important role in H pylori-mediated inflammation and disease.3

Mejías-Luque et al4 investigated NF-κB signalling in H felis-infected mice. Mice lacking MyD88, an adaptor molecule in the NF-κB pathway, had more severe gastric pathology. This effect was linked to activation of non-canonical NF-κB signalling, with increased CXCL9 and ICAM1 expression, STAT3 signalling and T-cell infiltration in the gastric mucosa of infected MyD88−/− mice.3

Dang et al investigated inflammatory signalling in gastric epithelial cells and showed that the proapoptotic Bcl-2 protein PUMA was upregulated by H pylori infection via Toll-like receptor 2 (TLR2) signalling and NF-κB activation. Severity of apoptosis and inflammation in H pylori-positive human gastric biopsies was associated with PUMA expression. The authors concluded that PUMA plays an important role in H pylori disease via apoptosis of epithelial cells and exacerbation of inflammation.5

Kong et al found elevated expression of the hormone adrenomedullin (ADM) in the H pylori-infected gastric mucosa of mice and humans, correlated with the severity of gastritis. Administration of ADM-blocking antibodies to infected mice significantly reduced gastric inflammation. ADM expression by gastric epithelial cells, mediated via CagA-dependent signalling, induced macrophages to express IL-12, which exacerbated inflammation.5

Lv et al reported that H pylori-induced matrix metallopeptidase-10 (MMP-10) expression by gastric epithelial cells, plays a role in enhancing H pylori colonisation and inflammation via ERK pathway signalling. Human gastric MMP-10 levels were associated with gastritis severity; colonisation densities and gastric inflammation were reduced in MMP-10−/− mice compared to WT mice.7 Kuo et al showed that expression of IL-33 and its receptor ST-2 was upregulated in H pylori-infected gastric epithelial cells. ST-2 localised into lipid rafts in cell membranes. In the gastric mucosa of infected mice, increased IL-33 and ST-2 expression exacerbated leukocyte infiltration and the severity of inflammation.8

2 | IMMUNOLOGY

2.1 | Lymphocytes and innate lymphoid cells

El-Zaatari et al found that expression of an inflammasome component called Absent in melanoma 2 (Aim2), was increased in the gastric mucosa of mice with H felis-induced premalignant changes. Aim2 is involved in the activation of IL-1β and IL-18, and was anticipated to contribute to spasmylocytic peptide-expressing metaplasia (SPEM) mucosal damage. Unexpectedly, infected Aim2−/− mice had worse gastritis than WT animals. Aim2−/− mice had more CD8+ T cells in their gastric mucosa, and these cells had lost homing receptors and gained markers of tissue-resident memory T (Trm) cells. These changes were induced by CXCL16 from B cells. The authors concluded that Aim2 regulates CD8+ T-cell infiltration independently of the inflammasome, and inhibits metaplasia via a novel mechanism.9

Lv et al7 also reported that gastric epithelial cell expression of MMP-10 led to CXCL16-mediated migration of CD8+ T cells into the H pylori-infected gastric mucosa.

During H pylori infection, T helper (Th) cell subsets are recruited to the gastric mucosa. These vary in function, with Th1 and Th17 being more inflammatory and regulatory T cells (Tregs) being immunosuppressive. Th cells may be converted into other phenotypes by the cytokine environment. Shamsdin et al compared frequencies of IL-9-secreting Th9 cells, IL-9-secreting cytotoxic T cells (Tc9), and IL-9-secreting Th17 and Tc17 cells (Th17/9 and Tc17/9) in peripheral blood from H pylori-positive and -negative patients. They found significantly increased Th17, Tc17, Th17/9, and Tc17/9 subsets, and higher serum IL-9, IL-23 and IL-21 concentrations in the infected group.10

Bagheri et al found significantly higher numbers of T-bet+ Th1 cells in gastric biopsies from peptic ulcer disease (PUD) patients, compared to those with gastritis.11 The same group also reported increased gastric Th22 cells in PUD patients. Th1 and Th22 cell numbers correlated with Th17 cells, and inversely correlated with Treg cells. They concluded that Treg cell responses modulate Th1, Th17 and Th22 responses. If imbalanced, heightened Th1, Th17 and Th22 activity worsens gastritis and favours PUD.12

Capitani et al found that H pylori lipoprotein HP1454 stimulated proliferation in CD4+ T cell clones from gastric tissues of H pylori-infected patients, and the infection induced a HP1454-specific response. The clones secreted TNFα, IFNγ and IL-17, indicative of Th1 and Th17 cells. The authors concluded that HP1454 is an important T cell antigen, with properties likely to promote gastric inflammation.13

IL-21 plays an important role in gastritis in H pylori-infected mice, and the mucosal Th1 and Th17 response is reduced in IL-21−/− mice. Yasmin et al showed significantly reduced IL-17A expression in the Peyer’s patches and mesenteric lymph nodes of infected IL-21−/− mice compared to WT mice. Exposure of H pylori-infected dendritic cells (DCs) to IL-21 inhibited expression of the costimulatory molecule CD40, and impaired the ability of DCs to induce antigen-specific Th17 cell proliferation. IL-21 may therefore have both pro-inflammatory and immunoregulatory activities, the balance of which may affect H pylori disease outcome.14

Innate lymphoid cells (ILCs) are important in regulating immune responses and homeostasis of the intestine. Bostick et al studied the effects of two intestinal Helicobacter species, Helicobacter apodemus and Helicobacter typhlonius, on ILC frequencies. These infections resulted in ILC activation and inflammatory damage to the gut mucosa, but suppressed the activity and proliferation of type 3 ILCs (ILC3s).15
2.2 | Neutrophils, macrophages and dendritic cells

Chu et al characterised the role of hepatoma-derived growth factor (HDGF) in *H pylori*-mediated gastritis. In human gastric biopsies, high expression was associated with *H pylori* colonisation and neutrophil scores. In infected HDGF−/− mice, gastric neutrophil infiltration, TNFα and cyclooxygenase-2 (COX-2) responses were reduced. Exposure of neutrophils to HDGF in vitro induced differentiation and chemotactic responses. Yang & Wang found upregulated expression of the neutrophil-associated antigen CD177 in the gastric mucosa of *H pylori*-infected mice, which correlated with gastritis severity. Gastritis scores were similar in infected WT and CD177−/− mice, indicating that CD177 expression is stimulated by inflammation.

Gobert et al found that *H pylori* activates the reverse transsulfuration pathway (RTP) in macrophages. RTP involves enzymes including cystathionine γ-lyase (CTH) to produce hydrogen sulphide from l-cysteine or homocysteine. CTH expression was induced in *H pylori*-infected macrophages, which interfered with macrophage activation via metabolic disruption, accumulation of putrescine, and histone modifications. They concluded that CTH is subverted by *H pylori* for immune evasion.

Two signals are needed for NLRP3 inflammasome activation and production of mature IL-1β by immune cells. Signal 1 induces expression of inflammasome components, and signal 2 provided by host and microbial factors (including adenosine triphosphate [ATP] and the microbial toxin nigericin) is required for inflammasome scaffold formation and activation. Pachathundikandi et al investigated NLRP3 regulation using human monocytes and macrophages. They confirmed that *H pylori* infection increased pro-IL-1β expression, but secretion of the mature cytokine was low. When ATP or nigericin was added to infected cells, the NLRP3 inflammasome became active and high mature IL-1β secretion occurred. *H pylori* cannot generate signal 2; therefore, other factors in the inflamed gastric mucosa provide this.

Chownerawong et al showed that expression of NLR family CARD domain-containing 5 (NLRC5) was significantly higher in gastric biopsies from *H pylori*-positive compared to -negative patients. NLRC5−/− macrophages expressed significantly higher levels of inflammatory cytokines. Mice with macrophages unable to express NLRC5, had more severe *H felis*-induced gastric pathology than WT mice, and there were more abundant B-cell follicles. NLRC5 therefore inhibits *Helicobacter*-induced gastritis and prevents the development of lymphoid follicles.

Zhao et al reported that MALT lymphoma patients and *H felis*-infected mice had elevated numbers of myeloid suppressor cells (MDSCs) in their gastric mucosa, accompanied by higher numbers of IL-17-producing gamma delta T-cells (γδT17), indicating their role in the development of gastric MALT lymphoma. A subset of MDSCs in the SPEM gastric mucosa of *H felis*-infected mice express the myeloid differentiation factor Schlafen4 (SLFN4). Ding et al compared the transcriptomes of SLFN4+ and SLFN4− gastric MDSCs, and microRNA MIR130b was highly expressed by SLFN4+ cells. They showed that MIR130b plays an essential role in the function of MDSCs. MIR130b co-localised with a human homologue of SLFN4 in human gastric cancer tissue, and elevated levels of MIR130b were also present in the serum. MIR130b was shown to activate NFκB p65, contributing to inflammation and metaplastic changes in the stomach.

Codo et al reported that *H pylori* reduces HLA-II expression in macrophages, by suppressing expression of the class II MHC transactivator (CIITA). This was dependent on upregulation of miRNAs let-7f-5p, let-7i-5p, mir-146b-5p, and -185-5p. These miRNAs were increased in mucosal biopsies from gastritis patients, suggesting their role in immune evasion. Arnold et al showed that BATF3−/− mice expressed lower levels of CXCR3 and CXCL9/10 chemokines, which indicated the role of BATF3−/− DCs in the migration of CXCR3+ T-effector and Treg cells to infected sites and their local expansion.

3 | VACCINES

3.1 | Immunogenic antigens: Old friends and new candidates

Among the old and well know vaccine candidates, *H pylori* urease is considered to be a good antigen. Liu et al developed a multivalent subunit *H pylori* vaccine containing *H pylori* urease subunit A (UreA), urease subunit B (UreB) and neutrophil-activating protein (HPNAP) with double-mutant heat-labile toxin (dmLT) from *Escherichia coli*. Oral immunisation of *H pylori*-infected mice significantly reduced gastric bacterial colonisation and induced antigen-specific serum IgG, mucosal IgA, and Th1, Th2 and Th17 responses. Guo et al tested a multivalent epitope vaccine FVpE, containing HP-NAP, three peptides from CagA and VacA, and a urease multi-epitope peptide (UE) from CTB-UE, administrated with a polysaccharide adjuvant (PA). In mice, FVpE and the multi-epitope vaccine CTB-UE could induce specific antibodies against *H pylori* urease. However, only FVpE could induce high levels of specific antibodies to CagA, VacA, and NAP. Oral therapeutic immunisation with FVpE plus PA significantly reduced the *H pylori* colonisation density in Mongolian gerbils. The protection of FVpE was related to the mixed Th cell responses and epitope-specific antibodies against various *H pylori* antigens.

A vaccine design targeting conserved or essential genes is of major interest for vaccine development. Hornburg et al developed a combined discovery-driven mass spectrometry and computational strategy to identify bacterial vaccine candidates and validate their immunogenicity using *H pylori*. They isolated surface antigens by enzymatic cleavage and identified 72 surface-exposed antigens. One candidate jhp_0775, encoding for a hypothetical protein, induced specific B and T cell responses and significantly reduced colonisation levels in mouse therapeutic vaccination studies. In infected humans, jhp_0775 was immunogenic and activated IFNγ secretion from peripheral T cells.
Liu et al analysed the protein composition and potential vaccine function of outer membrane vesicles (OMVs) derived from H pylori strain 713. C57BL/6 mice were immunised orally with OMVs or an H pylori whole-cell vaccine (WCV), with or without cholera toxin (CT) as an adjuvant. Oral immunisation with OMVs elicited stronger serum and mucosal antibody responses than WCV plus CT. The OMVs predominantly induced Th2-biased immune responses that reduced bacterial loads. OMV-based vaccines could therefore be of great value in controlling H pylori infection.

HpaA, an H pylori surface-located lipoprotein was proposed as a putative vaccine candidate against H pylori infection. Xue et al showed that LP2, which mimics the terminal structure of native HpaA, was able to activate TLR2. An H pylori peptide antigen with the sequence Met-Val-Thr-Leu-Ile-Asn-Asn-Glu (MVTLINNE) was also used by Espinosa-Ramos et al in prophylactic immunisation. This peptide protected 100% of the mice against H pylori infection, but the immune mechanisms remain to be identified.

3.2 | In silico vaccine design could help in identifying new vaccine candidates

Recent advances in bioinformatics have increased research on novel vaccine candidates. HPAG1_0576 from H pylori HPAG1, which shares 98% identity with TNF-α inducing protein (Tip-α) of H pylori, was chosen by Ashrafi et al Bioinformatics analyses suggested the presence of immunogenic CD8+ and CD4+ T-cell epitopes and a potential to induce IFNγ-mediated responses. Cho et al reported on structure-based molecular studies of H pylori FliD (hpFliD). Assays with anti-H pylori antibodies demonstrated that hpFliD-specific D4 and D5 domains are highly antigenic, providing a molecular basis for further vaccine development.

Khan et al explored the H pylori proteome and designed a B and T cell multi-epitope subunit vaccine: antigenic epitopes were predicted from CagA, OipA and GroEL. Urrutia-Baca et al described a multi-epitope vaccine composed of CTB fused to epitopes from eleven H pylori proteins. Surface-exposed membrane proteins were predicted from 826 proteins of 53H pylori strains by Pasala et al, who proposed novel vaccine cocktails and insights for T-cell driven subunit vaccine design. Validation of these proposed vaccines is needed to verify their safety and immunogenicity.

3.3 | Recombinant antigen delivery modes

CagL is highly conserved amongst pathogenic H pylori strains. Aliramaei et al suggested that recombinant CagL could be a suitable vaccine candidate, expressed by Lactococcus lactis. After oral immunisation of mice, a significant increase in CagL-specific serum IgA, IgG, IL-17 and IFNγ was observed. CagL-specific IgA was also detected in the faeces. This vaccine is therefore immunogenic and able to induce mucosal responses. Mice orally immunised with recombinant L lactis expressing H pylori Lpp20 antigen also had elevated serum IgG levels and reduced gastric urease activity following H pylori challenges.

3.4 | Immune response and vaccine efficacy

An effective vaccine should prevent initial acquisition of the infection and protect against reinfections. Longet al demonstrated that prophylactic intragastric immunisation with a whole-cell killed H pylori antigen administered together with the non-toxic oral adjuvant α-galactosylceramide (α-GalCer) induced effective immune protection against H pylori infection in mice. This vaccine formulation elicited strong intestinal and systemic Th1 responses as well as significant antigen-specific mucosal and systemic antibody responses. They also reported that the protective intestinal Th1 responses induced were dependent on CD1d, IL-1R as well as IL-17R signaling. Therefore, α-GalCer could be a promising adjuvant for inclusion in an oral vaccine.

Nemattalab et al designed a study to compare the effects of Th1, Th2, Treg and Th17 modulatory effects on the efficacy of H pylori vaccine. They used a bicistronic vector for simultaneous expression of oipA gene and modified mice IL-18, IL-17A, IL-22 and Foxp3 cytokines. Four weeks after bacterial challenge, potent clearance of H pylori infection was seen. Highest cell-mediated immunity cytokines were produced in the IL-17 receiving group in which the Treg responses were suppressed previously by the administration of the Foxp3 as an immunogen.

Liu et al showed that Trm cells are enriched at the sites of previous infection and required for enhanced protective immunity. They suggest that conventional vaccine strategies are unable to establish a measurable antigen-specific memory cell pool in the stomach. In comparison, gastric subserous injection of mice with a micro-dose of an alum-based H pylori vaccine can induce a pool of local CD4+ Trm cells. Upon Helicobacter infection, CD4+ Trm cells orchestrate a swift recall response with the recruitment of circulating antigen-specific Th1/Th17 cells to trigger a tissue-wide pathogen clearance. This study highlights the need to design a vaccine strategy against H pylori by establishing the protective CD4+ Trm cells.

Xu et al showed that vaccine-induced gastric CD4+ Trm cells proliferate in situ to amplify the immune response against H pylori. A decrease in H pylori colonisation was observed in the vaccinated mice which could be related to the presence of pro-inflammatory myeloid cells still in the stomach of the vaccinated mice. Akter et al aimed to find immune correlates to vaccine efficacy, the frequencies of neutrophils, eosinophils and inflammatory monocytes and CD4+ T-cell memory and mucosa homing integrin α4β7 cells. H pylori antigens and cholera toxin or the multiple mutant CT (mmCT) were administered via the sublingual (SL) and intragastric route (IG). They observed that in the blood of mice after SL or IG route of vaccination, changes in frequencies of innate and adaptive immune cell subsets were present compared to infection controls. In particular, the frequency of circulating mucosal homing α4β7+ C-D4+T cells after vaccination correlated with low bacterial load in the...
stomach of individual mice irrespective of the immunisation route. This study revealed that the innate and adaptive immune cell subsets can be measured in the blood after vaccination and that an increased frequency of α4(7+) CD4+ in the blood after immunisation could act as a predictor for vaccine efficacy.42

As reported by Sutton and Boag,43 there are currently no advanced vaccine candidates. No new clinical trials were reported.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.


INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is considered as the most prevalent human pathogen infecting more than 50% of the worldwide population. Its role in non-malignant infectious diseases of the human stomach is indisputable. The role of *H. pylori* in esophageal diseases remains controversial and is driven by clinical observations. The prevalence of *H. pylori* associated peptic ulcer diseases (PUD) is decreasing worldwide; however, within this context many clinical challenges still remain. Furthermore, molecular alterations induced by *H. pylori* infection in non-malignant upper gastrointestinal conditions remain underexplored and this field mandates further research.

This review summarizes the advances in epidemiologic, clinical, and basic science knowledge published during the period March 2019 to April 2020 in the field of *H. pylori* and non-malignant diseases of the upper gastrointestinal tract.

2 | ESOPHAGEAL DISEASES

2.1 | Gastroesophageal reflux disease

Conflicting data regarding the interaction of *H. pylori* infection and gastroesophageal reflux disease (GERD) were published during the last decades. The group of Yalaki et al studied the influence...
of different locations of *H. pylori* infection and the histopathological findings related to reflux disease. As expected, the incidence of *H. pylori* infection in patients with GERD was lower than in controls. A significantly lower amount of *H. pylori* infection was found in the cohort of subjects presenting with erosive reflux disease compared to controls whereas no differences were detected in antrum predominant gastritis and atrophic lesions. The authors concluded that hypochloridity driven by corpus predominant gastritis was the reason for these observations. Screening by esophagogastroduodenoscopy (EGD) before bariatric surgery is frequently applied to detect contraindications which may change the planned surgical procedure. Also, screening for *H. pylori* infection is recommended to reduce post-operative complications driven by the infection. AEIid et al analysed the findings in 356 patients suffering from obesity who were scheduled for bariatric surgery. Forty-one percent of their cohort tested positive for *H. pylori* infection and 4% presented reflux esophagitis.

An increasing number of patients take acid suppressive therapies in a regular manner. To determine the appropriateness of prescribing these drugs, Franchi et al analysed data from 101 medical and geriatric wards in Italy. Appropriateness was defined for specific clinical findings such as history of peptic ulcer, history of gastrointestinal hemorrhages, advanced age, use of specific medications for acid suppression or *H. pylori* infection. For nearly half of the patients (n = 1776, 45.6%, 95% confidence interval (CI): 44.0%-47.1%), acid suppressive drugs for a given indication were inappropriately prescribed or even not prescribed. Sixty percent of acid suppressive therapies were overprescribed and in the subgroup of non-prescribed patients; 22% required acid suppressive treatment in line with current guidelines.

The characteristics leading to the prescription of antisecretory drugs in patients without macroscopic lesions of the upper gastrointestinal tract and no evidence of *H. pylori* infection were analysed in 472 patients. Hiatus hernia, age and the intake of calcium channel blockers were associated with the new onset of acid suppressive drugs.

### 2.3 | Eosinophilic esophagitis

Eosinophilic esophagitis (EOE) remains the most common immune- and allergen-mediated disorder of the upper gastrointestinal tract. During the last decades, growing experimental and clinical data have suggested an inverse relationship between *H. pylori* infection and different immune-related and allergen-driven diseases such as asthma, inflammatory bowel diseases and also food allergies. Animal studies demonstrated a relationship between transmaternal or postpartum *H. pylori* exposure and the expansion of regulatory T-cell subsets expressing CXCR3 or retinoic acid-related orphan receptor *γt* and demethylation of the forhead box P3 (FOXP3) locus. As in non-IgE-related diseases, the mechanisms of food allergy in EOE are mediated by Th2 cell activation by food and environmental antigens. Varying data regarding the association between *H. pylori* exposure and risk of EOE or esophageal eosinophilia led Shah et al to perform a systematic review and meta-analysis including 11 observational studies with data from 377 795 individuals from different regions all over the world. Exposure to *H. pylori* caused a 37% reduction in EOE (OR: 0.63; 95% CI: 0.51-0.78) and a 38% reduction in esophageal eosinophilia (OR: 0.62; 95% CI: 0.52-0.76). Interestingly the association was significant only in prospective trials independent of location, cohort, time period or regional *H. pylori* prevalence. This analysis supports the concept of evolutionarily expected positive and protective effects of *H. pylori* exposure. This protection might be triggered by *H. pylori*-induced alterations of immunological pathways...
with weakening of Th1/17 and consecutive effects, eg on regulatory T-cell response.14

3 | GASTRIC DISEASES

3.1 | Peptic ulcer disease

The debate about the cost-effectiveness of a test and treat strategy for prevention of PUD and other H pylori associated diseases is still ongoing. Høgh et al performed a randomised controlled trial with a 13-year follow-up in order to evaluate the cost-effectiveness of H pylori population screening and eradication in Denmark. The study showed no long-term effect of Danish population screening for H pylori when compared to usual care – neither on quality of life, PUD incidence, dyspepsia prevalence, nor on use of health care resources.15 Nevertheless, the idea of population-based screening and eradication of H pylori remains an attractive option in some regions. The analysis of a 10 million asymptomatic 20-year-old Chinese cohort showed that this strategy saved 1854 deaths from PUD during life expectancy. The authors concluded that, compared with a no-screen strategy, H pylori screen-and-treat strategy for the general Chinese population is cheaper and more effective in preventing H pylori-associated diseases.16

An American study showed that among patients hospitalised for bleeding PUD, admission to the intensive care unit (ICU) was the factor most frequently associated with non-adherence to H pylori testing guidelines (66% of patients in the ICU were tested vs 90% patients not in the ICU, P < .01). Considering this problem, which may also occur in other countries, new strategies might be required for proper H pylori testing.17 Recent data suggest that the proportion of gastroduodenal erosions/ulcers associated with H pylori infection in the paediatric population is lower than expected. However, a Belgian retrospective study showed that the proportion between H pylori-positive and -negative ulcers in children did not significantly change over the past two decades.18 On the other hand, H pylori infection prevalence continues to fall in the general population. Sonnenberg et al once again showed this tendency in the US general population: between 2009 and 2018 the general H pylori infection prevalence fell significantly from 11% to 9% with a decline in the fraction of H pylori-positive gastric ulcers from 17% to 14% and H pylori-positive duodenal ulcers from 25% to 21%. A higher H pylori infection prevalence remains among East Asians and Hispanics.19 Guo et al identified all patients from Hong Kong who received the first course of clarithromycin-based triple therapy between 2003 and 2012, and divided them into three cohorts according to aspirin use: new users (aspirin commenced after H pylori eradication) (n = 6985), chronic users (n = 5545) and non-users (n = 48 908). Compared with chronic users, new users had a higher risk of GI bleeding (hazard ratio (HR): 1.89, 95% CI: 1.29-2.70). The study showed that the increased risk in new aspirin users was only observed in the first 6 months for all GI bleeding (HR: 2.10, 95% CI: 1.41-3.13) and upper GI bleeding (HR: 2.52, 95% CI: 1.38-4.60), but not for lower GI bleeding. Lower GI bleeding was more frequent than upper GI bleeding in aspirin users after H pylori eradication.20

3.2 | Helicobacter pylori gastritis

As gastric cancer is usually diagnosed at late critical stages, there is still the need for novel biomarkers and perception of molecular pathways involved in early detection of H pylori gastritis patterns that carry a high risk profile for malignant transformation. A group of scientists performed a study that demonstrated increased Th9, Th17/9, Th9 TCg, and Th9/17 TCg in H pylori infection and their interaction during the immunopathological responses. They stated that an increased number of these cells may be influential in the progression and severity of H pylori infection. In addition, increased levels of IL-9 and IL-4, and TCg17/9 and Th17/9 were observed in chronic active gastritis patients.21 INS-GAS mice showed a gender-specific miR-20b expression pattern following H pylori infection. There was a stepwise increase in miR-20b expression at the different time points from 12 to 50 weeks with the highest difference at 50 weeks, that appear to occur in parallel with the progression of H pylori gastritis.22 Another international research group demonstrated that levels of specific miRNA – miR130b increase in gastric myeloid-derived suppressor cells (MDSCs), which express Schlafen4 factor (SLFN4+). As miR130b plays an essential role in MDSC function and the levels in the blood correlate with metaplastic changes in the stomach, this miRNA has the potential to be an early non-invasive biomarker for gastric metaplastic changes and early-stage gastric cancer.23

The gastric microbiota has received a lot of attention over recent years and there are continuous emerging data on its role in H pylori gastritis.24 Sung et al investigated gastric microbiota changes one year after H pylori eradication and their relationship with gastric precancerous states. Their study showed that some bacteria (Peptostreptococcus, Streptococcus, Parvimonas, Prevotella, Rothia and Granulicatella) were associated with development and persistence of gastric atrophy and intestinal metaplasia after H pylori eradication.25 Gastric microbiota has been mainly analysed in adults, while paediatric populations have been less explored. In accordance with the results from adult studies, the diversity and richness of gastric microbiota were in inverse correlation with H pylori gastritis in children, while H pylori eradication was helpful to restore the shifted gastric microbiota.26

4 | FUNCTIONAL DISORDERS OF THE UPPER GI TRACT

A connection between H pylori infection and functional gastro-intestinal disorders is generally accepted since effects of H pylori eradication on dyspeptic symptoms have been shown in different clinical trials.27 Based on current recommendations, patients with H pylori-associated dyspepsia should be considered as a separate group of patients with functional dyspepsia. These patients should
receive *H pylori* eradication treatment as a proof of entity. Several guidelines and consensus reports are available guiding the management of functional dyspepsia and *H pylori* infection. Facing the fact that the majority of patients are treated by primary care physicians, McNicholl et al performed an open on-line survey to evaluate the adherence and also limitations, perceptions and attitudes to guidelines among Spanish primary care physicians. Analysing 1445 responses, only 40% of the physicians had read at least one Maastricht consensus report. Sixteen percent reported no access at all to validated *H pylori* diagnostics and 33% of the responders do not systematically refer to eradication confirmation tests.²⁸ In conclusion, there is a clear need to improve the perception/availability and adherence to current guidelines. To define a cost-effectiveness strategy to diagnose and treat dyspeptic patients, Beresniak et al compared a Test and Treat strategy with symptomatic treatment and upfront upper gastrointestinal endoscopy focusing on cost-effectiveness based on Spanish medical resources. The Test and Treat strategy based on urea breath test was shown to be the most cost-effective approach for the management of dyspeptic patients and also in prevention of *H pylori* complications.²⁹ Simplifications of participation in Test and Treat strategies are needed to improve test frequency in dyspeptic patients. Therefore, Canadian pharmacists in a community setting offered *H pylori* antibody screening using a rapid response serological test to patients under acid-suppressive therapy for longer than six weeks.

This Test and Treat strategy involving easily accessible structures such as pharmacies had a positive impact on the detected and treated of undiagnosed *H pylori* infections.³⁰ Depending on local structures, prevalence of *H pylori* infection or parasitic gastric infection and the prevalence of gastric cancer diagnostic pathways differ strongly. A Cambodian cohort with 1231 dyspeptic patients, analysed retrospectively and divided into three subtypes according to their symptom-profile, underwent upper gastrointestinal endoscopy: 1065 (86.5%) patients demonstrated normal findings whereas 13.5% revealed macroscopic findings including 19 upper gastrointestinal tumours which were not detected using the Test and Treat strategy.³¹ An updated guideline of diagnostic algorithms was published in 2019 in Korea. With a weak grade of recommendation, the use of the Test and Treat approach for *H pylori* is considered for dyspeptic patients under 40 years without alarm symptoms who are not responding to acid suppressants or prokinetics.³² Due to the variety of different management strategies for uninvestigated dyspepsia, Eusebi et al performed a systematic review and meta-analysis regarding the effectiveness of different management strategies. Identifying 15 eligible randomised controlled trials, data from 6162 patients were included. The authors ranked the Test and Treat first, but with a similar relative risk (RR) of remaining symptoms compared to the prompt endoscopy approach (RR 0.89 vs 0.90). In general, no approach tested was significantly less effective than Test and Treat. From the patients’ viewpoint, dissatisfaction with the management approach chosen was significantly lower in the prompt endoscopy cohort than in the Test and Treat cohort, and in patients empirically treated with acid suppressive agents.³³ To enlarge the therapeutic spectrum, a group from Iran studied the effect of ginger (*Zingiber officinale* Roscoe) on *H pylori* eradication and on dyspeptic symptoms. In this pilot study, 53.3% of *H pylori* infected patients had a negative stool antigen test after four weeks of ginger supplementation. Severe dyspeptic symptoms decreased significantly after treatment, eg fullness, early satiety, nausea, belching, gastric burn and gastric pain. The authors considered ginger as a potential complementary therapy for patients with *H pylori*-induced functional dyspepsia.³⁴

An association between eosinophilic infiltration of the duodenum and dyspeptic symptoms was reported, but the influence of *H pylori* infection on eosinophilic infiltrates in the duodenum has not been well studied. A group from Brazil enrolled 63 patients with and without dyspeptic symptoms and with and without evidence of *H pylori* infection. The duodenal eosinophilic count in five high power fields was significantly higher in *H pylori* infected subjects than in uninfected controls (*P* = .005) but similar in those with or without dyspeptic symptoms.³⁵

### 5 | HELICOBACTER PYLORI AND CELIAC DISEASE

The possible relationship between celiac disease (CD) and *H pylori* infection is still under debate for more than 20 years. Conflicting data regarding associations between *H pylori* infection and CD are published mainly from pediatric cohorts.³⁶-⁴⁰ Different mechanisms to explain a potential protective effect of *H pylori* against CD including altered T-cell response in *H pylori* infected subjects and regulatory T-cell involvement which leads to a reduced risk of allergic and atopic diseases.⁴¹,⁴²

Narang et al performed a clinical trial on 324 children with confirmed MARSH grade III CD and examined *H pylori* infection histologically. Compared to non-CD children the prevalence of *H pylori* was significant higher in non-CD children (50% vs 11.4%, *P* < .001). The inverse relationship shown in this study exclude *H pylori* to be a risk factor for CD. It might exist a protective effect of CD against *H pylori* infection but a pathophysiological explanation is not given.³⁴

### ACKNOWLEDGEMENTS

None.

### CONFLICTS OF INTEREST

The authors have no potential conflicts of interest to declare.

### REFERENCES


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INTRODUCTION

Cancer immunotherapy, which impairs cancer evasion from immunosurveillance, has been recently explored as an effective therapeutic strategy for cancer treatment. In fact, inhibition of immune checkpoint proteins, programmed death ligand 1 (PD-1) and its ligand (PD-L1), has created a paradigm shift in cancer treatment. Cancer cells manage to escape immunosurveillance by up-regulating immune checkpoint proteins, such as PD-L1. However, this escape mechanism can be prevented with immune checkpoint inhibitors (ICI), which block the physical interaction between PD-1 and PD-L1. ICI have been recently approved for the treatment of patients with advanced or metastatic gastric cancer (GC), whose tumours express PD-L1 detected by immunohistochemistry (IHC). Nevertheless, the response rates are low, highlighting the need to elucidate the mechanisms underlying treatment resistance. In this review, we focus on the articles published in the past year concerning the relationship between GC, its immune microenvironment and response to immunotherapy.

IMMUNE MICROENVIRONMENT

The expression of PD-1 and PD-L1 in GC and its association with patients-survival rate was recently addressed. Ji-Hyun et al reported that 60% of GC cases expressed PD-L1 and found the expression to be significantly higher in patients with advanced disease stages. The authors also found that patients with positive PD-L1 expression had longer disease-free survival time and poor prognosis.

Microsatellite instability (MSI) is also a predictor of response to ICI. Due to impaired DNA-mismatch repair, MSI tumours typically present an increased mutation burden, which can result in the emergence of mutation-associated neoantigens that will trigger an increase in tumour lymphocyte infiltration. However, MSI status has
recently been evaluated in the context of both systemic immune response and tumour lymphocyte infiltration. Shin et al. looked at the MSI status of a cohort of GC cases and its association with tumour immune infiltration and systemic immune response to evaluate its prognostic value. They found that the stroma of MSI tumours shows a significant increase in the number and activation status of cytotoxic T lymphocytes (CD8^+ T cells). On the other hand, only a weak association was observed between the systemic immune response and local immune infiltration. Pernot et al. also performed an analysis of tumour infiltrating and peripheral immune cell composition in a cohort of advanced GC cases. They found that the percentage of circulating natural killer (NK) and regulatory T cells (Treg) were significantly lower in patients with a diffuse/mixed histological type in a cohort of advanced GC cases. They found that the percentage of circulating natural killer (NK) and regulatory T cells (Treg) were significantly lower in patients with a diffuse/mixed histological type than in patients with an intestinal histological type. Regarding tumour-infiltrating cells, they found less CD8^+ T cells in the diffuse/mixed-type. Moreover, they observed that patients with an elevated number of circulating NK cells and patients with increased tumour infiltrating CD8^+ T cells had a significantly longer overall survival. These results suggest that the low number of tumour infiltrating CD8^+ T cells and circulating NK cells can account for the worse prognosis observed in diffuse/mixed-type GC.

Using transcriptome analysis and deconvolution algorithms, Li et al. found that increased tumour infiltration by CD8^+ T cells and memory CD4^+ T cells, independent of the tumour histological type, predicted a favourable prognosis in GC patients. However, the opposite result was observed for increased tumour infiltration by M2 macrophages. These observations between tumour infiltrating CD8^+ T cells and M2 macrophages and GC patients' prognosis were also observed and reported by another group. A different group described that tumour infiltrating CD8^+ T cells were present at higher densities but were less functional in the diffuse histological type when compared with the intestinal histological type. Moreover, the diffuse type-associated CD8^+ T cell pattern was characterised by levels of immunosuppressive mediators, such as transforming growth factor (TGF)-β1.

Gastric cancer is a heterogeneous disease, with several histological and molecular subtypes. Thus, effective stratification strategies and selection of predictive biomarkers for immunotherapy are required. To better dissect the immune infiltrate of GC patients according to their histological subtype, Kim et al. retrospectively investigated the immune infiltrate of 43 patients classified according to the classification of The Cancer Genome Atlas (TCGA), which identifies Epstein–Barr virus positive (EBV^+), MSI, genomically stable, and chromosomal unstable GCs. The most prevalent immune cells were CD8^+ T lymphocytes and CD68^+ macrophages. EBV and MSI tumours were the most infiltrated, harboring 30%-50% T cells and 20% macrophages. Intestinal tumours contained fewer T cells but disproportionately more macrophages. Diffuse tumours were the least infiltrated. PD-L1 was most frequently expressed in intestinal tumours, whereas 70% of EBV and MSI tumours expressed PD-L1. In another study, Kim et al. also demonstrated a correlation between the molecular subtype and the immune composition within the tumour microenvironment. The authors observed that EBV^+ and MSI molecular subtypes, considered subtypes with better prognosis, were significantly associated with increased tumour lymphocyte infiltration (namely CD3^+ and CD8^+ T cells).

Gullo et al. analysed 24 GC with lymphoid stroma (GCLS) samples, according to the 2010 World Health Organization classification. They found that 16 of these cases were EBV^+ MSS (microsatellite stable), 4 were EBV^+ MSI-high and 4 were EBV^+ MSS. All EBV^+ cases showed a significantly higher number of CD3^+ T cells and CD8^+ T cells. Additional characterisation of the T cell profile showed an enrichment of genes related to cytotoxic T cells, Th1 cells and pro-inflammatory factors, along with genes indicative of activation of regulatory T cells (Tregs) and immune inhibitory checkpoints in EBV^+ GCLS samples. They also found PD-L1 expression to be frequent in cancer and stromal immune cells and restricted to EBV^+ and MSI molecular subtypes. High levels of PD-L1 were also observed in EBV^+ GC cell lines, being responsible for the suppression of T cell proliferation through modulation via the IFN-γ signaling pathway, highlighting the role of EBV infection in promoting an inflamed tumour microenvironment, which may be a potential predictive biomarker of response to targeted immunotherapies. Another study examined the RNA expression profiles of 94 gastroscopic biopsies from 47 patients, including gastric precancerous lesions. They detected that the immune microenvironment was more active in early-stage GC than in precancerous lesions, with a significantly higher infiltration score. Among the immune cells present in the infiltrate, monocytes and M1 macrophages were the most abundant, although lymphocytes were also detected.

Increased levels of CXCL8 (also known as interleukin 8), predominantly secreted by macrophages, were identified to be indicative of a poor prognosis in GC patients. High CXCL8 secretion was associated with decreased CD8^+ T cell infiltration, proliferation and function by inducing the expression of PD-L1 on macrophages. The authors found that the small molecule CXR2 inhibitor (reparixin) drives the decreased PD-L1^+ macrophages and promotes antitumour immunity, providing potential therapeutic effects for GC patients.

As the tumour microenvironment (TME) is a critical component of tumour tissues and is not only composed of immune cells, recent research work emerged to clarify the impact of TME composition and prognosis stratification in GC. In a study by Wang et al., gene expression data were analysed to estimate tumour infiltration by stromal (non-immune) and immune cells, resulting in the generation of two signature scores: the stromal score and the immune score. The authors demonstrated that both stromal and immune scores were unfavourable factors for survival outcome and could be used to stratify patient prognoses and improve the prediction accuracy of the TNM staging system. In another study, Ren et al. explored the clinical significance of non-immune cells in the TME and their potential as biomarkers for PD-1/PD-L1 immunotherapy. The stromal score showed significant differences for different tumour stages and molecular subtypes. They observed that patients in the low stromal score group have a survival advantage and are the ones who could benefit most from ICI. Additionally, Zeng et al. estimated the TME composition of a large cohort of GC cases and correlated the TME
phenotypes with different genomic and clinicopathologic characteristics of tumours. From the three TME score groups defined by the authors, they observed that the low TME score subtype, characterised by activation of transforming growth factor β, epithelial-mesenchymal transition and angiogenesis pathways, was significantly associated with a worse prognosis.

In an attempt to systematically identify predictive molecular networks and key regulators that could identify biomarkers for immunotherapies in GC, several studies relied on high-throughput transcriptomic analysis of different cohorts. Ren et al evaluated the most differentially expressed genes (DEGs) from two datasets (tumour vs normal gastric tissue) of the Gene Expression Omnibus database. A Kaplan-Meier survival analysis was also performed to identify the genes with the strongest correlation with patient survival. GPNMB, which encodes a cell surface glycoprotein, emerged as one of the strongest candidates, being significantly associated with immunosuppressive cell-related markers. Further in vitro experiments revealed that GPNMB could activate the PI3K/AKT signaling pathway and induce CCL4 expression (a chemoattractant for NK cells). Overall, their results suggest the potential value of GPNMB blockade as a new immunotherapy against GC. Using single-cell gene expression analysis, Sathe et al demonstrated that GC TME suffers from a series of cellular changes compared to matched stomach mucosa. They reported an increased number of Tregs, which contribute to an immunosuppressive microenvironment and identified transcriptional cell states unique to GC in dendritic cells and two subclasses of exhausted cytotoxic T lymphocytes. More interestingly, they found that macrophages from the GC TME exhibited a heterogeneous gene expression profile that was not confined to a binary M1/M2 designation. Moreover, in this study, immune cell subtypes did not cluster clearly according to the samples’ MSI/MSS status. In a different study, Xiang et al identified IDO1 to be highly expressed in 4 GC cell lines (AGS, Hs746T, SGC-7901 and MKN-45) and to be positively associated with extracellular matrix formation and collagen processing. Expression of collagens in tumour tissues could not only ignite tumour invasion and metastasis but also prevent T cells from entering into the tumour tissue and impair a proper antitumour immune response.

### 3 | RESPONSE TO IMMUNOTHERAPY

In a study aiming at determining the predictive value of MLH1 and PD-L1 expression for prognosis and response to preoperative chemotherapy in gastric cancer, Hashimoto et al demonstrated that patients with resectable MSI GC, due to loss of mutL homolog 1 (MHL1), exhibit a good prognosis and negative response to fluorouracil-based chemotherapy. In a similar study, Di Bartolomeo et al showed that MSI status was independently associated with increased disease-free survival and overall survival in GC patients randomised for an intensive sequential chemotherapy regimen vs fluorouracil/leucovorin monotherapy as adjuvant treatment. It is noteworthy that MSI tumours are also the ones with a better response to current immunotherapies. However, there is an urgent need to identify additional predictive biomarkers to improve patient selection for ICI. Kim et al used a gene panel-based sequencing approach to demonstrate that, in addition to MSI and PD-L1 expression, the tumour mutation burden can be used as a predictive biomarker in GC patients treated with ICI. They showed that patients with a high tumour mutation burden have prolonged progression-free survival. Moreover, Jiang et al found mutated ARID1A to be associated with higher tumour mutation burden across different cancer types, and to confer prolonged overall survival in advanced cancers treated with ICI. These results suggest that ARID1A mutational status can be used to predict a survival benefit from immunotherapy across multiple cancer types, including GC.

New advances in high-throughput technologies have provided many insights into the molecular characterisation of GC. Nevertheless, reliable biomarkers for therapy response, especially in diffuse-type GC, are largely unknown. Based on transcriptome profiling analyses, Kim et al identified a molecular signature of distinct prognostic subtypes in diffuse-type GC: the intestinal-like (INT) and core diffuse-type (COD) subtypes. By integrating gene expression and mutational profiles, they showed that patients with the COD subtype benefit from standard chemotherapy, while patients with the INT subtype are responsive to ICI. With this approach, the authors unraveled a molecular signature that allows the identification of diffuse-type GC patients who will benefit the most from ICI.

Finally, in approximately 10% of advanced GC patients, the PD-1 blockade leads to rapid cancer progression, a phenomenon known as hyperprogressive disease (HPD). Kamada et al analysed tumour samples from HPD patients and observed that, in the majority of them, tumour-infiltrating FoxP3+CD45RA−CD4+ T cells (effector Treg) expressed PD-1 at equivalent levels as CD4+ or CD8+ effector/memory T cells. Comparison of GC tissue samples before and after anti–PD-1 therapy revealed that the treatment markedly increased proliferative (Ki67+) eTreg cells in HPD patients, contrasting with their reduction in non-HPD patients. These cells were highly activated, with a high expression of CTLA-4, and therefore highly suppressive. They also showed that genetic ablation or antibody-mediated blockade of PD-1 increased eTreg proliferation and suppressive capacity of antitumour immune responses in mice. This study suggests that the presence of actively proliferating PD-1+ eTreg cells in tumours is a reliable marker for HPD.

### 4 | CONCLUSIONS

Immunotherapy has been revolutionising the oncology field in recent years. Although GC was not among the first successful targets of this therapeutic approach, it has been slowly making its way into the realm of immunotherapy. In the past year, several studies were published showing that the expression of immune-related proteins by GC cells as well as the abundance and nature of the immune infiltrate influence the behaviour of GC. The expression of immune checkpoint proteins tends to correlate with worse survival, whereas
tumour infiltration with immune effector cells such as CD8⁺, CD4⁺, and NK cells tends to correlate with better survival. It is also interesting that MSI⁺ and EBV⁺ positive tumours tend to be associated with immunologically active tumours, indicating that these tumours might benefit from immunotherapy approaches.

Regarding GC response to immunotherapy the literature is scarcer. However, the few studies published are good indicators that the same biomarkers used successfully in other tumour types will also be effective for GC. Finally, several studies suggest that GC histological/molecular subtypes should be considered in therapeutic decisions, particularly for immunotherapeutic eligibility.

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CONFLICT OF INTERESTS
The authors have no competing interests.

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Review: Prevention and management of gastric cancer

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Abstract
Gastric cancer (GC) is still the fifth most frequently diagnosed cancer and the third leading cause of cancer deaths in both sexes worldwide. Although the incidence of GC is predicted to continue declining in a growing number of countries in the future, on a global scale the number of newly diagnosed GC cases will remain high, or increase even further, due to changes in population size and increasing risks observed in younger generations. In a retrospective cohort study, collecting data from the Veterans Health Administration, treatment of Helicobacter pylori infection decreased GC risk only if eradication was successful. In a German case-control study, among GC patients with autoimmune gastritis, pernicious anemia was associated with earlier detection of GC, which translated into a significantly better 5-year survival. In an updated meta-analysis, H. pylori eradication therapy in healthy individuals significantly reduced both GC incidence and mortality from GC with a number needed to treat of 72 and 135, respectively. In Korea, successful H. pylori eradication substantially reduced GC incidence in first-degree relatives of GC patients as well. A meta-analysis of four trials including 1,556 patients with resectable GC reported that the patient subgroup tumors with high microsatellite instability undergoing surgery did not benefit from perioperative or adjuvant chemotherapy.

KEYWORDS
autoimmune gastritis, epidemiology, gastric cancer, Helicobacter pylori, prevention, therapy

1 | INTRODUCTION

Helicobacter pylori infection is the principal risk factor for gastric cancer (GC). During the past year, new important epidemiological data indicated that the prevalence of GC is changing. Retrospective studies and randomized controlled trials (RCTs) stressed the importance of the success of H. pylori eradication for effective GC prevention. Microsatellite instability (MSI)-high status is gaining attention for its role as a prognostic, and possibly predictive biomarker in patients with GC. This review summarizes recent epidemiological aspects and clinical advances in the field of GC prevention and therapy published between April 2019 and March 2020.

2 | METHODS

The authors performed an independent search on PubMed on April 2020 for publications on GC during the previous year. The only filter applied to the searches was the “custom date range” for articles published between April 2019 and March 2020. The Boolean operator ‘AND’ was used to narrow the search results. The following search term combinations were used: “gastric cancer” [All Fields] AND “epidemiology” [All Fields]; “gastric cancer” [All Fields] AND “prevention” [All Fields]; “gastric cancer” [All Fields] AND “therapy” [All Fields]. Studies included and discussed in the present review were selected by the authors for their relevance and importance to the field of GC.
### 3 | EPIDEMIOLOGICAL ASPECTS

GC still ranks as the fifth most frequently diagnosed cancer and is the third leading cause of cancer death in both sexes worldwide. However, GC incidence is steadily decreasing globally, and in some populations is now regarded as a rare disease.

In a registry-based study, Arnold et al extracted data on GC incidence by year of diagnosis, sex, and age from 92 cancer registries in 34 countries, based on the International Classification of Diseases, 10th revision: C16. The numbers of new cases and age-standardized incidence rates per 10,000 by country, sex, and age, beginning in 2012, were extrapolated to 2035 and fitted to recent trends using a log-linear age-period-cohort model that levels off exponential growth and limits linear trend projection. According to their analysis, overall GC incidence rates will fall further in both high- and low-incidence countries (ie, Japan and Australia, respectively). They also predict that, by 2035, GC incidence rates will fall below the rare disease threshold (defined as 6 per 100,000 person-years) in 16 out of the 34 evaluated countries. In contrast, alarming incidence increases will be observed in younger age groups (below 50 years of age) in both low-incidence and high-incidence populations. On a global scale, they predicted that the number of newly diagnosed cases will remain high or increase even further. Changes in the population size and structure, as well as in the prevalence of risk factors, especially in those aged below 50 years, are likely to explain this development. Thus, while in some regions GC will become a rare disease, in others it will remain a major public health challenge.

*Helicobacter pylori* infection is the most well-known risk factor for GC. Based on a cohort of 371,813 patients (median age 62 years; 92.3% male) from the Veterans Health Administration in the United States, who received a diagnosis of *H. pylori* infection from 1994 to 2018, Kumar et al aimed to calculate the incidence of and risk factors for non-cardia gastric adenocarcinoma after detection of *H. pylori* and to identify how treatment and eradication affect cancer risk using a time to event (ie, diagnosis of cancer) with competing risk analysis (with death before cancer as a competing risk).

Patients with *H. pylori* infection were identified based on results of endoscopic pathology, stool antigen test, urea breath test, prescription for one of 11 accepted *H. pylori* eradication regimens, as recommended by the American College of Gastroenterology, or *H. pylori*-associated International Classification of Diseases (ICD), Revision 9/10 codes. For patients with multiple criteria, the criterion with the earliest date was used. Patients with non-cardia GC were identified using the Veterans Affairs Central Cancer Registry and/or ICD 9/10 codes.

With respect to non-cardia GC, the cumulative incidence of cancer was 0.37% at 5 years and increased to 0.5% and 0.65% after 10 and 20 years post-detection of *H. pylori* infection, respectively. Older age and a history of smoking both slightly increased the cancer risk, with a sub-hazard ratio [SHR] and 95% confidence interval [CI], of 1.13 (1.11-1.15) and 1.38 (1.25-1.52), respectively. The risk of GC was roughly doubled in racial and ethnic minorities, with a SHR and respective 95% CI of 2.52 (1.64-3.89), 2.00 (1.80-2.22), and 1.59 (CI, 1.34-1.87) in Asian, African American, and Hispanic, or Latino ethnic groups, respectively. The non-cardia GC risk was still slightly increased in patients who received *H. pylori* eradication treatment (SHR: 1.16; 95% CI: 0.74-1.83) but substantially reduced in the subgroup of patients with successfully confirmed *H. pylori* eradication (SHR: 0.24; 95% CI: 0.15-0.41).

*H. pylori* infection increases the risk of non-cardia GC unequivocally, whereas its protective role against esophageal adenocarcinoma and proximal GC is debatable. In a retrospective study, Kumar et al aimed to identify, in the aforementioned Veterans Health Administration cohort of patients with previously diagnosed *H. pylori*, the risk factors for future esophageal adenocarcinoma and cardia GC. Compared with whites as the reference population, the risk of future esophageal adenocarcinoma or cardia GC was similar among African Americans (SHR: 0.87, 95% CI: 0.57-1.43) and American Indians (SHR 1.31; 95% CI, 0.18-9.60) but substantially reduced in Asians (no cases among 213 *H. pylori*-positive) or native Hawaiian origin (no cases among 295 *H. pylori*-positive). Increasing age and smoking were confirmed as risk factors for esophageal adenocarcinoma and cardia GC (SHR: 1.17, 95% CI: 1.09-1.25 and SHR: 2.06, 95% CI: 1.33-3.18, respectively). Neither prescription of *H. pylori* treatment, nor eradication status, were associated with future esophageal adenocarcinoma or cardia GC.

Although *H. pylori* gastritis is the main risk factor for GC, patients with autoimmune gastritis (AIG) may develop GC as well. In a case-control study from the German centers of the staR project on GC research, Weise et al assessed the characteristics and outcomes of GC patients with AIG. From 2013 through 2017, they recruited 759 patients with GC and documented presenting symptoms using a self-administered questionnaire. Histological assessment of gastric mucosa was available for 572 of 759 GC patients. Overall, 28 (4.9%) GC patients had AIG (mean age 67.9 years, female-to-male ratio 1.3:1). Patients with AIG were matched in a 1:2 fashion for age and gender to GC patients with no AIG. Paraffin-embedded specimens of gastric mucosa from GC patients with and without AIG were assessed centrally by a reference gastrointestinal (GI) pathologist. In patients with AIG, GC was more likely to be localized in the proximal stomach (ie, cardia, fundus, corpus) (OR: 2.7, 95% CI: 1.0-7.1). In GC patients with AIG, pernicious anemia was the leading clinical sign (OR: 22.0, 95% CI: 2.6-187.2) and represented the most common indication for esophagogastroduodenoscopy (OR: 29.0, 95% CI: 7.2-116.4). GC patients with AIG were more likely to present without distant metastases (OR: 6.2, 95% CI: 1.3-28.8) and to be treated with curative intention (OR: 3.0, 95% CI: 1.0-9.0). The 5-year survival rates with 95% CI in GC patients with and without AIG were 84.7% (83.8%-85.6%) and 53.5% (50.9%-56.1%), respectively (OR: 0.25, 95% CI: 0.08-0.75, P = .001). The authors concluded that in AIG patients, pernicious anemia was associated with earlier detection of GC, which was associated with significantly better clinical outcomes.

### 4 | PREVENTION STRATEGIES

New data have accumulated on the effect of *H. pylori* eradication in preventing GC in *H. pylori*-positive healthy individuals and in patients...
with gastric neoplasia undergoing endoscopic mucosal resection. In an updated meta-analysis, with a literature search date through to February 2020, Ford et al identified 10 RCTs, comparing the effect of *H. pylori* eradication therapy vs. placebo or no treatment on future GC incidence.⁵ Participants were comprised of 8,323 healthy *H. pylori*-positive adults and 1,841 *H. pylori*-positive patients with gastric neoplasia undergoing endoscopic mucosal resection. All but one of the trials were conducted in East Asia. Follow-up lasted 2 years or longer. In the pooled analysis, *H. pylori* eradication therapy reduced GC risk among first-degree relatives of patients with GC to receive either eradication therapy (lansoprazole (30 mg), amoxicillin (1000 mg), and clarithromycin (500 mg), each taken twice daily for 7 days) or placebo.⁶ Overall, a significant reduction in future GC incidence was also demonstrated in patients already harboring gastric neoplasia (RR = 0.49; 95% CI: 0.34-0.70, NNT = 21) but undergoing *H. pylori* eradication therapy after endoscopic mucosal resection.

First-degree relatives of GC patients with *H. pylori* infection are themselves at increased risk of developing GC. In a single-center, double-blind RCT, Choi et al demonstrated that successful *H. pylori* eradication therapy reduced GC risk among first-degree relatives of patients with GC. They randomly assigned *H. pylori*-infected first-degree relatives of patients with GC to receive either eradication therapy (lansoprazole (30 mg), amoxicillin (1000 mg), and clarithromycin (500 mg), each taken twice daily for 7 days) or placebo.⁶ Overall, 1,676 participants were included in the modified intention-to-treat population. During a median follow-up of 9.2 years, GC developed in 10 participants (1.2%) in the treatment group and in 23 (2.7%) in the placebo group (HR: 0.45; 95% CI: 0.21-0.94; *P* = .03 by log-rank test). In the treatment group, five out of the 10 participants in whom GC developed (50.0%) had persistent *H. pylori* infection. Thus, GC developed in 0.8% of participants (5 of 608) with confirmed *H. pylori* eradication and in 2.9% of participants (28 of 979) with persistent infection (HR: 0.27; 95% CI: 0.10-0.70).

### 5 | Treatment

Mismatch repair deficiency (dMMR)/-microsatellite instability (MSI)-high status is gaining attention for its possible role as a prognostic and possibly predictive biomarker in patients with GC. However, GCs with dMMR/MSI-high status represent only 9% to 22% of all diagnosed GC cases, and thus large data sets are needed to draw robust evidence concerning its prognostic/predictive value.

In an individual patient data meta-analysis of four prospective trials, Pietrantonio et al investigated the value of MSI as a biomarker in 1,556 patients with resectable GC.⁷ Briefly, in the MAGIC and CLASSIC trials, patients were randomized to receive or not receive perioperative or adjuvant chemotherapy, respectively, whereas in the ITACA-S and ARTIST trials, patients were randomized to receive two different schedules of adjuvant chemotherapy and chemotherapy, with or without concurrent irradiation, respectively. The 5-year overall survival (OS) of patients with MSI-high tumors was significantly higher compared with patients with MSI-low/microsatellite stable (MSS) tumors (77.5% vs. 59.3%, respectively). A 9% increase in 5-year OS was confirmed in the subgroup of MSI-low/MSS GC patients receiving

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Biomarker-driven approved strategies for patients with advanced inoperable or metastatic gastric/esophagogastric junction cancer*</th>
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<tbody>
<tr>
<td><strong>Group 1</strong> HER-2 negative, MMRp/MSS, PD-L1 CPS &lt; 1</td>
<td><strong>Group 2</strong> HER-2 positive, MMRp/MSS, PD-L1 CPS &lt; 1</td>
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<tr>
<td><strong>First-line</strong></td>
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<tr>
<td>• FP + Platin (doublet), or</td>
<td>• FP + Cisplatin +Trastuzumab</td>
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<tr>
<td>• FP + Irinotecan (doublet), or</td>
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<tr>
<td>• FP + Platin +Docetaxel (triplet)</td>
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<tr>
<td><strong>Second-line</strong></td>
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<tr>
<td>• Paclitaxel + Ramucirumab, or</td>
<td>• Treatment as in group 1</td>
</tr>
<tr>
<td>• Docetaxel, or</td>
<td></td>
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<tr>
<td>• Irinotecan, or</td>
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<tr>
<td>• Paclitaxel, or</td>
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<tr>
<td>• Ramucirumab</td>
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<tr>
<td><strong>Third-line</strong></td>
<td></td>
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<tr>
<td>• Trifluridine/tipiracil, or</td>
<td>• Treatment as in group 1</td>
</tr>
<tr>
<td>• Irinotecan (if not received in previous lines)</td>
<td></td>
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<tr>
<td>• Nivolumab*²</td>
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</tbody>
</table>

Abbreviations: CPS: combined positive score, defined as the number of PD-L1-positive cells (tumor cells, lymphocytes and macrophages) out of the total number of tumor cells x 100; FP, fluoropyrimidine (infusional 5-FU, capecitabine or S-1); HER-2, human epidermal growth factor receptor 2 (also HER2/neu or ERBB2); MMRp/MMRd, mismatch repair proficient/deficient; MSS/MSI-H, microsatellite stability/high microsatellite instability; PD-L1: programmed cell death 1 ligand 1.

*For patients who have no satisfactory alternative treatment options.

*Application for healthy insurance reimbursement may be required. Studies with pembrolizumab in earlier treatment lines are ongoing.

*Approval status may differ according to regulatory authorities for drug safety.
chemotherapy plus surgery, compared with surgery alone (62% versus 53%, respectively, HR: 0.75, 95% CI: 0.60 - 0.94). Conversely, patients with MSI-high GC did not benefit from chemotherapy (5-year OS: 75% versus 83%, respectively). Thus, for patients undergoing surgery with curative intention for GC, MSI is a robust prognostic marker that should be adopted as a stratification factor in future trials. Prospective trials investigating the role of perioperative chemotherapy omission and/or immune checkpoint blockade in MSI-high GCs are warranted.

No new treatment emerged for patients with advanced non-resectable GC in the last year (Table 1).8,9

6 | CONCLUSIONS

Although in the near future GC incidence rates will fall below the rare disease threshold in many countries, on a global scale the number of newly diagnosed GC cases will remain high, or increase even further, due to changes in population size and increasing risks observed in younger generations. Successful eradication of *H. pylori* is the key to GC prevention. Accumulating evidence suggests that, in patients with MSI-high resectable GC, chemotherapy may be omitted. Precision oncology has become the standard of care for a selected group of patients, but no advances were made in systemic therapy for the majority of patients with advanced GC during the last year, and thus, more efforts in this field are warranted.

CONFLICT OF INTEREST

MV is involved in speakers’ bureau or consulting: Nordic Pharma, Merck Serono, Bayer Vital, Lilly, and Sirtex and is a member of the advisory boards of Ipsen, Lilly, Nordic Pharma, BMS, MSD, Eisai, and Amgen. PM is involved in speakers’ bureau or consulting: Biocodex, Biohit, Danone, Mayoly-Spindler. AC F. and TR declare no conflict of interest.

REFERENCE


INTRODUCTION

In the last decades, a multitude of investigations have reported a link between Helicobacter pylori infection and a variety of extra-gastroduodenal manifestations. However, for several of these, an aetiological role of such bacteria has not been confirmed, due to epidemiological limitations of the studies and the multifactorial aetiology of the investigated diseases.

This review reports the most relevant studies on this topic, published between April 2019 and March 2020, identified by searching for the term “Helicobacter” in the MEDLINE/Pubmed database. Consistent data emerged from studies investigating metabolic syndrome and ischaemic cardiovascular diseases. Other reported fields of investigation were hepatology, especially focused on non-alcoholic steatohepatitis, neurology, including Parkinson's disease and Alzheimer's disease, as well as dermatology. Inflammatory bowel disease (IBD), that comprises Crohn's disease and ulcerative colitis, may originate from a dysregulation of the host's immune response to commensal bacteria in individuals with genetic predisposition. The reduction of biodiversity and other specific imbalances in the faecal microbiome composition of IBD patients compared to that of healthy controls support this hypothesis. In this context, an inverse correlation between H pylori infection and IBD prevalence has been confirmed. Similar results were found in patients with kidney diseases and allergic manifestations. There are indications of the possible involvement of H pylori infection in metabolic syndrome and ischaemic cardiovascular diseases. However, due to a series of factors linked to study designs and the multifactorial pathogenesis of some diseases, further studies are needed.
2 | METABOLIC DISORDERS

2.1 | Metabolic syndrome and obesity

Metabolic syndrome (MS), described in 1988 as insulin resistance (IR) syndrome or syndrome X, includes obesity, dyslipidaemia, hyperglycaemia, high blood pressure and IR. Clustering of these components is associated with increased risk of both diabetes mellitus (DM) and coronary heart disease (CHD). In a Korean multicentre study, including 21 106 participants enrolled for a health check-up, H pylori seropositivity was associated with a higher total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), and lower high-density lipoprotein (HDL-C) levels than seronegativity (P < .05). The prevalence of MS was higher in H pylori-seropositive subjects than in negative ones (27.2% vs 21.0%; P < .05), and H pylori seropositivity increased the likelihood of MS (odds ratio [OR], 1.19; 95% confidence interval [CI], 1.09-1.31; P < .001). This association disappeared in subjects ≥65 years old. Another study showed that H pylori infection, detected with the urea breath test (UBT), was significantly associated with MS. In contrast, in a cross-sectional, longitudinal study, no association among H pylori seropositivity, body mass index (BMI) and metabolic diseases (type 2 DM, hypertension and dyslipidaemia, gout or increased uric acid) or between H pylori seropositivity and incident metabolic diseases/risk factors was found. A systematic review evaluated the relationship between H pylori infection and obesity among Chinese adults. The prevalence of H pylori was 42% (95% CI, 37-47) and mean difference of BMI between subjects with and without H pylori infection was 0.94 (95% CI, −0.04 to 1.91). The OR value was 1.20 (95% CI, 1.13-1.28), confirming that H pylori infection could be a risk factor for obesity.

2.2 | Diabetes

A systematic review identified 41 case-control studies investigating the relationship between H pylori infection and DM. The pooled OR was 1.27 (95% CI, 1.11-1.45; P = .0001), significant only among patients with type 2 DM (OR, 1.43; 95% CI, 1.11-1.85) and more evident in Asians. In agreement, a meta-analysis including 35 studies with 4401 diabetic patients, showed that glycated haemoglobin (HbA) levels were significantly higher in patients with H pylori infection compared to uninfected subjects (weighted mean difference = 0.50; 95% CI, 0.28-0.72; P < .001), without any difference related to the type of DM or study design. In Saudi Arabia, a study included 212 type 2 DM patients aged ≥ 40 years and 209 age-matched non-diabetic subjects. H pylori prevalence was significantly higher in overweight and obese non-diabetic subjects (P = .013). Obese participants in both groups had the highest prevalence of infection (57.9% and 54.5%). Thus, in this cohort, H pylori infection was associated with BMI but not with DM. Similar results were obtained in a Korean retrospective study on 16 091 non-diabetic persons. During the follow-up, 1338 subjects developed DM, with an incidence unrelated to H pylori seropositivity (Hazard ratios [Hrs], 1.01; 95% CI, 0.88-1.16; P = .854). The latter was not associated with impaired glucose tolerance (P = .566), diabetic nephropathy (P = .952) or poor glycaemic control (P = .535).

In a cross-sectional study, including 58 482 Chinese adults, H pylori-positive participants had a higher DM rate (7.3% vs 5.2%; P < .001), fasting plasma glucose (P < .001) and glycated HbA1c (P < .001) than uninfected ones. Multivariate regression analysis confirmed that H pylori infection was related to DM (OR, 1.25; 95% CI, 1.15-1.35), especially in participants aged ≥ 44 years. Interestingly, in Japan, the risk of DM decreased from 1.36 (95% CI, 1.10-1.67) to 0.92 (95% CI, 0.79-1.07) after H pylori eradication, concordant with the findings that H pylori eradication decreased HbA1c levels in diabetic patients.

3 | CARDIOVASCULAR AND CEREBROVASCULAR DISEASES

Although knowledge about the classical risk factors for CHD has increased over time, it fails to explain all of the epidemiological and clinical differences observed. After the first report in 1994, on the association between H pylori infection and CHD, several studies with controversial results were published. Defining whether the bacterium plays a pathogenic role during acute coronary syndrome (ACS) or during the gradual, progressive increase of atherosclerotic stenosis is a crucial step. Hitherto, the first pathway seems more relevant. Indeed, long-term inflammation may raise cytokine (interleukin (IL)-6) and tumour necrosis factor (TNF)-α levels in the bloodstream, and consequently activate fibroblast and smooth muscle cell proliferation.

Fang et al, in a systematic review with meta-analysis, found that H pylori infection was associated with an increased risk of ACS (OR, 2.03; 95% CI, 1.66-2.47), higher for developing countries than for developed countries (OR 2.58 vs OR 1.69). Choi et al, using an evaluation of cardio-ankle vascular index (CAVI), correlated arterial stiffness, a predictor of cardiovascular events, to anti-H pylori IgG antibody. Median age (P < .001), systolic blood pressure (P = .027), LDL-C levels (P = .016), median CAVI value and proportion of subjects with high CAVI value (both P < .001) were significantly higher in case of H pylori seropositivity. In 198 487 Koreans treated for hypertension, H pylori eradication reduced the risk of mortality due to cerebrovascular disease (P = .007) but not to CHD.

Testerman et al evaluated the role of H pylori in the development of atherosclerosis in pre- and post-menopausal cynomolgus monkeys. Ninety-four pre-menopausal animals were fed an atherogenic diet (lactalbumin (CL)) or high isoflavone soy (SOY) for 36 months followed by ovariectomy and an additional 36 months on the same or the alternate diet. H pylori infection was found in 46% of the monkeys with coronary atheromas, and correlated with an increased intimal plaque area and thickness independent of the menopausal status and regardless of diet (P < .01). Additionally, the plasma lipid
profile was altered in infected animals, independently of diet or hormonal status.\textsuperscript{21}

In a prospective Chinese study, including 17,613 patients screened with both carotid ultrasound examination and \textsuperscript{13}C-UBT, \textit{H pylori} infection was an independent risk factor for prevalent and incident carotid atherosclerosis only in males under 50 years ($P = .009$ and $P = .028$, respectively).\textsuperscript{22}

To support the role of \textit{H pylori} infection in the pathogenesis of CHD via endothelial damage, Li et al showed that the presence of this microorganism increased the expression of miR-25 in gastric epithelial cells as well as in human peripheral blood. The exosome-associated miR-25, through targeting of Kruppel-like factor 2 (KLF2) in vascular endothelial cells, was involved in the regulation of the nuclear factor (NF)-\kappaB signaling pathway, resulting in increased expression of IL-6, monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1), all markers of endothelial damage.\textsuperscript{23}

4 | HEPATOBILIARY DISEASES

Different \textit{Helicobacter} species can colonise the hepatobiliary tract as well as cause chronic active hepatitis and induce a striking increase in hepatocellular tumours.\textsuperscript{24} Interestingly, sequences belonging to \textit{Helicobacter} species were found in liver samples from humans with cirrhosis and hepatocellular carcinomas (HCC).\textsuperscript{24}

Recently, Cao et al in a preclinical study in mice confirmed that \textit{H hepatitis} infection was associated with the development of chronic hepatitis and liver cancer. Necrosis or cirrhosis developed in the \textit{H hepatitis}-infected livers at 24 weeks post-infection. Moreover, expression of pro-inflammatory mediators and signaling pathways of inflammation were significantly induced by \textit{H hepatitis}.\textsuperscript{25}

Considering the recent marked reduction in viral hepatitis and the increase in non-alcoholic fatty liver disease (NAFLD) prevalence,\textsuperscript{26} the association between \textit{H pylori} infection and NAFLD has also been evaluated. Four recently published meta-analyses\textsuperscript{27-30} showed a significant association between \textit{H pylori} infection and NAFLD. This finding was confirmed in a cross-sectional study including 646 patients. NAFLD (diagnosed by both ultrasonography and Fibroscan with controlled attenuation parameter) was significantly higher in the \textit{H pylori}-positive group than in \textit{H pylori} negative group and this difference remained after linear regression.\textsuperscript{21} In a study including 91 patients with NAFLD, overweight/visceral obesity and dyslipidaemia were closely associated with the severity of NAFLD and \textit{H pylori} infection. The latter correlated with the severity of steatosis by altering the occurrence of MS.\textsuperscript{32} In another study, including obese patients who were candidates for bariatric surgery, \textit{H pylori} infection was independently associated with NASH ($P = .002$), severe NASH ($P = .018$) and presence of fibrosis ($P = .001$).\textsuperscript{33} Nevertheless, in an Iranian case-control study, \textit{H pylori} tests (both IgG antibodies in serum and faecal antigen) did not reveal significant differences between NAFLD patients and controls ($P = .37$).\textsuperscript{34}

In cirrhotic patients, the incidence of complications, such as portal vein thrombosis and HCC, has been related to \textit{H pylori} infection through increased inflammatory markers and vascular mediators. Bacterial eradication significantly reduced this incidence.\textsuperscript{35}

The link between \textit{H pylori} infection, biliary stones and biliary tract cancers was also recently evaluated in Morocco. Eighty-nine cases were enrolled and bile duct specimens were investigated for \textit{H pylori}. The bacterium was detected in 54% of the samples and was significantly associated with the presence of stones ($P < .001$).\textsuperscript{36}

5 | NEUROLOGIC AND PSYCHIATRIC DISEASES

An Iranian study evaluated the association of \textit{H pylori} infection, gastroesophageal reflux disease (GERD), gastric ulcer and duodenal ulcer (DU) with migraine in 341 patients who underwent upper gastrointestinal (GI) endoscopy due to refractory dyspepsia. \textit{H pylori} infection ($P < .001$), GERD ($P < .001$) and DU ($P = .001$) were significantly associated with migraines.\textsuperscript{37}

Previous studies suggested that \textit{H pylori} infection could be a risk factor for Parkinson's disease (PD) and Alzheimer's disease (AD). In a meta-analysis, including studies on the relationship between intestinal disorders and PD or AD, the OR for \textit{H pylori} infection in PD and AD patients was 1.65 (95% CI, 1.43-1.91) and 1.40 (95% CI, 1.12-1.76), respectively.\textsuperscript{38}

Cardenas et al examined data on participants ≥60 years old, in the Third National Health and Nutrition Examination Survey (NHANES), to assess the relationship between \textit{H pylori} infection and results of the Mini-Mental State Examination (MMSE). Moreover, they examined performance on the digit-symbol substitution test (DSST) of participants in the 1999-2000 NHANES according to their \textit{H pylori} infection status. In 1988-1991, older adults infected with CagA strains of \textit{H pylori} had a borderline increased level of cognitive impairment, as measured by MMSE scores (OR: 1.5, 95% CI, 1.0-2.0). In 1999-2000, older adults infected with \textit{H pylori} scored 2.6 points lower in the DSST than uninfected adults. Thus, \textit{H pylori} infection might be a risk factor for cognitive decline in the elderly, associated with low cobalamin and elevated homocysteine levels.\textsuperscript{39}

Guillain-Barré's syndrome (GBS) is a demyelinating disorder of peripheral nerves. About 30% of GBS cases have been attributed to \textit{Campylobacter jejuni}, leading to a search for other infectious agents. A recent meta-analysis included studies assessing anti-\textit{H pylori} IgG positivity in the cerebrospinal fluid or in the serum of GBS patients. Anti-\textit{H pylori} IgG were significantly more prevalent in patients compared to controls, in both cerebrospinal fluid (CSF) ($P < .00001$) and serum ($P = .004$).\textsuperscript{40}

6 | SKIN DISEASES

A systematic review with meta-analysis evaluated the relationship between \textit{H pylori} infection and psoriasis. The prevalence of \textit{H pylori}}
infection was significantly higher in the psoriasis group vs controls (OR, 1.19; 95% CI, 1.15-2.52; \( P = .008 \)). Moreover, \( H \) pylori prevalence increased significantly in patients with moderate and severe psoriasis (OR, 2.27; 95% CI, 1.42-3.63).\(^{41} \)

Another recent meta-analysis found that in \( H \) pylori-positive patients, antibiotic treatment induced chronic spontaneous urticaria remission independently of \( H \) pylori eradication.\(^{42} \)

In a prospective study, 57 consecutive patients (27 pityriasis versicolor, 30 telogen effluvium) screened for anti-\( H \) pylori and anti-CagA IgG, showed significantly higher rates of \( H \) pylori positivity in the pityriasis versicolor group than in the telogen effluvium group (\( P < .05 \)). The number of patients with dyseptic complaints was also higher in the pityriasis versicolor group than in the telogen effluvium group.\(^{43} \)

### 7 | INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD), which includes Crohn’s Disease (CD) and ulcerative colitis (UC), are chronic disorders of the digestive system, still with an unclear aetiology. The presence of dysbiosis, with a significant reduction of biodiversity and other specific imbalances in the faecal microbiome composition of IBD patients compared to that of healthy controls, supports the hypothesis that IBD may originate from a dysregulation of the host’s immune response to commensal bacteria in genetically predisposed individuals.\(^{44} \)

The role of \( H \) pylori infection as a potential IBD risk factor was initially proposed due to the similarities of their immuno-biological pathogenesis.\(^{45} \) Indeed, preclinical studies provide evidence that non-\( H \) pylori enterohemorrhagic \( Helicobacter \) species can enhance an IBD-like bowel inflammation.\(^{46} \) An association between the presence of \( H \) pylori in the intestinal mucosa, identified by 16S rRNA gene sequencing, and a UC-like pattern of patients with CD was observed.\(^{47} \)

A pilot study reported that 40% and 56.6% of the histologic samples from the colon of newly diagnosed patients with UC were positive for \( H \) pylori by Giemsa and immunohistochemistry staining, respectively. Results were significantly higher when compared to the control group.\(^{48} \)

However, more recent evidence shows an inverse correlation between \( H \) pylori and IBD, independently of the type of IBD considered, suggesting a potential protective role of this bacterium.\(^{49} \)

For instance, a lower frequency of IBD was observed among individuals with a positive result for IgG antibodies against \( H \) pylori when compared to subjects with negative results.\(^{50} \) A meta-analysis, including 80 789 individuals, reported a significant negative association between \( H \) pylori infection and IBD, regardless of age, nationality, \( H \) pylori detection test and IBD subtype, with a pooled OR of 0.43 (\( P < .01 \)); of interest, enterohemorrhagic \( Helicobacter \) spp were associated with an increased IBD risk.\(^{52} \) These data support a likely protective effect of \( H \) pylori on IBD as reported by other meta-analyses,\(^{51-53} \) possibly caused by the bacterium’s ability to promote immune system maturation and prevent the onset of aberrant immune responses. Indeed, \( H \) pylori exerts immunomodulatory effects by increasing the number of regulatory T cells, which suppress excessive inflammatory T-cell responses through the secretion of IL-10 and TGF-\( \beta \).\(^{54} \) However, \( H \) pylori may protect against IBD development through mucus production enhanced by IL-18 release and NLRP3 inflammasome activation, as assessed by a study on mouse models.\(^{55} \)

\( H \) pylori may also counteract intestinal inflammation by suppressing type I IFN and IL-12 responses from human plasmacytoid dendritic cells.\(^{56} \) Moreover, higher gut microbiota diversity and complexity, which are pivotal for the intestinal barrier integrity, were reported among \( H \) pylori infected individuals vs uninfected subjects.\(^{57} \)

In accordance, widespread use of eradication therapy and the reduced incidence of \( H \) pylori infection in countries with improved hygienic conditions have followed the trend of the increased IBD incidence during the last decades.\(^{58} \)

### 8 | KIDNEY DISEASES

A systematic review with meta-analysis, including 47 studies, recently reported a prevalence of \( H \) pylori infection in 48% of patients with chronic kidney disease (CKD) compared to 59% of healthy controls, with an OR of 0.64 (95% CI, 0.52-0.79).\(^{58} \) In a cross-sectional study including 293 Iranian patients undergoing kidney transplant, 50% of them were \( H \) pylori positive by gastric biopsy testing.\(^{59} \) Interestingly, \( H \) pylori infection could be a potential risk factor for renal damage in patients with peptic ulcer, as a positive association between this bacterium and altered albumin-to-creatinine ratio (\( P = .025 \)) was found, and a significant decrease of the latter occurred after \( H \) pylori eradication (\( P < .01 \)).\(^{60} \)

### 9 | IMMUNE-MEDIATED DISEASES

#### 9.1 | Autoimmune diseases

Autoimmune diseases (ADs) are multifactorial disorders in which an abnormal immune response against self-antigens occurs because of a break in immunological tolerance mechanisms. Several infectious agents, together with environmental and hormonal factors, in subjects with predisposing genetic background, may trigger autoimmunity by different strategies. These include molecular mimicry, microbial super-antigens, MHC class II molecules expression on non-immune cells, immune complex formation, bystander activation, epitope spreading, and polyclonal activation.\(^{61,62} \)

\( H \) pylori may be involved in the induction of gastric autoimmunity, since this bacterium is associated with an atrophic pattern which is similar to autoimmune gastritis (AIG). Indeed, it is plausible that \( H \) pylori can trigger a mechanism of molecular mimicry with formation of serum anti-parietal cell auto-antibodies (PCA) targeting the gastric \( H^+ \), \( K^+ \)-adenosine triphosphatase.\(^{63} \) Accordingly, following \( H \) pylori eradication, a significant decrease in anti-PCA in patients suffering from AIG with corpus atrophy was observed.\(^{64} \)
Interestingly, there is evidence supporting a role of *H pylori* in the pathogenesis of various autoimmune extragastric disorders. For instance, in the case of immune thrombocytopenia purpura (ITP) an increase in the platelet count of patients affected by ITP was observed after *H pylori* eradication therapy. A molecular mimicry between platelet surface glycoproteins (IIb/IIIa or Ib) and *H pylori* CagA and VacA proteins could be responsible for ITP induction. Even if the associative mechanism is still under debate, literature reviews and meta-analyses have shown that *H pylori* eradication has a significant effect in increasing platelet counts, with variable responses, in patients with ITP.

Environmental influencers, such as bacteria and viruses, are capable of mimicking the antigenic profile on the thyroid cell membrane, suggesting a plausible role in the onset of autoimmune thyroid diseases. Indeed, a significantly increased rate of *H pylori* infection was reported among patients with Graves' disease, with a correlation between CagA positive *H pylori* strains and this disease. Even if *H pylori* infection, especially CagA positive strains, has been also associated with Hashimoto's thyroiditis (HT), and the prevalence of anti-thyroid peroxidase (TPO) antibodies seems higher in subjects with *H pylori* infection, a solid correlation between *H pylori* infection and HT was not confirmed by more recent evidence.

A possible role of *H pylori* in autoimmune pancreatitis (AIP) has also been proposed. Molecular mimicry through a sequence similarity between *H pylori* α-CA and human CA type II antigen located in the ductal epithelium of the pancreas, and between *H pylori* plasminogen-binding protein and human ubiquitin-protein ligase E3 component n-recognin 2, present in pancreatic ductal and acinar cells, may trigger autoimmunity. Moreover, a homology between α-2 carbonic anhydrase of *H pylori* and human carbonic anhydrase II (the auto-antibodies play a possible pathogenic role in AIP) has been observed.

*H pylori* infection has been associated with various systemic rheumatic diseases. This is in line with the capacity of *H pylori* urease to stimulate B-1 cells in vitro to produce self-reactive antibodies, eg IgG3, IgM-type rheumatoid factors, and anti- single stranded DNA (ssDNA), thus promoting AD onset. Patients with rheumatoid arthritis (RA) have a higher prevalence of *H pylori* infection than controls. Moreover, the eradication of this pathogen can induce a significant long-lasting improvement in clinical and laboratory indices.

*H pylori* infection may also be involved in the pathogenesis of systemic sclerosis (SSc), albeit with contrasting results. A recent meta-analysis reported an increased rate of a previous *H pylori* infection in patients affected by SSc. In this context, a possible role of *H pylori* in enhancing production of antibodies against mycobacterial heat shock protein (HSP)65 was proposed as a trigger of autoimmunity.

A meta-analysis including 619 patients with Sjögren's syndrome (SS) reported an *H pylori* infection prevalence of 63.65% in patients with SS, which was significantly higher compared to controls (49.29%). A 60 kDa HSP, enhanced by *H pylori* infection, could play a role in the development of SS.

**9.2 | Allergic disorders**

Recently, the association between asthma or other allergic diseases and *H pylori* has been intensively investigated. In a case-control study, including more than 10,000 patients, *H pylori* infection was found in 31%, asthma in 10.4%, and allergic rhinitis in 16% of the patients, but without any significant association; however, in patients with abdominal obesity, *H pylori* infection was associated with a 30%-40% reduced OR of asthma and a 25% reduced OR of allergic disorders. Moreover, in a case-control study of 27 paediatric patients with asthma and 54 controls, an inverse association between *H pylori* and asthma was found (OR 0.1, 95% CI, 0.039-0.305; *P* = .026). In a cohort study, 16% of children who were uninfected at 2 and 10 years of age developed asthma at 16 years vs none of those with *H pylori* infection at 2 years of age. In another study, including 274 patients with various allergies or non-allergic rhinitis with eosinophilic syndrome and 75 controls, a significantly positive association was found between *H pylori* and allergies in adults but not in children. These conflicting findings warrant larger prospective studies.

The role of *H pylori* has also been investigated in other allergic-like disorders. In a systematic review with meta-analysis, including 11 studies and 377,795 patients, *H pylori* was found to be associated with a decreased risk of eosinophilic oesophagitis (OR 0.63; 95% CI, 0.51-0.78).

The relationship between allergy and *H pylori* was also investigated in preclinical models. Perinatal exposure to *H pylori* extract or to VacA was found to protect mice against allergic airway inflammation. Moreover, *H pylori* extract was also able to decrease mucus production and inflammation in mice challenged with house dust mites.

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**CONFLICT OF INTEREST**

We declare no conflicts of interest.

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1 INTRODUCTION

*Helicobacter pylori* infection in children differs from adults in that children rarely develop complications of infection. Furthermore, treatment options are more limited. Therefore, *H pylori*-focused research in the pediatric population provides a unique opportunity to address these potential differences with respect to pathogenesis and to optimize management. The objective of this review is to provide an update of the relevant pediatric literature focused on *H pylori* infection that was published from April 2019 to March 2020.

2 EPIDEMIOLOGY OF INFECTION

Studies published in the past year continue to demonstrate a decrease in prevalence of *H pylori* infection in children. A comprehensive review of data on Japanese children and adolescents between 1997 and 2017 showed that the prevalence of *H pylori* infection was approximately 10% in individuals born in 1985 but decreased to 3% in individuals born in 2011. This declining prevalence was explained by the steady improvements in sanitation and living conditions from the 1950s and onwards, and it was speculated that the decreasing birth rate might have led to fewer children per family, thus decreasing the risk of intra-familial infection. In a study from Korea, the seroprevalence of *H pylori* infection decreased from 50%-70% in children aged 5-7 years in 1988-1989 to 15% in children aged 5-14 years in 2014-2015. In Hong Kong, only 10.3% of children with gastritis and 39.5% of children with peptic ulcer disease (PUD) had an *H pylori* infection. In Africa, the seroprevalence rates of *H pylori* infection in 360 Nigerian children aged 6 months to 5 years and 304 Uganda children aged 1 to 15 years were 32.8% and 24.3%, respectively. Similarly, studies of children with gastrointestinal symptoms in Bulgaria and Poland showed a reduction in *H pylori* infection rates over time. Potential explanations for this decrease include overall socioeconomic improvement, recognition of the relationship between *H pylori* infection and gastric cancer, as well as early diagnosis and treatment in adults.
3 | PATHOPHYSIOLOGY OF DISEASE IN CHILDREN

Children rarely develop complications of infection such as PUD. In addition, epidemiological studies show an inverse association between infection and allergy/atopy suggesting that the host immune response during infection in children may differ from adults. Although studies in pediatric patients are limited, a few publications addressing the effect of infection on host immune responses in children were published this past year. A study of 29 Chilean children undergoing upper endoscopy compared serum dendritic and T regulatory cells in children with and without infection. The authors found an increased expression of the high-affinity IgE receptor in circulating dendritic cells, and FoxP3 and latency-associated peptide in circulating T regulatory cells in serum from children with an H. pylori infection. Furthermore, incubation of H. pylori ATCC 26695 strain with monocyte-derived dendritic cells generated from pediatric patients resulted in upregulation of high-affinity IgE receptor and IL-10 secretion, in comparison with untreated cells. However, co-culture of H. pylori-treated dendritic cells with naive CD4 + T cells was not able to generate an increase in T regulatory cells. The exact mechanisms responsible for these observations and the effect of eradication in these patients were not delineated. In addition, whether these observations differ in H. pylori-infected adults was not determined.

Previous studies suggest that innate toll-like receptor (TLR) polymorphisms may influence disease outcome. A cross-sectional study of Romanian children with gastritis found no association with specific TLR4 polymorphisms and the presence of H. pylori infection. However, the study included a small number of children. Therefore, additional studies are needed to determine if these TLR4 polymorphisms are involved in susceptibility to infection.

4 | H. PYLORI AND MICROBIOTA

Previous studies in both adults and children demonstrate changes in the gastric microbiota in association with H. pylori infection. A small study of symptomatic Chinese children undergoing upper endoscopy investigated the effect of H. pylori infection and the presence of ulceration on the gastric microbiome. The authors found a reduction in bacterial diversity in the presence of H. pylori infection in comparison with children with gastritis alone, which was not influenced by the presence of duodenal or gastric ulceration. In a small subset who underwent follow-up endoscopy 4 weeks after receiving a variety of eradication therapies, the gastric bacterial diversity was closer to that observed in the group of uninfected children with gastritis. These findings are consistent with previous studies demonstrating alterations in the gastric microbiome associated with infection. A Chinese study investigated the effect of gastritis and H. pylori on the fecal microbiome. The fecal microbiome obtained from both children with gastritis alone and H. pylori-related gastritis was altered in comparison to healthy controls suggesting that gastric inflammation may impact the gut microbiota.

5 | CLINICAL MANIFESTATIONS OF H. PYLORI

5.1 | Gastrointestinal manifestations

With the decrease in H. pylori prevalence, a reduction in H. pylori-related ulcers has been suggested. However, in a retrospective Belgium study of 5,618 children undergoing diagnostic endoscopy, the proportion of H. pylori-associated lesions remained stable over the time periods from 1990 to 2012. Thus, H. pylori remains an important risk factor for duodenal ulcers and duodenal and gastric erosions in children.

A number of studies identified correlations of H. pylori infection in children with a variety of gastrointestinal diseases including autoimmune gastritis, celiac disease, and parasitic infection. However, association does not prove causation and confounding factors likely account for these associations.

5.2 | Extra-intestinal manifestations

Previous epidemiologic studies suggest that early acquisition of H. pylori may be protective against allergy and atopic diseases. Three studies examined this relationship in this time period with conflicting results. In a case-control study from Greece, 11% of children with asthma had a positive H. pylori stool antigen test (SAT), while 29.6% of nonasthmatic children were found to be infected (P = .026). A Norwegian study assessed whether the presence of H. pylori IgG at 2-10 years of age was associated with asthma in adolescence. Of 197 children, none of the 12 seropositive children developed asthma in adolescence. In contrast, 16% of patients with negative serology had asthma by 16 years old. However, these findings were not statistically significant. A cross-sectional study in Istanbul enrolled 274 patients 3-76 years old to estimate H. pylori prevalence in patients with allergic and nonallergic nasal conditions compared to controls. No association between H. pylori infection and prevalence of allergic disease was detected in the pediatric group.

The current European Society for Paediatric Gastroenterology, Hepatology and Nutrition and the North American Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN/NASPGHAN) guidelines conclude that there is no association with iron deficiency anemia (IDA), and a weak association between refractory IDA and H. pylori infection. A recent study of Iranian children found no association with H. pylori infection and IDA, supporting previous findings. Based on weak evidence, current ESPGHAN/NASPGHAN guidelines suggest that chronic idiopathic thrombocytopenic purpura (ITP) is an indication to test for H. pylori infection and treat if positive. A study from Turkey assessed the mean platelet volume in children undergoing upper endoscopy for dyspeptic symptoms and found no difference between H. pylori-infected patients and controls.

Studies continue to investigate the association between H. pylori infection and stunted growth. Studies in Riyadh, Saudi Arabia, and
Lagos, Nigeria,24 show a correlation between *H pylori* infection and short stature. Similarly, a large cross-sectional study carried out in China on 6,896 apparently healthy patients 6-36 months old determined that *H pylori* seropositive children were more likely to be vitamin D deficient and had a lower weight and height.25 However, these studies do not provide evidence of causation. Both short stature and infection are increased with poor socioeconomic status which is associated with poor nutritional status.

### 6 | DIAGNOSIS

The current ESPGHAN/NASPGHAN guidelines recommend that diagnosis be based on culture or histopathology along with one other positive biopsy-based test and at least six gastric biopsies.8 The guidelines also recommended follow-up testing with either the urea breath test (UBT) or SAT. A variety of studies addressed diagnostic testing for *H pylori* infection in the last year. A retrospective study of Romanian children compared the endoscopic and histopathologic findings in 166 *H pylori*-negative children and 82 *H pylori*-positive children and showed a good agreement between endoscopic and histologic findings.26 However, some *H pylori*-infected children had a normal appearance of the gastric mucosa on endoscopy.

Various noninvasive diagnostic tests are available for post-treatment evaluation of *H pylori* infection. *H pylori* SAT (HpSA) is one of the noninvasive tests recommended for follow-up post-treatment. Several studies assessed the performance of the test in this time period.27-29 In a study cohort of 101 Algerian children aged 5-15 years, the IDEIA HpSTAR SAT (Oxoid, Cambridge, UK) was shown to have high sensitivity (93.6%) and specificity (100%) in both pre-treatment and post-treatment conditions using culture or histology and rapid urease test (RUT) as reference.27 In a prospective cross-sectional study with 303 symptomatic children, the sensitivity values of antral nodularity (AN), HpSA, and RUT were 62%, 69%, and 87%, and the specificity values of AN, HpSA, and RUT were 88%, 89%, and 65%, respectively, using culture as the reference method.28 A study comparing the results of endoscopic biopsy and the results of the HpSA, salivary IgG, serum IgG, and serum IgM tests in a group of 37 Indonesian children confirmed that serologic and salivary assays should not be used for detection of *H pylori* due to poor specificity.29

In summary, diagnosis of *H pylori* infection should be based on endoscopic biopsies in children who are highly suspected of *H pylori*-related gastroduodenal disease. Noninvasive tests can be chosen according to the clinical setting or study purpose. Further comparative studies of high methodological quality are required to obtain more reliable evidence of relative accuracy of the tests used for diagnosing *H pylori* infection in children.

### 7 | TREATMENT

*H pylori* eradication in children should be supported by a clear benefit. For those with PUD, chronic ITP, or refractory IDA, testing for *H pylori* is recommended, and treatment should be advised for confirmed cases.8 According to the updated ESPGHAN/NASPGHAN guidelines, therapy should be based on the results of antimicrobial susceptibility testing (AST) where available. If antibiotic sensitivity is unknown, then a 14-day bismuth-based therapy is the first choice for *H pylori* eradication in children where bismuth is available or high-dose triple therapy if bismuth is not available.8 The recommended dose of antibiotics and proton pump inhibitor are based on weight, while for bismuth the recommended dose is based on age (<10 years old 262 mg QID, >10 years old 524 mg QID). In Europe and North America, treatment failure, side effects, and alteration of the gut microbiome have been highlighted as potentially outweighing the possible benefit of preventing future peptic ulcers or gastric cancer.8

The therapeutic strategies for children and adolescents with *H pylori* infection developed in Europe and North America may not be appropriate for treating children and adolescents in all countries in the world because of the differences in the epidemiological characteristics of *H pylori* between regions.8 In Japan, adolescents were included in the guidelines revised in 2016 for the management of *H pylori* infection to prevent gastric cancer and to prevent intra-familial transmission, and they have also been included in the screening test-endoscopy-treatment strategy.30,31 In Korea, pediatric gastroenterologists treat children with *H pylori* infection according to the guidelines of Europe and North America and treat children when the parents want their child treated.32

Treatment failure is expected to increase worldwide because of a general increase in the antimicrobial resistance of *H pylori*. Korean guidelines recommend an endoscopy be performed to determine AST if treatment fails. Based on results of susceptibility testing, two appropriate antibiotics are administered at maximal tolerable doses with the double-dose proton pump inhibitor (PPI) bid and bismuth qid for at least 14 days.32 The existence of clarithromycin-resistant *H pylori* is an important factor involved in eradication failure. In 222 *H pylori* strains isolated from 1,887 Swedish children from 2005 to 2016, 21% (46/222) were clarithromycin-resistant.33 In a prospective, open, comparative, and cross-sectional study of 228 Chinese children aged 6 to 18 years, the eradication rates were 74.1% for standard triple therapy, 69.5% for sequential therapy (41/59), 89.9% for bismuth-based quadruple therapy (53/59), and 84.6% for concomitant therapy (44/52). Bismuth-based therapy was superior to triple therapy, whereas sequential therapy and concomitant therapy were not superior to triple therapy.34

The efficacy of addition of probiotics as an adjunct to therapy was investigated in a recent meta-analysis. Lactobacilli, as an adjunct to triple therapy, increased *H pylori* eradication rates by approximately 13% as well as reduced the incidence of therapy-related diarrhea in children.35 The eradication rates increased significantly in the high-dose group (>5 × 10⁹ CFU/day) and long-term group (>4 weeks), but not in the low-dose group (<5 × 10⁹ CFU/day) and short-term group (≤2 weeks), which indicated that a higher dose and a longer duration of Lactobacilli supplementation may improve eradication efficacy. However, these studies involved a variety of different probiotics. Therefore, it is not possible to make...
any specific recommendation regarding addition of probiotics as an adjunct to therapy.

In summary, clarithromycin resistance is increasing worldwide, and the use of bismuth-based triple or quadruple therapy instead of the PPI-based triple therapy is increasingly used as a first-line therapy. Additional studies are required to determine specific probiotics that might be helpful in increasing the eradication rate and in decreasing the side effects of eradication therapy. Physicians should understand the mechanisms underlying eradication therapy and explain drug prescription in detail to both parents and children with a view to increasing adherence.

8 CONCLUSIONS

Recent publications of H pylori infection in children highlight the distinct clinical and pathophysiologic features associated with infection, the decreasing prevalence of infection, and increasing challenge with eradication rates due to antibiotic resistance. Ongoing studies in children will continue to unravel key factors involved in disease. Larger well-designed studies will be required to identify optimal treatment options.

DISCLOSURES OF INTEREST

The authors have no disclosures of interest.

REFERENCES


INTRODUCTION

The past year has been another busy period for research publications on the treatment of Helicobacter pylori. This review summarizes important studies regarding H pylori therapy published from April 2019 to April 2020. The main themes that emerge involve studies assessing antibiotic resistance, and there is also growing momentum behind the utility of vonoprazan as an alternative to proton pump inhibitor (PPI) therapy and also bismuth-based regimens as a first-line regimen. Antibiotic resistance is rising wherever it is being assessed, and clarithromycin resistance in particular has reached a point where it may no longer be a viable therapy without previous testing in many regions of the world. The evidence for the efficacy of a bismuth-based quadruple therapy as a first-line therapy is now very clearly established, and there is substantial evidence that it is the best performing first-line therapy. The utility of vonoprazan as an alternative to PPI therapy, especially in resistant and difficult-to-treat groups, has also been considered in great detail this year, and it may offer an opportunity in the near future to reduce the problem of antibiotic resistance.

TRIPLE THERAPY

Triple therapy (TT) remains the standard of care in the published international guidelines of the European Helicobacter and Microbiota Study Group (EHMSG) in areas of low clarithromycin resistance. Two studies from the Americas looked at the outcomes and both showed poor eradication rates albeit with divergent results for TT with clarithromycin compared to levofloxacin. In the US, 78% of patients receiving clarithromycin-based TT achieved eradication, compared to 49% with levofloxacin-based TT. On the other hand,
in Argentina 75% of patients were cured with clarithromycin TT but 93% achieved eradication with levofloxacin TT. Interestingly, a Japanese study, where eradication rates with clarithromycin TT were evaluated over time during the period 2013-2018, showed that treatment success improved markedly over that time period, coinciding with the use of the potassium competitive acid blocker (P-CAB), vonoprazan, becoming the preferred means of acid inhibition over PPI. This will be explored in more detail later in this review.

Increasing treatment duration has been proposed as a means of improving eradication rates with TT but this was not borne out in a Korean trial comparing 7- with 14-day regimens which reported similar poor success rates of 64% and 66%, respectively. A meta-analysis of 45 studies from Turkey was in agreement with just 60% achieving eradication with both 7- and 14-day regimens. In Indonesia, however, a 14-day TT regimen was significantly more effective than a 10 day TT (87% vs 68%). Two meta-analyses addressed the question of whether TT with clarithromycin or metronidazole was more effective. Both concluded that the regimens were equally, poorly efficacious with a trend in more recent years in favour of metronidazole TT since it is more effective following the rise in clarithromycin resistance. A study of 7896 subjects from Israel examined the different PPIs used for eradication therapy and found esomeprazole to be associated with a greater proportion of successful eradication than other PPIs (85% vs. 77%). Data from Tanzania suggested that antibiotic resistance and poor adherence are the two factors most closely associated with TT failure. Again in Africa, in Ethiopia, a study showed that developing an adverse drug reaction on TT reduced the chances of eradication, most likely via an inhibiting effect on adherence to therapy. The presence of type 2 diabetes was shown in another study to also be associated with TT failure with 74% eradication in the diabetes mellitus group compared to 85% in the non-diabetic group.

## 4 | HYBRID, SEQUENTIAL AND CONCOMITANT NON-BISMUTH THERAPIES

Original research on concomitant, sequential, and hybrid therapies has been sparse this year. In Egypt, one study showed very high eradication rates of: 90% with 10-day sequential therapy, 97% with 14-day sequential therapy, and 63% for standard 7-day TT. Elsewhere, in Myanmar, 10-day sequential therapy was compared to 14-day concomitant therapy and found to be equally efficacious (82% vs 79% eradication) with lower costs. Again with a view to foresee cost control, safety and adherence, a study from one Italian group who pioneered sequential therapy showed 10-day regimens to be of equal efficacy to 14-day regimens (87% vs 90% eradication). Data from Korea collated over the course of the last decade suggested a much lower eradication rate of 70% although it is notable that this rate did not decline in spite of rising antibiotic resistance rates. A non-bismuth concomitant quadruple therapy (QT) where three antibiotics are given at once for 10 days was used in Greece and found to have an 87% eradication rate, which did not improve when treatment was extended to 14 days. The reverse hybrid therapy (PPI plus amoxicillin for 14 days, with clarithromycin plus metronidazole added for the initial 7 days) is considered to be a means of combining the benefits of the sequential and concomitant regimens. A trial from Taiwan this year compared this to concomitant therapy and reported comparable eradication rates (95% vs 93%) with a lower frequency of adverse events. Ten-day concomitant therapy performs well on cost-effectiveness analysis with esomeprazole, as characterised by the lowest cost-effectiveness analysis ratio (CEAR) 179€, followed by the same regimen using pantoprazole (183€) compared to a hybrid regimen which, although equivalent in eradication rate, had a slightly higher CEAR (187€). In contrast, the sequential regimen was not considered cost-effective (CEAR: 216€).

## 5 | ANTIBIOTIC RESISTANCE

An unprecedented number of studies last year reported on the important topic of antimicrobial resistance of H pylori strains. These are outlined in Table 1. A great degree of divergence was observed in antibiotic resistance rates throughout the world with an unacceptably high level of clarithromycin resistance, however, being a recurring theme, with only 8 of 33 studies reporting a resistance rate less than the 15% threshold at which the Maastricht guidelines recommend clarithromycin-based TT to be abandoned. A meta-analysis of 27 studies, including 4825 patients treated with both clarithromycin- and metronidazole-containing regimens illustrated the clinical importance of monitoring resistance rates, noting low overall eradication rates for both regimens, 75% for clarithromycin and 72% for metronidazole. In areas with low metronidazole and high clarithromycin resistance rates, metronidazole had a significantly higher eradication rate (92% vs. 71%), while even in areas with high metronidazole and low clarithromycin resistance rates, the eradication rate with clarithromycin-based TT was only 73%. Together, these data call into question the continuing viability of clarithromycin as a mainstay of H pylori eradication treatment without previous testing.

## 6 | PERSONALISED TREATMENT

A series of articles published in the journal Helicobacter this year addressed the question of personalised or tailored treatment, which had hitherto been considered in a second-line context but is now gaining interest as a first-line intervention. A meta-analysis looked at 2,890 patients submitted to endoscopy and H pylori culture, reporting cure rates using the antibiotic to which susceptibility was detected, with 72% in patients harbouring clarithromycin-susceptible strains, 93% in patients harbouring metronidazole-susceptible strains, and 84% in patients harbouring a levofloxacin-susceptible strain. In Korea, a study used personalised treatment, after testing
### TABLE 1  
*Helicobacter pylori* resistance to antibiotics in the studies published during the last year worldwide

<table>
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<tr>
<th>Author</th>
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<th>CLA %</th>
<th>MET %</th>
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Abbreviations: AMO, amoxicillin; CIP, ciprofloxacin; CLA, clarithromycin; FUR, furazolidone; LVX, levofloxacin; MET, metronidazole; MOX, moxifloxacin; RIF, rifabutin; SIT, sitafloxacin; TET, tetracycline.

*Meta-analysis.*
with dual priming oligonucleotide (DPO) polymerase chain reaction (PCR) and asking for a previous antibiotic exposure in order to predict resistance. The tailored first-line treatment based on antibiotic susceptibility, reported an eradication rate of 92%. Another first-line study also conducted in Korea involved administration of either a 7-day clarithromycin-containing TT, a 7-day moxifloxacin-containing TT, or a 7- or 14-day bismuth quadruple therapy (BQT) based on the MIC of the various antibiotics and reported 93% eradication with common adverse events such as epigastric pain, nausea, and vomiting occurring less commonly than with empirical therapies.

A multicentre study of 467 *H pylori*-positive patients assigned to receive tailored therapy or empirical therapy, revealed eradication rates for tailored TT, traditional bismuth-containing QT, and tailored bismuth-containing QT of 67%, 64%, and 86%, respectively. Beyond susceptibility-guided treatment, another means of personalising treatment is based on the CYP2C19 status. Genetic differences in the activity of the enzyme CYP2C19 (the homozygous EM, heterozygous EM (HetEM), and poor metabolizer) dictate how effective PPI will be. A group in Colombia found cure rates to improve from 84% to 92% in a group consisting mainly of rapid metabolisers when omeprazole doses were chosen based on the CYP2C19 polymorphism, which may be useful particularly in populations with a broad spread of CYP2C19 polymorphisms.

**7 | BISMUTH-BASED THERAPY**

Bismuth-based therapies were looked at in great detail this year both as primary and second-line therapies. Most of the studies examined bismuth as part of a BQT regime with a PPI, a tetracycline-class antibiotic and another antibiotic. One study of 118 patients treated with a BQT regimen containing amoxicillin and doxycycline reported 90% eradication with 97% adherence to therapy. A retrospective analysis of American patients suggested a 14-day regimen with bismuth and tetracycline to be the most effective first-line eradication treatment in that country, with an 87% eradication rate. A three-in-one single capsule formulation containing bismuth subcitrate, metronidazole and tetracycline (Pylera®) may be used with PPI for *H pylori* eradication purposes, and a Spanish study of 200 patients using this treatment achieved cure in 91% of patients with 96% adherence. Another systematic review and meta-analysis on Pylera® concluded that a 10-day treatment achieved an effective eradication rate of approximately 90% both in first- and second-line therapy, regardless of the type and dose of the PPI, in patients with clarithromycin- or metronidazole-resistant strains, and in those previously treated with clarithromycin. It has been proposed that H₂-receptor antagonists induce less bismuth absorption and, as a consequence, less systemic toxicity than PPIs. With this in mind, a pilot randomised clinical trial was conducted looking at eradication rates for patients receiving ranitidine rather than a PPI alongside Pylera® and found similar eradication rates (91%) compared to 94% in those receiving PPI although intolerance levels were the same in this small pilot cohort.

Two studies compared such therapies with standard concomitant QTs and found comparable results between the two regimes. A study from Spain compared a 10-day BQT with a 14-day standard QT arm, and found a slightly superior eradication for the bismuth group, 88% vs 86%. A Korean cohort where both arms received a 14-day therapy reported 88% eradication in the bismuth group and 79% in the QT group. Two trials compared BQT to susceptibility-guided treatment and revealed very high levels of eradication in both arms in both trials, ie a study of 150 patients in Korea reporting 96% eradication for patients both with a tailored treatment and a BQT, and a separate group in China reported 97% for both regimens. A study in China looking at minocycline in combination with amoxicillin vs metronidazole found the best cure rates for amoxicillin with 86% eradication in a 14-day BQT compared to 77% when metronidazole was used and 72% when amoxicillin and clarithromycin were used in BQT without minocycline. The duration of therapy has obvious implications in matters like cost of and adherence to therapy, and a study in Turkey showed that BQT could be shortened from 14 to 10 days without weakening the success rate. Another factor influencing adherence is whether or not the tablets can be taken once daily, and in the ONCE trial in Thailand, a once-daily treatment regimen containing levofloxacin, modified-release clarithromycin, bismuth and PPI reported 94% eradication when used for 14 days compared to 84% for a 7-day therapy.

A novel and very interesting use of bismuth has been as an adjunct to standard TT. A large, high-quality study from the European Registry on *H pylori* Management (Hp-EuReg) on 1,141 patients receiving this regimen showed cure in 93% of the patients, with 36% reporting adverse events, three-quarters of which were mild and self-limiting. A smaller Chinese study on 216 patients compared standard TT with and without bismuth and reported similar eradication rates, 98% for the group with bismuth and 95% without.

Bismuth remains a useful second-line option and a study in Korea looking at 15 years of data for the drug in treatment failures suggests an overall eradication rate of 79% with no significant changes over the period in question (2003 to 2018) and adverse events in 57% of patients. Another Korean study showed even better results with 93% of second-line patients using bismuth-based therapy with twice-daily dosing being equally efficacious as four times daily dosing. In China, a study on BQT used as a rescue therapy comparing amoxicillin plus berberine vs. tetracycline plus furazolidone found similar eradication rates (76% vs 77%) with a significantly lower rate of adverse events in the amoxicillin and berberine group. The European Registry on *H pylori* Management (Hp-EuReg) reported on penicillin-allergic patients and found excellent eradication rates for bismuth as both first-line (91%), second-line (78%) and third-line (75%) therapies in this cohort. A Chinese study also on penicillin-allergic patients reported 87% eradication for bismuth-based therapy with tetracycline and metronidazole. The antibiotic furazolidone, to which resistance remains uncommon, may also be used as part of a QT with a Chinese group reporting 10-day and 14-day regimens achieving eradication rates of 94% and 98% respectively, with adverse drug reactions seen in 8.2%.
Vonoprazan is the first clinically available P-CAB. Vonoprazan can attain more potent gastric acid inhibition in comparison to PPIs. The pH ≥4 and ≥5 holding-time ratios achieved by vonoprazan (20 mg twice daily on day 7 of the treatment) were 100% and 99%, respectively. Moreover, vonoprazan can attain pH 7 in the stomach within approximately 3 hours of the initial dosing of 20 mg. Therefore, vonoprazan can create the ideal pH condition in the stomach for eradication of H. pylori from day 1 of the eradication therapy. Since 2015, when vonoprazan was used clinically in Japan, the eradication therapy has dramatically changed. The current most popular standard regimen for H. pylori eradication is the triple regimen with vonoprazan (20 mg b.i.d.), amoxicillin (750 mg b.i.d.) and clarithromycin (200 mg or 400 mg b.i.d.) for 7 days, while regimens used outside of Japan have been changed from the TT to non-bismuth quadruple therapies or BQTs.

As the first-line therapy, Ashida et al reported that the eradication rate attained by a triple regimen with vonoprazan (20 mg), amoxicillin (750 mg), and clarithromycin (200-400 mg) twice daily for 7 days was 91% (427/468). Kusunoki et al reported an eradication rate of 92% (384/415) with the same vonoprazan-containing regimen, while eradication rates attained by the PPI-containing regimens were 85% (57/67) when esomeprazole was used, 85% (384/454) when lansoprazole was used, and 82% (341/415) when rabeprazole was used. Saito et al compared vonoprazan and esomeprazole as first-line therapies and reported that the vonoprazan-based regimen attained higher eradication rates than the esomeprazole-based regimen. Takara et al also reported the superiority of vonoprazan as first-line therapy in comparison to PPIs. Lyu et al performed a systematic search and reported that the efficacy of vonoprazan-based TT was superior to that of PPI-based TT for first-line H. pylori eradication. Ierardi et al summarised three prospective studies comparing vonoprazan and PPI as first-line therapies and confirmed that vonoprazan was better than conventional PPIs for H. pylori treatment in every case. Deguchi et al demonstrated that a change of acid inhibitor from PPI to vonoprazan increased the eradication rates of H. pylori in Japan based on the analysis of a nationwide claims database including >1.6 million patients. In summary, it can be concluded that vonoprazan is superior to PPI as first-line therapy with amoxicillin and clarithromycin. Finally, Shinmura et al reported that the eradication rates by the vonoprazan-based regimens could be further improved when antimicrobial agents were selected based on susceptibility testing.

Concerning second-line therapy, Ashida et al reported that the eradication rate attained by the triple regimen including vonoprazan (20 mg), amoxicillin (750 mg), and metronidazole (250 mg) twice daily for 7 days was 95% (42/44). However, Saito et al demonstrated that there was no significant difference between vonoprazan and esomeprazole in the second-line eradication rate. Sue et al compared a vonoprazan-based third-line therapy consisting of vonoprazan (20 mg), amoxicillin (750 mg), and sitafloxacin (100 mg) b.i.d. for 7 days with a PPI-based third-line therapy and found that the vonoprazan therapy was superior. In summary, it can be concluded that vonoprazan is equal or superior to PPIs in second- and third-line therapies.

Dual therapy with PPI and amoxicillin has been recently proposed to improve eradication rates. In this dual therapy, amoxicillin and PPI are prescribed three (t.i.d.) or four (q.i.d.) times daily for at least two weeks. However, b.i.d. dosing of vonoprazan (20 mg) could attain the potent acid inhibition from day 1 as noted above. Furuta et al reported that dual therapy with vonoprazan (20 mg) b.i.d. and amoxicillin (500 mg) t.i.d. for 1 week attained a 93% eradication rate, which was not inferior to the TT with vonoprazan (20 mg), amoxicillin (750 mg) and clarithromycin (200 mg) b.i.d. for 1 week. Suzuki et al showed that dual therapy with vonoprazan (20 mg) and amoxicillin (750 mg) b.i.d. for 7 days attained almost the same eradication rate as vonoprazan (20 mg), amoxicillin (750 mg), and clarithromycin (200 mg) b.i.d. for 7 days. Therefore, when vonoprazan is used as the acid inhibitor, dual therapy with amoxicillin can be one of the standard therapies in Japan.

There are clinically important merits for dual therapy with vonoprazan and amoxicillin, especially because it allows to avoid clarithromycin. First, because clarithromycin is a well-known inhibitor of p-glycoprotein (p-Gp) and cytochrome P450 3A4 (CYP3A4), the interaction between clarithromycin and substrates of P-Gp and CYP3A4 is of concern. Furthermore, clarithromycin increases plasma levels of the substrates of CYP3A4 and MDR1, such as statins, cyclosporine, warfarin and triazolam. Clarithromycin is also known to increase the risk of elongation of the QT interval, which constitutes a risk of sudden death by arrhythmia. Thirdly, macrolide antibiotics are known to stimulate the intestinal peristalsis, which may lead to diarrhoea during the eradication treatment. Although these adverse events are relatively infrequent, the avoidance of clarithromycin might contribute to their reduction.

As noted above, the potent acid inhibition attained by vonoprazan improves the eradication rates of H. pylori infection in comparison with PPIs. However, current data about vonoprazan have come only from Eastern Asia. Therefore, its strong power needs to be confirmed outside this geographic area in Western countries and should be related to the different local antibiotic resistance rates.

Usefulness of vonoprazan in other regimens should also be tested.

Reinfection

A large-scale multicentre, prospective open cohort observational study in China reported an annual reinfection rate after successful eradication treatment of 1.5% per person-year and this reinfection was independently associated with several risk factors, namely membership of a minority group, a lower level of education, a family history of gastric cancer, and residence in Western or Central China.

Probiotics and Other Adjuncts

In H. pylori eradication regimens, probiotics are proposed to decrease side effects, improve compliance and thereby increase eradication...
rates. A network meta-analysis of 40 studies with 8924 patients performed this year showed a higher eradication rate and lower incidence of total side effects in the probiotic group compared to controls. Further analysis showed that prolonged use of probiotics before, throughout and after treatment improved eradication rates and that probiotics combined with BQT was the best combination.115 Lactobacilli were shown in that meta-analysis to be the best choice of probiotic strains and two studies this year even investigated *Lactobacillus reuteri* along with PPI as an alternative to antibiotics for *H pylori* eradication. One trial in Romania, conducted on 23 patients with functional dyspepsia, reported an eradication rate of 65%.116 However, in a group in Italy, cure was only achieved in 3 out of 24 patients (12%).117 Aside from Lactobacilli, two other studies this year showed small beneficial adjuvant effects for a two-bacterial-strain formula, containing *Bifidobacterium animalis* lactic BB12 and *Enterococcus faecium* L3, and another for *Saccharomyces boulardii*.118,119

## 11 | CONCLUSION

There have been many studies pertaining to *H pylori* eradication treatment in the published literature over the last 12 months, often with diverse results, although several broad themes have emerged. Bismuth-based therapies continue to show a clear advantage for first-line therapy. In addition, there is a crisis in rising antibiotic resistance rates. Clarithromycin resistance rates in almost all regions have now passed the point where clarithromycin-based TT cannot be considered without previous testing and it is time for global clinical practice to reflect this. Vonoprazan is a very promising emerging option for several reasons including the fact that it may offer the opportunity to improve the resistance problem and should be trialled in more regions in the coming years.

## DISCLOSURES

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A few articles describing the presence of gastric non-Helicobacter pylori Helicobacter species (NHPH) in humans were published this year. Mohammadi et al investigated the association between NHPH infection and gastroduodenal diseases in Iran by comparing biopsy samples from patients positive for H. pylori with those from H. pylori-negative subjects. Of the 70 H. pylori-negative patients (36%), half of them had gastritis and 91% of them were positive for Helicobacter heilmannii. No mixed Helicobacter infections were found. The authors further highlighted the importance of zoonotic H. heilmannii infections in humans and emphasised the clinical relevance to test H. pylori-negative patients with gastric complaints for the presence of NHPH.1 Another report compared the clinicopathological characteristics and the presence of NHPH in different types of mucosa-associated lymphoid tissue (MALT) lymphoma.2 Although gastric MALT lymphomas are often associated with H. pylori infection, nodular gastritis-like MALT lymphomas seem to be typically linked with NHPH and more specifically with Helicobacter suis infection.
in which eradication therapy may be the first-choice treatment. As NHPH are difficult to culture from the human stomach, diagnosis is often based on microscopic examination and molecular analysis of biopsies. Due to the scarce data regarding antimicrobial resistance among NHPH, infected patients are usually treated with antibiotics, like clarithromycin. A report by Pichon et al described for the first time a mutation associated with clarithromycin resistance in an H. suis isolate from a 43-year-old obese woman who underwent a gastric sleeve surgery. Histological analysis confirmed the presence of atrophic gastritis with moderate intensity and the presence of spiral-shaped bacteria, ie H. suis. Eradication of this pathogen was successful with a metronidazole-based treatment. This study highlighted the benefit to use genotypic detection of resistance to improve therapeutic management of NHPH infections. NHPH infections can also be associated with the aggravation of extra-gastric manifestations, like neurodegenerative disorders. Augustin et al evaluated the pathological significance of H. suis in patients with Parkinson’s disease (PD). They concluded that this zoonotic agent, for which pigs and non-human primates are the natural reservoirs, is associated with all-cause mortality in PD. Surprisingly, this phenomenon was not seen for H. pylori. Another study investigated the role of a Helicobacter felis infection in the onset of Alzheimer’s disease using a mouse model. They showed that infection with this pathogen induced a severe gastritis and an increased neuroinflammation but without brain amyloid deposition or systemic inflammation.

Enteroheliotic Helicobacter (EHH) infections have been associated with several diseases in humans such as acute gastroenteritis, inflammatory bowel disease (IBD) and hepatobiliary diseases. Although they are frequently detected in clinical samples by molecular methods, these fastidious organisms are rarely isolated by traditional culture technique, resulting in an underestimation of the prevalence and clinical importance of these pathogens. A study compared media and growth conditions for culturing EHH, ie Helicobacter canicola, Helicobacter apodemus, Helicobacter bilis, Helicobacter canis, Helicobacter equorum and other Helicobacter species. Regardless of the use of hydrogen, Columbia or Brucella media can be used for the EHH isolation. All isolates were resistant to trimethoprim but the antibiotic concentrations included in commercial supplements for Campylobacter culture are suitable for EHH culture. Among EHH, Helicobacter cinaedi is most frequently associated with human diseases. Helicobacter cinaedi was primarily identified in bloodstream infections of immunocompromised patients and is now becoming an emerging pathogen in humans, with an increasing number of reported cases of infections. A case of ovarian abscess caused by H. cinaedi was reported this year in a 38-year-old nulligravid woman with endometriosis. Helicobacter cinaedi was isolated from cultures of blood and ovarian abscess fluid after oophorectomy. Eradication of H. cinaedi was performed successfully with intravenous ampicillin/sulbactam for 2 weeks, followed by oral amoxicillin for an additional 2 weeks. Multilocus sequence typing (MLST) and antimicrobial susceptibility testing (AST) of H. cinaedi were evaluated among 16 successive H. cinaedi blood clinical isolates. The most common risk factors associated with these H. cinaedi infections included the use of steroids (75.0%) and immunosuppressant drugs (37.5%). Symptoms of H. cinaedi bacteraemia included colitis (37.5%) and cellulitis (31.3%). MLST allowed the classification of the strains into five clusters, but none of these clusters were related to clinical symptoms, situations at onset, and prognosis. Helicobacter cinaedi infection recurred in three of seven patients who underwent antimicrobial therapy for less than 10 days. Currently, no recommended guideline is available for the treatment of H. cinaedi bacteraemia. This study proposed a long-term antibiotic therapy, excluding ciprofloxacin and clarithromycin, to ensure healing of symptoms and prevent recurrence. Another study investigated whether recurrent H. cinaedi infections are relapses of former infections or reinfections with different clones. Ten colonies were isolated from the blood cultures of a 69-year-old woman during each of the two episodes of a recurrent H. cinaedi bacteraemia-associated cellulitis after a 51-day interval. The AST of a representative isolate was tested against 14 antimicrobials. Whole-genome sequencing (WGS) revealed six single-nucleotide polymorphisms (SNPs) among the 20 isolates but none of these SNPs was associated with additional antibiotic resistance. Based on the six SNPs detected, five genotypes were identified, all detected in the first infection. However, only two genotypes were detected in the second infection. WGS showed that the second infection was a relapse of the first infection and highlighted the importance of long-term antibiotic treatment to eradicate H. cinaedi to prevent the recurrence of this difficult-to-treat infection. H. cinaedi culture and identification are challenging. Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) is currently used in clinical practice and is efficient to identify some Helicobacter species from humans, dogs, cats, and hamsters. MALDI-TOF MS was successfully applied to 54 human clinical isolates to identify H. cinaedi.

2 | PRESENCE OF NON-Helicobacter PyLORI HELICOBACTER SPECIES IN ANIMALS

Gruntar et al described three novel Helicobacter species isolated from the gastric mucosa of red foxes shot by hunters in the surroundings of Ljubljana, Slovenia, for which the names Helicobacter labacensis sp nov., Helicobacter mehlei sp nov. and Helicobacter vulpus sp nov. have been proposed. The canine and feline Helicobacter bizzozeronii and Helicobacter baculiformis were the most closely related species. It was shown before that H. suis in pigs originates from non-human primates via a possible host jump from macaques to pigs sometime between 100 000 and 15 000 years ago, after which domestication has had a significant impact on the spread of this pathogen among pigs. De Witte et al further unravelled the ancestral source of H. suis and concluded that Cynomolgus macaques but not Rhesus macaques are the common ancestor of the pig-associated H. suis population. An article from the same research group performed AST among H. suis isolates from pigs and macaques using a combined agar and broth dilution method. Two porcine isolates
showed acquired resistance to fluoroquinolones which was due to the presence of SNPs in the gyrA gene. Furthermore, one porcine isolate showed resistance to tetracycline, one porcine and two primate isolates to lincomycin and one primate isolate to spectinomycin. Minimum inhibitory concentrations (MICs) of ampicillin, tetracycline and doxycycline were also higher for porcine isolates from which SNPs were detected in genes encoding penicillin binding and ribosomal proteins. Based on this study, it should be noted that porcine isolates with a zoonotic importance may thus be intrinsically less susceptible to beta-lactam antibiotics and tetracyclines.  

3 | ANIMAL MODELS

Enterohepatic Helicobacter induce inflammation in rodents akin to human IBD. Numerous immunocompetent or compromised murine models are used to study inflammation induced by EHH. A gnotobiotic model of *H. bilis* infection confirmed that this bacterium causes portal hepatitis in outbred Swiss Webster (SW) mice but stimulated gut-associated lymphoid tissue (GALT) with an anti-inflammatory bias. Thus, *H. bilis* exhibits both anti- and pro-inflammatory effects on GALT that deserve to be further evaluated in mouse models of human disease.  

Helicobacter saguini was first isolated in 1999 from a cotton-top tamarin, a rare non-human primate species, with a family history of IBD. Infection of germfree IL-10−/− mice with *H. saguini* is naturally transmissible from parent to offspring and associated with multigenerational IBD. WGS of *H. saguini* among mouse generations showed that the bacterium undergoes genomic adaptation during multigenerational transmission and chronic colonisation in its hosts, attested by microevolutions in environmental interaction, nutrient metabolism, and virulence factor genes. This suggests bacterial adaptation and survival in new hosts and chronic inflammatory environments. These findings highlight the possible importance of multigenetic transmitted pathogenic microbiota in the aetiology of IBD in humans.  

Intestinal innate lymphoid cells (ILCs) are a subset of immune cells, which maintain homeostasis and contribute to protective immunity. The interplay of ILCs and the microbiota is naturally transmissible from parent to offspring and associated with multigenerational IBD. WGS of *H. saguini* among mouse generations showed that the bacterium undergoes genomic adaptation during multigenerational transmission and chronic colonisation in its hosts, attested by microevolutions in environmental interaction, nutrient metabolism, and virulence factor genes. This suggests bacterial adaptation and survival in new hosts and chronic inflammatory environments. These findings highlight the possible importance of multigenetic transmitted pathogenic microbiota in the aetiology of IBD in humans.

Helicobacter pylori infects the stomach and colon and is associated with peptic ulcer disease, gastric adenocarcinoma, and with the development of liver carcinoma in mice, leading to discrepant results. Indeed, hepatitis virus-induced tumourigenesis of hepatocellular carcinoma (HCC) could be aggravated by bacteria. Hepatitis B virus (HBV) transgenic C57BL/6 mice were infected with *H. hepaticus* to evaluate its hepatic carcinogenic effect. Compared with C57BL/6 and HBV-infected mice without neoplasm, *H. hepaticus* more often colonised the lower colon of HBV-infected mice with HCC and potentiated tumour formation in the liver. ILC-derived cytokines contribute to *H. hepaticus*-associated HCC development in HBV transgenic mice. Additional data showed that *H. hepaticus* infection promoted a detrimental immune microenvironment via the IFN-γ/STAT1 axis, which can induce tumourigenesis via HBV by recruiting innate lymphoid cells.

4 | PATHOGENESIS OF NON-HELICOBACTER PYLORI HELICOBACTER INFECTIONS

During the past year, further evidence has emerged regarding the pathogenicity of NHPH. De Sousa et al evaluated the expression of...
HER-2 in feline gastric epithelial cells infected with *H. heilmannii*. A statistically significant association between *H. heilmannii* infection and HER-2 expression in the lateral membrane of gastric surface cells was noted. As *H. heilmannii* has a tropism for parietal cells as well, HER-2 expression was also abundantly present in the plasma membrane of this cell type. Another study highlighted that long-term *H. suis* infection in mice does not only induce gastric MALT lymphoma, but also hepatic and pulmonary lymphoma. Furthermore, they showed a relationship between substance P and the maintenance of MALT lymphoma. A study by De Witte et al. offered more insight into the influence of a naturally acquired *H. suis* infection on the microbiota of the non-glandular part of the porcine stomach and the pathogenic potential of *Fusobacterium gastritis*. Infection with *H. suis* influenced the relative abundance of several taxa, whereas *F. gastritis*, which supports gastric ulceration, induced death of gastric and oesophageal epithelial cells.

Several other reports investigated the pathogenic role of host factors in *H. felis*-induced gastric pathologies. The *H. felis* mouse model remains a good and reproducible model to study host immune responses to gastric Helicobacters, as well as to examine factors that promote gastric carcinogenesis. Chonwerawong et al. showed that NLR family CARD domain containing 5 (NLRC5) is a negative regulator of gastric inflammation and MALT lymphoma formation by promoting B-cell lymphomagenesis during *H. felis* infection. Zhao et al. highlighted the contribution of myeloid-derived suppressor cells (MDSCs) and γδT17 cells in the development of *H. felis*-induced gastric MALT lymphoma by dysregulating the immune balance. The myeloid differentiation factor Schlafen 4 (Sfln4) marks a subset of gastric MDSCs during Helicobacter-induced spasmolytic polypeptide expressing metaplasia (SPEM). The study by Ding et al. identified gene products expressed by Sfln4-MDSCs and promoting SPEM. Moreover, they showed that MIR130b plays an essential role in supporting metaplastic transformation and MDSC function. El-Zaatari et al. demonstrated a novel function of absent in melanoma 2 (Aim2) that regulates gastric metastasis lesions via CXCL16-mediated CD8+ T cells during *H. felis* infection. Another study investigated how β-catenin signaling regulates the tumour immune microenvironment in the stomach using a murine gastric cancer model established by *H. felis*. They showed that the β-catenin-CCL28-Treg cell axis serves as an essential mechanism for immune suppression of the gastric tumour microenvironment. NF-κB signaling plays an essential role during Helicobacter infection and malignant transformation. Deficiency of the adaptor molecule myeloid differentiation primary response 88 (MyD88), which signals via NF-κB, accelerates the development of gastric pathology. Mejias-Luque et al. further investigated this phenotype. They showed that, in the absence of MyD88, *H. felis* infection enhances the activation of non-canonical NF-κB that is associated with an increased expression of Cxcl9 and Icam1 and CD3+ lymphocyte recruitment. In addition, the absence of MyD88 induced STAT3 activation. Copper is essential for all living organisms. A study by Esposito et al. explored the effect of deprivation on *H. felis* infection in mice. They showed that this bacterium takes advantage of gastric copper, reducing the availability for the host. Furthermore, they also highlighted that copper levels determine the outcome of infection. Finally, Hong et al. developed a live-based cell assay based on, amongst others, *Helicobacter mustelae* a1-2 fucosyltransferase to analyse host cell glycan-mediated influenza virus infection. They highlighted that *H. mustelae* a1-2 fucosyltransferase is a useful tool for this application.

Enterohpatic Helicobacter, such as *Helicobacter pullorum* and *H. hepaticus*, are associated with several intestinal and hepatic diseases. Their main virulence factors are a type six secretion system (T6SS) and a cytolethal distending toxin (CDT). *Helicobacter pullorum* is an emerging zoonotic pathogen that causes digestive diseases in humans ingesting contaminated meat. Haemolysin coregulated protein (Hcp) plays a key role in the structure of the T6SS pilus and acts as effector protein in certain bacteria. The presence of the Hcp gene was screened via PCR in *H. pullorum* isolated from 156 caeca of broiler chickens, of which 29.7% possessed the T6SS gene. During co-culture experiments, Hcp was not associated with hepatocyte cell death but with greater haemolytic activity of infected erythrocytes. Further studies are required to characterise the importance of T6SS and Hcp in the virulence of *H. pullorum*.

Cytolethal distending toxin is widespread among EHH *H. hepaticus* CDT upregulates the MAFB gene encoding the MAFB transcription factor, as well as MAFB target genes. MAFB silencing changed the cellular response to CDT with the formation of narrower lamellipodia, a reduction of the increase in nucleus size and the formation of less γH2AX foci, the biomarker for DNA double-strand breaks. These data, obtained in intestinal and hepatic cell lines, demonstrated that the cellular and nuclear remodeling induced by Helicobacter CDT, produced by *H. pullorum* and *H. hepaticus*, involves the MAFB oncoprotein. These authors unveiled that the nuclear remodeling following DNA damage induced by CDT can be associated with the formation of deep cytoplasmic invaginations in the nucleoplasm of giant nuclei. The core of these structures, also known as nucleoplasmic reticulum (NR), concentrates protein production machinery of the cell, as well as controlling elements of protein turnover. Additional data showed that insulation and concentration of these adaptive ribonucleoprotein particles within CDT-induced NR are dynamic, transient, reversible and allow the cell to pause and repair the DNA damage caused by CDT in order to maintain cell survival.

A new type of alanine dehydrogenase (ADH; EC.1.4.1.1) with high pyruvate reduced activity was isolated from *Helicobacter aurati* and expressed in *Escherichia coli* for further biochemical characterisation.

## 5 | OMICS OF NON–HELICOBACTER PYLORI HELICOBACTER SPECIES

The complete genome sequence of *H. suis* strain SNTW101c isolated from a patient with nodular gastritis was recently sequenced. The genome size was 1 680 021 bp comprising 1744 coding sequences (CDSs), 5 ribosomal RNAs and a GC content of 40%. Two putative plasmids encoding 6 CDSs each were also identified. As suggested
before, the main H. pylori virulence factors were absent indicating that other factors contribute to the pathogenicity of this zoonotic agent. Another study performed WGS to support Helicobacteraceae taxonomy. They showed that, a strain (CNRCH 2005/566H) isolated in a faecal sample from a patient with a HCC and gastroenteritis, and another strain (48 519) isolated in blood sample from a patient with bacteremia represented novel EHH species for which the names “Helicobacter burdigalis” and “Helicobacter labetoulli,” respectively, were proposed.

Berlamont et al gained more insights into the interactions of the zoonotic H. suis and H. heilmannii species with the human gastric epithelium by unraveling their transcriptomic signatures upon adherence. The differentially expressed genes of H. suis and H. heilmannii upon adherence belonged to multiple functional classes, indicating that adhesion of both species to the gastric epithelium evoked pleiotropic adaptive responses. Furthermore, the authors concluded that distinct pathways are involved in human gastric colonisation of H. suis and H. heilmannii.

Wastewater is a continuous source for agricultural irrigation. Poor management prior to water reuse may cause risks for the environment but might also negatively impact human health. Hortelano et al investigated the presence of pathogens in wastewater samples using metagenomic approaches. They unveiled the presence of bacteria belonging to the Helicobacter genus. More specifically, the molecular techniques used in this study identified the presence of H. pylori, H. suis, H. pullorum and H. hepaticus DNA in wastewater samples, even after disinfectant treatment.

DISCLOSURES OF INTERESTS

The authors declare no conflict of interests.

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Electronic Poster Round 2: Treatment of Helicobacter infection
Electronic Poster Round 3: Gastric cancer
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Missing abstracts within the consecutive presentation numbers represent withdrawn papers.
ABSTRACTS

ELECTRONIC POSTER PRESENTATIONS

ELECTRONIC POSTER ROUND 1: DIAGNOSIS OF HELICOBACTER INFECTION

EP1.01 | “Test-and-treat” strategy with urea breath test: A cost-effective approach for the management of Helicobacter pylori-related dyspepsia and the prevention of peptic ulcer in the United Kingdom-results of the Hp-Breath initiative

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Background: Clinical data comparing strategies used for the management of H. pylori-associated diseases are limited. In the UK, Stool Antigen Test (SAT) seems to be the most frequently used non-invasive test for H. pylori detection. Cost-effectiveness studies might help to identify optimal strategies.

Aim: To assess cost-effectiveness of the “Test-and-Treat” strategy with Urea Breath Test (UBT) versus other strategies, in patients with H. pylori-associated dyspepsia and peptic ulcer in the UK.

Methods: Cost-effectiveness models compared four strategies: “Test-and-Treat” including either UBT or SAT, “Endoscopy and Treat” and “Symptomatic Treatment”. Advanced simulations were performed over a 4 week time horizon for the endpoint “Probability of dyspepsia symptoms relief” and over 10 years for the “Probability of peptic ulcer avoided”. Models were developed according to UK routine medical practice and costs.

Results: For the “Probability of dyspepsia symptoms relief” endpoint, “Test-and-Treat” strategies with either UBT or SAT were the most cost-effective (respectively £459 and £452/success) versus “Endoscopy and Treat” and “Symptomatic Treatment” (respectively £1,115 and £897/success). For the “Probability of peptic ulcer avoided” endpoint, “Test-and-Treat” strategies with either UBT or SAT were also the most cost-effective (respectively £182 and £167/peptic ulcer avoided/year) versus “Endoscopy and Treat” and “Symptomatic Treatment” (respectively £625 and £568/peptic ulcer avoided/year).

Conclusion: “Test-and-Treat” strategies with either UBT or SAT are the most cost-effective medical approaches for the management of H. pylori-associated dyspepsia and the prevention of peptic ulcer in the UK. “Test-and-Treat” strategy with UBT has comparable cost-effectiveness outcomes to the strategy utilising SAT.

D.M. Pritchard: F. Consultant/Advisory Board; Modest; Mayoly Spindler Laboratories. J. Bornschein: F. Consultant/Advisory Board; Modest; Mayoly Spindler Laboratories. I.L.P. Beales: F. Consultant/Advisory Board; Modest; Mayoly Spindler Laboratories. H. Salhi: A. Employment (full or part-time); Modest; Mayoly Spindler Laboratories. A. Beresniak: F. Consultant/Advisory Board; Modest; Mayoly Spindler Laboratories. P. Malfertheiner: F. Consultant/Advisory Board; Modest; Mayoly Spindler Laboratories.

EP1.02 | Comparative estimation of results of the new selective rapid urease test and detection of H. pylori in stool: A pilot study

N. V. Baryshnikova1,2; Y. P. Uspenskiy1,3; A. N. Suvorov2; M. A. Dmitrienko1
1Pavlov First St-Petersburg State Medical University, St-Petersburg, Russian Federation; 2Institute of Experimental Medicine, St-Petersburg, Russian Federation; 3Pediatric State St-Petersburg Medical University, St-Petersburg, Russian Federation; *Association of Medicine and Analytic, St-Petersburg, Russian Federation

Background: According to Maastricht-5, detection of H. pylori in stool is one of the main recommended method in clinical practice. Also we know many rapid urease tests for diagnosis of Helicobacter pylori (Hp) infection. It is fast, simple and cost-effective methods but efficacy of them is different with some percent of false-positive results. We begin to use the new selective rapid urease test for selective analysis of urease activity of H. pylori.

The Aim: Comparative estimation of results of the new selective rapid urease test and detection of H. pylori in stool.

Materials and Methods: We investigated gastric biopsies from antrum and fecal samples of eight patients to compare efficacy of the new selective rapid urease test (“Association of medicine and analytics, Saint-Petersburg, Russia) and detection of H. pylori in stool by enzyme immunoassay. Patients during at least 4 weeks before diagnostics did not take any medications (PPIs, antibiotics, antacids and bismuth), which could change the results of both tests. Statistical processing was performed using the SPSS8.0 software package.

Results: Concordance of results the new selective rapid urease test and detection of H. pylori in stool was 100%: 4 patient were H. pylori positive and 4 patient – H. pylori negative by results of both tests.

Conclusion: The new selective rapid urease test shows the high concordance with results of detection of H. pylori in stool and can be recommended as express invasive test for diagnostic of H. pylori infection. Next studies are needed for further investigation of this test.

EP1.03 | Screening for Helicobacter pylori infection and Clarithromycin resistance using real-time polymerase chain reaction

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1Department of Gastroenterology, University Hospital Tsaritsa Ioanna-ISUL, Medical University-Sofia, Sofia, Bulgaria; 2Department of Biotechnology, Faculty of Biology, Sofia University “St. Kliment Ohridski”, Sofia, Bulgaria

Background: Helicobacter pylori (H. pylori) infection is spread worldwide and affects at least half of the world’s population. Infected people are at increased risk of several diseases development including gastric adenocarcinoma.

Aims: The aim of this study was to screen patients with dyspeptic symptoms for H. pylori infection and assess Clarithromycin resistance prevalence among the infected patients.

Methods: Screening for H. pylori infection was performed in all patients using molecular test based on real-time polymerase chain reaction (RT-PCR) in feces after RNA-DNA extraction. Stool samples from all participants were collected 1-3 days after patients’ hospitalization. The positive results were furthermore assessed for confirmation by breath test and stool antigen test. By point mutations detection in 23S rRNA gene was possible to detect Clarithromycin resistance. Statistical analysis was performed via SPSS 22.0.

Results: This study enrolled 50 patients (18 males) at mean age 46.46 ± 15.10 years. Using molecular test based on RT-PCR in feces we identified H. pylori infection in 24 patients (48.00%). Clarithromycin resistance was observed in 7 of them (29.17%). None of those patients was eradicated before. There was no significant difference by age and gender between infected and non-infected patients. Gastrointestinal symptoms were more often reported in infected patients (P = 0.02). The molecular test showed 85.71% sensitivity and 100% specificity, with a diagnostic accuracy of 92.00%.

Conclusions: H. pylori screening by molecular test based on RT-PCR in feces might be beneficial as the test’s accuracy is high and include Clarithromycin resistance assessment, which could improve the outcome of eradication therapy.

M. Kovacheva-Slavova: None; H. Valkov: None; T. Angelov: None. R. Tropcheva: None. B. Vladimirov: None.

EP1.04 | Accuracy of the Helicobacter pylori diagnostic tests in patients with peptic ulcer bleeding- The results of a network meta-analysis

N. Vörhendi; P. Hegyi; B. Erőss

Institute for Translational Medicine, Medical School, Pécs, Pécs, Hungary

Introduction: Peptic ulcer (PU) being the most frequent source of gastrointestinal bleeding and Helicobacter pylori is a main etiologic factor for it. Some studies suggest accuracy of the diagnostic tests is decreased in PU bleeding. The international guidelines are vague on the method of testing in the setting of acute peptic ulcer bleeding. However, detection of H. pylori would be essential as it reduces the risks of untoward outcomes.

Aims: Our aim was to update the most recent meta-analysis which included studies until 2006 and to assess the accuracy of one or a combination of more diagnostic tests for H. pylori in patients with bleeding peptic ulcer.

Methods: A comprehensive literature search was carried out from inception to November 2019 to perform a network meta-analysis. We collected the raw data of diagnostic tests such as true positive, true negative, false positive and false negative values. All statistical calculations performed by R programming language using anova arm-based model by Nyaga et al., 2018. We ranked the methods, index tests according to the diagnostic odds ratio and/or superiority index.

Results: We analyzed 7 arbitrary gold standards in 7 network against single and combination of diagnostic tests. None of the calculated superiority indices proved that any of the tests have better diagnostic accuracy than the individual index tests.

Conclusion: The results from are extensive network metaanalysis showed that none of the current diagnostic tests for H. pylori are better or worse in the diagnosis of the infection in the context of peptic ulcer bleeding.

N. Vörhendi: None. P. Hegyi: None. B. Erőss: None.

EP1.05 | Helicobacter pylori screening in patients with acute myocardial infarction

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Introduction: Potent antithrombotic therapy has significantly improved prognosis for patients with acute myocardial infarction (MI), however, at a price of increased bleeding risk. Chronic gastric infection with Helicobacter pylori (HP) commonly causes upper gastrointestinal bleeding and is proposed as a risk factor for MI. The prevalence of Hp in a current MI population and the feasibility of Hp screening as part of routine clinical care are unclear.

Aims & Methods: In this multicenter, open-label, clinical trial, all patients admitted for acute MI during the study period were eligible for enrollment. After written informed consent patients were tested for Hp infection with a bedside urea breath test (UBT [Mayoly Spindler]) incorporated into routine care during the hospitalization period.

Results: 274 consecutive MI patients (median age 67 years, 24% women) were enrolled. Overall, the HP prevalence was 20% (95% CI, 15.5-25.3). The proportion of proton pump inhibitors (PPI) users was significantly higher in Hp negative compared to Hp positive patients (38% vs 16%; P = 0.003). After censoring of the 93 subjects with PPI exposure preceding the UBT, the HP prevalence was 25%. After adjusting for age and sex, smoking was found to be significantly
ABSTRACTS

Diagnostic accuracy of HpSA test was determined using 13C-urea breath test as reference standard. Baseline comorbidities were analyzed for factors which were associated with the accuracy of HpSA test. Results: 316 participants were enrolled, 193 in the pre-treatment group (77.2 ± 7.8 years old) and 123 in the post-treatment group (78.7 ± 8.3 years old). High accuracy (91.5%, 91.2% and 91.9%) and specificity (97.6%, 98.7% and 96.0%) were obtained in all, pre- and post-treatment groups respectively. However, the sensitivity was only 68.7%, 65.1% and 75.0%, respectively. In the pre-treatment group, constipation was associated with decreased sensitivity (76.7% vs 38.5%, P = 0.039), while colorectal polyps with increased sensitivity (45.0% vs 82.6%, P = 0.010). Multivariate analysis indicated that constipation (OR = 0.115, 95% CI: 0.020-0.666) and colorectal polyps (OR = 9.095, 95% CI: 1.656-49.955) were independent factors for the sensitivity of HpSA in the pre-treatment group.

Conclusions: Immunochromatographic assay-based HpSA test achieved high accuracy, with high specificity but suboptimal sensitivity in this elderly male cohort. Constipation was negatively while colorectal polyps was positively associated with HpSA sensitivity in pre-treatment group.

Y. Han; W. Dai; F. Meng; X. Gan; M. Liu; X. Deng; Y. Li; G. Wang
Chinese PLA General Hospital, Beijing, China

Background: The diagnostic role of Helicobacter pylori stool antigen (HpSA) test in elderly subjects remains unclear. The objective of this study was to assess the diagnostic accuracy of immunochromatographic assay-based HpSA test in a male elderly cohort and to identify factors that may affect the accuracy.

Materials and Methods: Data of asymptomatic elderly male citizens (≥65 years old) who conducted health check at Chinese PLA General Hospital between July 2007 and November 2018, were collected. Diagnostic accuracy of HpSA test was determined using 13C-urea breath test as reference standard. Baseline comorbidities were analyzed for factors which were associated with the accuracy of HpSA test.

Results: 316 participants were enrolled, 193 in the pre-treatment group (77.2 ± 7.8 years old) and 123 in the post-treatment group (78.7 ± 8.3 years old). High accuracy (91.5%, 91.2% and 91.9%) and specificity (97.6%, 98.7% and 96.0%) were obtained in all, pre- and post-treatment groups respectively. However, the sensitivity was only 68.7%, 65.1% and 75.0%, respectively. In the pre-treatment group, constipation was associated with decreased sensitivity (76.7% vs 38.5%, P = 0.039), while colorectal polyps with increased sensitivity (45.0% vs 82.6%, P = 0.010). Multivariate analysis indicated that constipation (OR = 0.115, 95% CI: 0.020-0.666) and colorectal polyps (OR = 9.095, 95% CI: 1.656-49.955) were independent factors for the sensitivity of HpSA in the pre-treatment group.

Conclusions: Immunochromatographic assay-based HpSA test achieved high accuracy, with high specificity but suboptimal sensitivity in this elderly male cohort. Constipation was negatively while colorectal polyps was positively associated with HpSA sensitivity in pre-treatment group.


EP1.06 | Diagnosis of Helicobacter pylori infection in the elderly by immunochromatographic assay-based stool antigen test

Y. Han; W. Dai; F. Meng; X. Gan; M. Liu; X. Deng; Y. Li; G. Wang
Chinese PLA General Hospital, Beijing, China

Implementation of the program of the Scientific Society of Gastroenterologists of Russia “Physicians without helicobacteriosis” in Chita

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1Chita State Medical Academy, Chita, Russian Federation; 2Moscow State University of Medicine and Dentistry named after A. I. Evodikomov, Moscow, Russian Federation

Objective: To identify the prevalence of Helicobacter pylori (HP) in doctors in Chita, morphological changes in gastric mucosa, carry out eradication treatment and assess its effectiveness, to determine HP resistance to clarithromycin.

Materials and Methods: 70 doctors were examined. HP antigen in feces was determined by immunochromatographic method before and 6-8 weeks after the end of eradication treatment. In 27 biopsy specimens of the gastric mucosa HP DNA and mutations A2142G, A2143G, T2717C in the bacterial genome were determined. 29 biopsies of the stomach mucous membrane from 5 points for histological examination and evaluation by OLGA system were taken.

Results: A positive result of AG HP in feces was recorded in 71.4% doctors. The efficiency of eradication treatment was 72.7% (table). During histological examination there was a high degree of inflammation in stomach mucosa, indicating high risk of erosive complications in the majority of doctors (86.1%). Different stages of atrophy were detected in 89.6%. Type III colonic metaplasia was detected in 6 (20.7%) people. One doctor demonstrated intraepithelial indefinite neoplasm. In 10 biopsies (37%) A2142G, A2143G mutations in the HP genome, ensuring its resistance to clarithromycin, were revealed. Mutations of T2717C were not determined.

Conclusion: Doctors in Chita demonstrate a high level of HP infection, insufficient eradication treatment effectiveness, high level of genotypic HP resistance to clarithromycin, increased prevalence of precancerous changes in gastric mucosa.

<table>
<thead>
<tr>
<th>Treatment regimen</th>
<th>Patients, n</th>
<th>Efficiency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esomeprazole, bismuth potassium dicitrate, probiotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>30</td>
<td>73</td>
</tr>
<tr>
<td>Josamycin</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
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<td></td>
</tr>
<tr>
<td>Metronidazole</td>
<td>7</td>
<td>43</td>
</tr>
</tbody>
</table>

E. Luzina: None. L. Lazebnik: None. N. Lareva: None. N. Chartorizhskaya: None. A. Dutova: None.
EP1.08 | "Helicobacter pylori and mast cells in the mucous membrane of the stomach"

N. Samodurova; D. Atiakshin
Voronezh State Medical University named after N. N. Burdenko, Voronezh, Russian Federation

Relevance: Mast cells are able to regulate the state of the gastric mucosa using mediators with high activity. Despite the obvious pathogenetic role of mast cells in the development of gastritis induced by H. pylori (HP), the features of their functional and morphological co-localization in the gastric mucosa are still poorly understood.

Materials and Methods: The biopsy samples of the stomach of patients infected with H. pylori were studied, followed by analysis of the results of immunohistochemical staining.

Results: The test material showed a high correlation between the abundance of mast cells and HP in the stomach mucosa. We noted frequent co-localization of tryptase+ mast cells with HP within paracrine influences the biological effects of specific proteases. Situations of direct contact of mast cells with HP cluster loci are particularly important. Activation of mast cell secretory pathways into a specific tissue gastric environment of patients infected with HP was also observed. An issue is also the resistance of the mucosa to HP in the presence of mast cells.

Conclusion: The study is a step forward in understanding the mechanisms of HP infection progression. HP in the gastric mucosa leads to an increase in mast cell tryptase expression, increased inflammation and the development of biological effects of specific proteases. N. Samodurova: None. D. Atiakshin: None.

EP1.09 | "Immunomorphological aspects of evaluating the interaction of mast cells and Helicobacter pylori in the stomach mucus"

N. Samodurova; D. Atiakshin
Voronezh State Medical University named after N. N. Burdenko, Voronezh, Russian Federation

Relevance: Morphological identification of Helicobacter pylori (HP) in the gastric mucosa, including coecal forms of the spiral bacterium, helps to verify the diagnosis and refine the prognosis of the disease. The use of multiple immunomarking technology opens up new prospects for the informative value of histochemical analysis due to the possibility of simultaneous identification of HP and mast cells.

Materials and Methods: Rabbit monoclonal antibodies (# ab172611, dilution 1:500) were used as primary antibodies for immunohistochemical staining of HP, and mast cell tryptases were murine monoclonal antibodies (# ab2378, dilution 1:3000). Goat Anti-Mouse IgG H&L antibodies (# ab97035) conjugated with Alexa Fluor 488 and Goat Anti-Rabbit IgG H&L antibodies (# ab150077) conjugated with Cy3 were used as secondary antibodies in multiple immunomarking. Next, the nuclei were stained with DAPI (5 μg/mL PBS; Sigma) for 15 seconds, washed with PBS and the sections were enclosed in an anti-fluorescent mounting medium. Stained micropreparations of the stomach were studied with a ZEISS Axio Imager.A2 microscope.

Results: Localization of HP in the gastric mucosa led to an increase in the number of mast cells, their frequent colocalization with spiral bacteria, increased expression of tryptase and activation of its secretory pathways with the development of biological effects.

Conclusion: Immunomorphological approaches provide new molecular aspects of the features of the interaction of mast cells and HP, expanding the interpretation of the mechanisms of the formation of a pro-inflammatory background, the characteristics of immunogenesis and remodeling of the own plate of the gastric mucosa. N. Samodurova: None. D. Atiakshin: None.

EP2.01 | Quality assessment of meta-analyses: Probiotics and eradication of Helicobacter pylori infection

G. M. Buzás; J. Józan
Ferencváros Health Service Non-Profit Ltd, Budapest, Hungary

Background: Meta-analyses are believed to represent the highest level of medical evidence (Grade A).

Aim: To assess the quality of meta-analyses published on the effect of adding probiotics to eradication regimens for Helicobacter pylori infection.

Methods: The full text of meta-analyses regarding the effect of probiotics on the eradication rates of regimens given for Helicobacter pylori infection were retrieved from MEDLINE and Google Scholar databases. The methodological and reporting quality were determined using the Assessment of Multiple Systematic Reviews-2 (AMSTAR 2) questionnaire. The correlation between the AMSTAR score as a dependent variable and the number of authors, number of databases used, impact factor and citation rate as independent variables was calculated. The rate of using the PRISMA checklist, PROSPERO registration and evidence grading was also noted.

Results: The literature search produced 20 meta-analyses published between 2007 and 2019. The mean AMSTAR score was 15.7 ± 0.74 (95% CI: 14.1-17.3), corresponding to a moderate quality. There was no correlation between number of authors (r = 0.20, P = 0.39), impact factor (r = 0.18, P = 0.47), number of databases searched (r = 0.02, P = 0.91), number of studies included (r = 0.03, P = 0.89), number of cases studied (r = 0.04, P = 0.86) and the AMSTAR score. The PRISMA checklist was used in 5 studies (25%), and no research protocol was registered in PROSPERO. The grading of evidence was explicitly stated only in 8 (40%) publications.

Conclusions: Meta-analyses published so far on the proposed topic are of rather moderate quality. Meta-analysis methodology must be improved to obtain more conclusive data on use of probiotics. G.M. Buzás: None. J. Józan: None.
EP2.02 | Rifabutin triple therapy for first-line and rescue treatment of Helicobacter pylori infection: A systematic review and meta-analysis

Y. Niv; R. Gingold-Belfer; Z. Levi; D. Boltin
Rabin Medical Center, Petah Tikva, Israel

**Background:** Due to the increasing resistance of *H. pylori* there is a need for novel antibiotic treatment protocols.

**Aims:** To perform a meta-analysis of clinical trials in order to determine the effectiveness and safety of rifabutin triple therapy for *H. pylori* infection.

**Methods:** We selected prospective clinical trials with a treatment arm consisting of proton pump inhibitor, amoxicillin and rifabutin, and a recorded outcome measure.

**Results:** Thirty-three studies including 44 data sets were included. The pooled eradication success for rifabutin triple therapy was 73.2% (95% CI 0.710-0.753) and the pooled OR for rifabutin triple therapy vs control was 1.40 (95% CI 1.103-1.775, \(I^2 = 73.55, P = 0.006\)). Treatment was more likely to be successful when given first line vs rescue (82.4% vs 71.3%, \(P = 0.0001\), in Asian vs non-Asian populations (81.0% vs 72.4% \(P = 0.001\)) and when drug dose or duration were augmented (80.6% vs 66.0% \(P = 0.0001\)). The overall event rate for adverse effects was 25.3% (95% CI 0.23-0.28) and the pooled OR for adverse effects in the treatment vs control group was 0.79 (95% CI 0.57-1.09, \(I^2 = 61.58, P = 0.15\)).

**Conclusion:** Rifabutin is a relatively safe and effective option for first-line and rescue treatment for *H. pylori* infection in adults.


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EP2.03 | European registry on Helicobacter pylori management (Hp-EuReg): First-line therapy in Israel

D. Boltin; Z. Beniashvili; A. Lahat; J. Hirsch; O. P. Nyssen; F. Mégraud; C. O’Morain; J. P. Gisbert; Y. Niv

**Background:** The antibiotic resistance profile of *H. pylori* is constantly changing. Up-to-date and reliable data for the effectiveness of first-line *H. pylori* treatments protocols for are necessary in order to provide evidence-based best-practice guidelines.

**Objectives:** We aimed to determine the effectiveness, compliance and safety of first-line treatment for *H. pylori* in Israel.

**Methods:** An observational, prospective, multicenter study was carried out in tertiary referral centers in Israel, as part of the European registry on *H. pylori* management (Hp-EuReg). *H. pylori*-infected patients were included from 2013 to March 2020. Data collected included demographics, clinical data, diagnostic tests, previous eradication attempts, current treatment, compliance, adverse events and treatment outcome.

**Results:** In total, 242 patients were registered including 121 (50%) who received first-line therapy, of whom 41% received clarithromycin based triple therapy and 58.9% received a four-drug regimen. The overall effectiveness of first-line therapy was 85% and 86% by modified intention-to-treat and per protocol analyses, respectively. The effectiveness of both sequential and concomitant therapies were 100% while clarithromycin-based triple therapy achieved an eradication rate of 79%. Treatment eradication was higher among patients who received high dose PPI compared to those treated with low dose PPI (100% vs 81.5% respectively, \(P < 0.01\)). No difference in treatment effectiveness was found between 7, 10 and 14-day treatment.

**Conclusion:** The effectiveness of clarithromycin-based triple therapy is suboptimal. First-line treatment of *H. pylori* infection should consist of four drugs, including high doses PPI, in accordance with international guidelines.


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EP2.04 | Eradication rate of Helicobacter pylori infection in Chinese duodenal ulcer patients treated with Vonoprazan 20 mg versus Lansoprazole 30 mg with bismuth containing quadruple therapy

J. Wang; X. Hou; F. Meng; W. Sha; L. Gu; K. Kudou; L. Dong; L. Xie; S. Zhang

1China-Japan Union Hospital, Jilin University, Changchun, China; 2Huzhong University of Science and Technology, Wuhan, China; 3Beijing Friendship Hospital, Capital Medical University, Beijing, China; 4Guangdong General Hospital (Guangdong Academy of Medical Sciences), Guangdong Geriatrics Institute, Guangdong, China; 5Takeda Development Center Asia, Shanghai, China; 6Takeda Pharmaceutical Company, Osaka, Japan; 7Takeda Pharmaceutical Company, Beijing, China

**Background:** Vonoprazan shows superior healing of erosive oesophagitis than lansoprazole. However, the clinical efficacy of vonoprazan in eradicating *Helicobacter pylori* (Hp) infection in Chinese patients with duodenal ulcers (DU) is not established.

**Methods:** This phase 3, multicentre, randomised, double-blind, double-dummy study aimed to demonstrate non-inferiority of vonoprazan (20 mg) to lansoprazole (30 mg) in treating subjects with DU (stratified by Hp status). Eradication of Hp was the secondary end point. Hp-positive patients received drugs twice daily for the first 2 weeks in addition to bismuth-containing quadruple therapy. Hp eradication status was assessed using 13C-Urea Breath Test. Hp eradication rates (%) and 2-sided 95% confidence intervals were calculated along with...
eradication rate difference between the groups. Non-inferiority margin was 10%.

Results: Totally, 433 Hp-positive Chinese patients with DU were randomised to vonoprazan (N = 215) and lansoprazole (N = 218), of which 394 were available for evaluation at 4 weeks post-treatment. Baseline characteristics were comparable between the 2 groups. Hp eradication rate was 91.5% and 87.6% in vonoprazan and lansoprazole groups, respectively, with a treatment difference of 4.0% (Table 1). Incidence of treatment-emergent adverse events was comparable between the groups.

Conclusion: Vonoprazan was non-inferior to lansoprazole when used in quadruple combination therapy for Hp eradication, with similar safety profiles; however, vonoprazan-based quadruple therapy is clinically meaningful with a Hp eradication rate of more than 90% in Chinese patients with DU.

**TABLE 1.** Percentage of Hp-positive patients with successful Hp eradication after 4 or 6 weeks of treatment

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Vonoprazan+bismuth-containing quadruple therapy* (N = 215)</th>
<th>Lansoprazole+bismuth-containing quadruple therapy* (N = 218)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of positive patients</td>
<td>201</td>
<td>193</td>
</tr>
<tr>
<td>Hp negative at 4 weeks post-treatment, (F-2 visit) n (%)</td>
<td>184 (91.5)</td>
<td>169 (87.6)</td>
</tr>
<tr>
<td>Hp positive at 4 weeks post-treatment, (F-2 visit) n (%)</td>
<td>17 (8.5)</td>
<td>24 (12.4)</td>
</tr>
<tr>
<td>95% CI</td>
<td>86.804, 94.996</td>
<td>82.064, 91.867</td>
</tr>
<tr>
<td>Difference in eradication rate vs lansoprazole (%)</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>−2.062, 10.071</td>
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</tbody>
</table>

**Methods:** The European Registry on H. pylori Management (Hp-EuReg) collecting the data of H. pylori diagnostics, prescribed treatment and outcomes. Data from 2013 to 2019 were analysed. The eradication success was calculated using the modified intention-to-treat (mITT) analysis.

**Results:** In total, 1,408 patients from Lithuania were included in the Hp-EuReg. Overall, triple-therapy (proton pump inhibitor (PPI) + 2 antibiotics) was administered in 93.5% of the cases. For the first-line treatment, triple therapy with PPI, clarithromycin and amoxicillin (PPI+C+A) was prescribed in 93.6% of the cases. The most frequent second-line treatment was combination of PPI, amoxicillin and levofloxacin (PPI+A+L) – in 57.1% of the cases. The frequencies of other combinations is presented in Table 1. The confirmation of Hp eradication was assessed only in 298 (21.2%) cases. The effectiveness of first-line PPI+C+A regimen was 86.3% and of second-line PPI+A+L regimen was 86.2%. The overall effectiveness of H. pylori treatments was 86.9%.

**Conclusions:** The number of cases with confirmatory tests post treatment is extremely low. The effectiveness of most common eradication regimens remains suboptimal.

**Background:** It is important to administer effective H. pylori eradication and perform testing for the eradication success.

**Aim:** To evaluate the H. pylori treatment effectiveness in Lithuania with regards to Maastricht V guidelines.
<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>% of all cases</th>
<th>No. of patients tested post-treatment</th>
<th>mITT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. pylori eradication regimen (first-line treatment)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPI+C+A</td>
<td>1,137</td>
<td>93.6%</td>
<td>234</td>
<td>86.3%</td>
</tr>
<tr>
<td>PPI+C+A+B</td>
<td>27</td>
<td>2.2%</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>PPI+C+M</td>
<td>23</td>
<td>1.9%</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
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<td>7</td>
<td>0.6%</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
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<td>0.6%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPI+A+L</td>
<td>6</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPI+A+M</td>
<td>4</td>
<td>0.28%</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>PPI+A+C+T Sequential</td>
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<td>0.08%</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>PPI+C+M+B</td>
<td>1</td>
<td>0.08%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPI+C+L</td>
<td>1</td>
<td>0.08%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPI+C+A+M</td>
<td>1</td>
<td>0.08%</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td><strong>H. pylori eradication regimen (second-line treatment)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPI+A+L</td>
<td>84</td>
<td>57.1%</td>
<td>29</td>
<td>86.2%</td>
</tr>
<tr>
<td>PPI+C+A</td>
<td>18</td>
<td>12.2%</td>
<td>8</td>
<td>62.5%</td>
</tr>
<tr>
<td>PPI+A+L+B</td>
<td>16</td>
<td>10.9%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPI+A+M</td>
<td>9</td>
<td>6.1%</td>
<td>3</td>
<td>100%</td>
</tr>
<tr>
<td>PPI+A+M+B</td>
<td>7</td>
<td>4.8%</td>
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</tr>
<tr>
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<td>4</td>
<td>2.7%</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>PPI+C+A+B</td>
<td>4</td>
<td>2.7%</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>PPI+A+C+T sequential</td>
<td>3</td>
<td>2%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPI+C+M+B</td>
<td>1</td>
<td>0.7%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPI+C+L+B</td>
<td>1</td>
<td>0.7%</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

PPI, proton pump inhibitor; C, clarithromycin; A, amoxicillin; L, levofloxacin; M, metronidazole; T, tinidazole; B, bismuth; mITT, modified Intention-To-Treat.

EP2.06 | Helicobacter pylori eradication treatment in Lithuania during 2013-2019: Trend analysis of the European Registry on H. pylori management (Hp-EuReg)

L. Jonaitis1; J. Kupcinskas1; G. Kliudelis1; P. Jonaitis1; R. Venciene2; L. Kupcinskas3; O. P. Nyssen4; I. Puig5; C. O’Morain6; F. Megraud7; J. P. Gisbert8; On behalf of the Hp-EuReg Investigators

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Background: In recent years eradication of Helicobacter pylori is shifting from triple to quadruple therapy and a treatment duration from 7 to 10-14 days.

Aim: To evaluate the trends of H. pylori eradication regimens and duration in Lithuania.

Methods: We analysed the data from the European Registry on H. pylori Management (Hp-EuReg) collected from 2013 to 2019. H. pylori eradication regimens were defined as triple or quadruple regimens and by length (7, 10 or 14 days).

Results: 1,408 patients were included. Between 2013 and 2019, triple therapies were mostly prescribed. Most of the quadruple therapies were introduced from 2018. (The bismuth introduced in the Lithuanian market only in 2018). The most common duration of treatment between the years 2013-2017 was 7 days however, since 2018 the most common duration was 10-14 days. This could be influenced by the Maastricht V guidelines in 2016 (7 days therapies not recommended). The most commonly prescribed eradication regimen in Lithuania (PPI, clarithromycin and amoxicillin) for 7 days was the preferred choice between 2013 and 2017; however, since 2015 the number of 10-14 days duration treatments increased and was most frequent in 2018. The detailed data are in Table 1.

Conclusions: Although triple therapy remains the most common treatment regimen in Lithuania, quadruple therapies have increased since 2018. During the 2013-2019, a clear shift from 7 to 10 or 14 days duration was observed.

### Table 1

<table>
<thead>
<tr>
<th>Year of visit and treatment duration by days</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment regimen</td>
<td>7</td>
<td>10</td>
<td>14</td>
<td>7</td>
<td>10</td>
<td>14</td>
<td>7</td>
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<tr>
<td>Triple C+M</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Triple C+A</td>
<td>63</td>
<td>5</td>
<td>136</td>
<td>2</td>
<td>–</td>
<td>136</td>
<td>2</td>
</tr>
<tr>
<td>Triple A+L</td>
<td>–</td>
<td>7</td>
<td>1</td>
<td>12</td>
<td>–</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Triple A+M</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Triple C+L</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Seq A+C+T</td>
<td>–</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple C+A+M</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple C+M+B</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple C+A+B</td>
<td>–</td>
<td>2</td>
<td>18</td>
<td>–</td>
<td>7</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple A+M+B</td>
<td>–</td>
<td>4</td>
<td>2</td>
<td>–</td>
<td>7</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple A+L+B</td>
<td>–</td>
<td>1</td>
<td>12</td>
<td>–</td>
<td>4</td>
<td>18</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple C+L+B</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple Total</td>
<td>64</td>
<td>13</td>
<td>141</td>
<td>15</td>
<td>144</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>Quadruple Total</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>8</td>
<td>32</td>
<td>–</td>
<td>20</td>
</tr>
</tbody>
</table>

Background/Aims: Eradication rate of standard triple therapy for H. pylori has declined to unacceptable level, and alternative regimens such as concomitant and sequential therapy have been introduced. We aimed to assess the current trend of eradication rates of concomitant and sequential therapies as for the first-line H. pylori eradication in Korea.

Methods: A nationwide multicenter retrospective study was conducted including 18 second or tertiary medical centers from January 2008 to December 2017. We included 3,940 adults who had test to confirm H. pylori eradication within 1 year after first-line concomitant or sequential therapy. Results: First-line concomitant and sequential therapy was prescribed for 2,609 and 1,331 patients, respectively. The overall eradication rate of concomitant therapy was significantly higher than sequential therapy (91.6% vs 85.7%, P < 0.001). In time trend analysis, concomitant regimen also showed higher eradication rate from 2015 to 2017 with an increasing trend. Among 289 patients with first-line eradication failure, second-line bismuth-based quadruple therapy and quinolone-based triple therapy was given for 202 (69.9%) and 71 (24.6%) patients, respectively. Bismuth-based quadruple therapy showed significant higher eradication rate than quinolone-based triple therapy.

Conclusion: Concomitant therapy was superior to sequential therapy as the first-line H. pylori eradication, showing consistent higher eradication rate with an increasing trend over the last 9 years. In patients with eradication failure after concomitant or sequential therapy, bismuth-based quadruple therapy is preferable than quinolone-based triple therapy in Korea.


TABLE 1. Helicobacter pylori eradication rates

<table>
<thead>
<tr>
<th></th>
<th>BCQT</th>
<th>MBST</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eradication rate</td>
<td>93.3% (84/90)</td>
<td>85.4% (76/89)</td>
<td>0.069</td>
</tr>
<tr>
<td>95% CI</td>
<td>85.5-97.3%</td>
<td>76.0-91.7%</td>
<td></td>
</tr>
<tr>
<td>PP analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eradication rate</td>
<td>96.6% (84/87)</td>
<td>88.5% (76/87)</td>
<td>0.025</td>
</tr>
<tr>
<td>95% CI</td>
<td>89.5-99.1%</td>
<td>78.1-93.2%</td>
<td></td>
</tr>
</tbody>
</table>
| BCQT, 7-day bismuth containing quintet therapy; CI, confidence interval; ITT, intention-to-treat; MBST, 14-day moxifloxacin-based sequential therapy; PP, per-protocol.

Background: The best approach for Helicobacter pylori management remains unclear. An audit process is essential to ensure clinical practice is aligned with best standards of care.

Design: International multicentre prospective non-interventional registry starting in 2013 aimed to evaluate the decisions and outcomes in H. pylori management by European gastroenterologists. Patients were registered in an e-CRF by AEG-REDCap up to April 2020. Variables included: demographics, previous eradication attempts, prescribed treatment, adverse events, and outcomes. Modified intention-to-treat (mITT) and per-protocol (PP) analyses were performed and data were subject to quality review to ensure information reliability.

Results: In total 36,319 patients from 29 European countries were evaluated and 24,882 (70%) first-line empirical H. pylori treatments were included for analysis. Triple therapy with amoxicillin and clarithromycin was most commonly prescribed (40%), followed by concomitant treatment (19%) and bismuth quadruple (Pylera®) (10%) achieving 83%, 91% and 95% mITT eradication rate, respectively. Over 90% effectiveness was obtained only with 10 and 14-day bis-

muth quadruple or 14-day concomitant treatment (Table). Longer treatment duration, higher acid inhibition and compliance were associated with higher eradication rates.

ABSTRACTS

EP2.10 | First-line H. pylori eradication therapy in Europe: Results from 24,882 cases of the European Registry on H. pylori Management (Hp-EuReg)

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**Conclusions:** Management of *H. pylori* infection by European gastroenterologists is heterogeneous. Only quadruple therapies lasting at least 10 days are able to achieve over 90% eradication rates.

**TABLE 1.** Effectiveness (by modified intention-to-treat and per-protocol analyses) of first-line empirical treatments in Europe

<table>
<thead>
<tr>
<th>First-line treatment</th>
<th>Length (days)</th>
<th>mITT, N (%)</th>
<th>95% CI</th>
<th>PP, N (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triple-C+A</td>
<td>7</td>
<td>1,903 (83)</td>
<td>81-84</td>
<td>1,886 (83)</td>
<td>81-85</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3,057 (83)</td>
<td>82-85</td>
<td>3,015 (84)</td>
<td>82-85</td>
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<td></td>
<td>14</td>
<td>2,264 (89)</td>
<td>88-90</td>
<td>2,238 (89)</td>
<td>88-91</td>
</tr>
<tr>
<td>Triple-A+M</td>
<td>7</td>
<td>118 (81)</td>
<td>74-89</td>
<td>117 (81)</td>
<td>74-89</td>
</tr>
<tr>
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<td>163 (85)</td>
<td>79-90</td>
<td>161 (85)</td>
<td>79-91</td>
</tr>
<tr>
<td>Triple-C+M</td>
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<td>724 (84)</td>
<td>82-87</td>
<td>721 (85)</td>
<td>82-87</td>
</tr>
<tr>
<td></td>
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<td>114 (65)</td>
<td>56-74</td>
<td>112 (66)</td>
<td>57-75</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>80 (70)</td>
<td>59-81</td>
<td>80 (70)</td>
<td>59-81</td>
</tr>
<tr>
<td>Triple-A+L</td>
<td>7</td>
<td>178 (79)</td>
<td>72-85</td>
<td>176 (78)</td>
<td>72-85</td>
</tr>
<tr>
<td></td>
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<td>142 (85)</td>
<td>79-91</td>
<td>136 (86)</td>
<td>80-92</td>
</tr>
<tr>
<td>Sequential-C+A+M/T</td>
<td>10</td>
<td>596 (83)</td>
<td>80-86</td>
<td>556 (85)</td>
<td>82-88</td>
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<tr>
<td>Quadruple-C+A+M/T</td>
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<td>2,378 (88)</td>
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<td>2,316 (89)</td>
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<td></td>
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<td>92-94</td>
<td>2,180 (93)</td>
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<td>Quadruple-C+A+B</td>
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<td>394 (86)</td>
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<td>390 (86)</td>
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<td></td>
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<td>1,194 (91)</td>
<td>89-93</td>
<td>1,178 (91)</td>
<td>90-93</td>
</tr>
<tr>
<td>Quadruple-M+Tc+B</td>
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<td>130 (94)</td>
<td>89-98</td>
<td>130 (94)</td>
<td>89-98</td>
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<tr>
<td>Pylera® (M+Tc+B)</td>
<td>10</td>
<td>2,267 (95)</td>
<td>94-96</td>
<td>2,223 (95.5)</td>
<td>95-96</td>
</tr>
</tbody>
</table>

A, amoxicillin; B, bismuth salts; C, clarithromycin; L, levofloxacin; M, metronidazole; mITT, modified intention-to-treat; PP, per-protocol; T, tinidazole; Tc, tetracycline.

EP2.11 | Room for improvement in the treatment of Helicobacter pylori infection: Lessons from the European Registry on H. pylori Management (Hp-EuReg)

O. P. Nyssen; D. Vaira; B. Tepes; L. Kupcinskas; D. Bordin; Á. Pérez-Aisa; A. Gasbarrini; M. Castro-Fernández; L. Bujanda; A. Garre; A. Lucendo; L. Vologzhanina; N. Bruglez Jurecic; L. Rodrigo-Sáez; J. Huguet; I. Voynovan; J. Jorge Perez Lasala; P. Mata Romero; M. Vujasinovic; R. Abdulkhakov; J. Barrio; L. Fernandez-Salazar; L. Jonaitis; M. Espada; F. Megraud; C. O’Morain; J. P. Gisbert; On behalf of the Hp-EuReg Investigators

Background: Managing Helicobacter pylori infection requires constant decision-making, and each decision is open to possible errors.

Aim: To evaluate common mistakes in the eradication of H. pylori, based on the European Registry on Helicobacter pylori management (Hp-EuReg).

Methods: International multicentre prospective non-interventional registry evaluating the decisions and outcomes of H. pylori management by European gastroenterologists in routine clinical practice.

Results: Countries recruiting over 1,000 patients were included (26,340 patients). The most common mistakes (percentages) were:

1) To use the standard triple therapy where it is ineffective (46%).
2) To prescribe eradication therapy for only 7-10 days (69%) (Table 1).
3) To use a low dose of proton pump inhibitors (48%) (Table 2).
4) In patients allergic to penicillin, to prescribe always a triple therapy with clarithromycin and metronidazole (38%).
5) To repeat certain antibiotics after eradication failure (>15%).
6) To ignore the importance of compliance with treatment (2%).
7) Not to check the eradication success (6%).

Time-trend analyses showed progressive greater compliance with current clinical guidelines.

Conclusion: The management of H. pylori infection by European gastroenterologists is heterogeneous, frequently suboptimal and discrepant with current recommendations. Clinical practice is constantly adapting to updated recommendations, although this shift is delayed and slow.
TABLE 1. Use and effectiveness of 7, 10 and 14-day triple regimens in Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>7 or 10 days Mistake (%)</th>
<th>14 days mITT (%)</th>
<th>14-days use, N (%)</th>
<th>mITT, N (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>71</td>
<td>71</td>
<td>1,429 (29)</td>
<td>1,297 (86)</td>
<td>83-87</td>
</tr>
<tr>
<td>Russia</td>
<td>63</td>
<td>77</td>
<td>984 (37)</td>
<td>790 (90)</td>
<td>88-92</td>
</tr>
<tr>
<td>Slovenia</td>
<td>62</td>
<td>85</td>
<td>1,070 (38)</td>
<td>722 (91)</td>
<td>89-93</td>
</tr>
<tr>
<td>Italy</td>
<td>93</td>
<td>84</td>
<td>28 (7)</td>
<td>21 (67)</td>
<td>43-85</td>
</tr>
<tr>
<td>Lithuania</td>
<td>84</td>
<td>75</td>
<td>182 (16)</td>
<td>1 (100)</td>
<td>1.3-99</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>81</td>
<td>3,693 (31)</td>
<td>2,831 (88)</td>
<td>87-89</td>
</tr>
</tbody>
</table>

CI, confidence interval; mITT, modified intention-to-treat; N, total number of patients; PP, per-protocol. 1% of mistake accounted for 7 or 10 day-treatment durations.

TABLE 2. Acid inhibition potency of proton pump inhibitor use in triple regimens in Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>Low, N (N %)</th>
<th>% mistake</th>
<th>95% CI</th>
<th>Standard, N (%)</th>
<th>95% CI</th>
<th>High, N (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>1,368 (23)</td>
<td>41</td>
<td>39-42</td>
<td>1,223 (36)</td>
<td>35-38</td>
<td>782 (23)</td>
<td>22-25</td>
</tr>
<tr>
<td>Russia</td>
<td>1,173 (36)</td>
<td>56</td>
<td>54-58</td>
<td>754 (36)</td>
<td>34-38</td>
<td>169 (8)</td>
<td>6.8-9.2</td>
</tr>
<tr>
<td>Slovenia</td>
<td>1,385 (38)</td>
<td>52</td>
<td>50-54</td>
<td>50 (2)</td>
<td>1.3-2.4</td>
<td>1,241 (46)</td>
<td>44-48</td>
</tr>
<tr>
<td>Italy</td>
<td>106 (11)</td>
<td>85</td>
<td>78-91</td>
<td>14 (11)</td>
<td>5.3-17</td>
<td>5 (4)</td>
<td>1.3-9.1</td>
</tr>
<tr>
<td>Lithuania</td>
<td>480 (46)</td>
<td>46</td>
<td>43-49</td>
<td>326 (31)</td>
<td>28-34</td>
<td>234 (23)</td>
<td>20-25</td>
</tr>
<tr>
<td>Total</td>
<td>4,512 (26)</td>
<td>48</td>
<td>47-49</td>
<td>2,367 (25)</td>
<td>24-26</td>
<td>2,431 (26)</td>
<td>25-27</td>
</tr>
</tbody>
</table>

CI: confidence interval; Low dose: 4.5 to 27 mg omeprazole equivalents; Standard dose: 32 to 40 mg omeprazole equivalents; High dose: 54 to 128 mg omeprazole equivalents. 1% mistake accounted for all PPIs dose less than 32 mg omeprazole equivalent (as the PPI given twice daily).


O. P. Nyssen1; D. Bordin2,4; B. Tepes5; A. Pérez-Aisa6; D. Vaira7; M. Caldas8; L. Bujanda9; M. Castro-Fernandez2; F. Lerang10; M. Leja11; L. Rodrigo12; T. Rokkas13; L. Kupcinskas14; J. Pérez-Lasala15; L. Jonaitis16; O. Shvets17; A. Gasbarrini18; H. Simsek19; A. R. T. Axon20; G. M. Buzás21; J. Machado22; Y. Niv23; L. Boyanova24; A. Goldis25; V. Lamy26; A. Tonkic27; K. Przytulski28; C. Beglinger29; M. Venerito30; P. Bytzer31; R. Abdulkhakov32; J. Barrio33; L. Fernandez-Salazar: None. L. Jonaitis: None. M. Espada: None. F. Méraud: None. C. O’Morain: None. J.P. Gisbert: None.

EP2.12 | European Registry on H. pylori Management (Hp-EuReg): Empirical first-line treatment use and effectiveness trends in Europe in the period 2013-2020

O. P. Nyssen; D. Bordin; B. Tepes; A. Pérez-Aisa; D. Vaira; M. Caldas; L. Bujanda; M. Castro-Fernández; F. Lerang; M. Leja; L. Rodrigo; T. Rokkas; L. Kupcinskas; J. Pérez-Lasala; L. Jonaitis; O. Shvets; A. Gasbarrini; H. Simsek; A. R. T. Axon; G. M. Buzás; J. Machado; Y. Niv; L. Boyanova; A. Goldis; V. Lamy; A. Tonkic; K. Przytulski; C. Beglinger; M. Venerito; P. Bytzer; R. Abdulkhakov; J. Barrio; L. Fernandez-Salazar; None. L. Jonaitis: None. M. Espada: None. F. Méraud: None. C. O’Morain: None. J.P. Gisbert: None.

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Background: The impact of consensus, prescription choices and efficacy trends on clinical practice over time has not been studied in depth.

Methods: International multicenter prospective non-interventional registry aimed to evaluate the decisions and outcomes of H. pylori management by European gastroenterologists. All infected adult patients were registered at AEG-REDCap e-CRF up to April 2020. Modified intention-to-treat (mITT) and time trend analyses were performed.

Results: So far 24,882 first-line empirical prescriptions from 29 European countries have been included. Overall, the most common prescribed treatments in 2013-20 were triple therapies; however, a shift in antibiotic regimens was identified. Triple therapies decreased from >50% of prescription in 2013/15 to less than 20% in 2018/20; concomitant therapy decreased from 21% in 2013/14 to 13% in 2019/20, while Pylera® increased from 0-1% in 2014/2015 to 18% in 2018/20. An increase in the average duration of treatments from 10.9 days in 2013 to 12.0 in 2020, and of the daily dose of PPI was identified. No trend was identified regarding the effectiveness of each specific treatment (data now shown); however, an overall 5% improvement in first-line mITT effectiveness was observed (Table 1).

Conclusions: European gastroenterological practice is constantly adapting to the newest published evidence and recommendations (reducing the use of triple therapies and increasing the duration of treatment and the dose of PPIs), with a subsequent improvement in overall effectiveness.
### TABLE 1. Prescriptions and effectiveness trends of first-line empirical treatments in Europe in 2013-2020

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadruple-C+A+B</td>
<td>0.5%</td>
<td>0.9%</td>
<td>5.2%</td>
<td>17.2%</td>
<td>10.2%</td>
<td>15.3%</td>
<td>5.2%</td>
<td>7.5%</td>
</tr>
<tr>
<td>Pylera®</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.5%</td>
<td>12.0%</td>
<td>24.5%</td>
<td>22.3%</td>
<td>19.6%</td>
<td>17.8%</td>
</tr>
<tr>
<td>Quadruple-M+Tc+B</td>
<td>2.3%</td>
<td>1.9%</td>
<td>0.4%</td>
<td>0.2%</td>
<td>0.3%</td>
<td>0.4%</td>
<td>1.6%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Quadruple-C+A+M/T</td>
<td>20.0%</td>
<td>21.4%</td>
<td>26.9%</td>
<td>22.3%</td>
<td>21.2%</td>
<td>10.6%</td>
<td>11.8%</td>
<td>14.4%</td>
</tr>
<tr>
<td>Sequential-C+A+M/T</td>
<td>8.1%</td>
<td>3.4%</td>
<td>1.8%</td>
<td>0.9%</td>
<td>0.3%</td>
<td>0.5%</td>
<td>0.2%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Triple-A+L</td>
<td>2.1%</td>
<td>2.2%</td>
<td>3.2%</td>
<td>1.9%</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Triple-A+M</td>
<td>4.1%</td>
<td>3.0%</td>
<td>1.7%</td>
<td>0.9%</td>
<td>0.9%</td>
<td>0.6%</td>
<td>2.3%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Triple-C+M</td>
<td>3.9%</td>
<td>6.4%</td>
<td>9.0%</td>
<td>6.6%</td>
<td>1.4%</td>
<td>1.0%</td>
<td>1.0%</td>
<td>4.0%</td>
</tr>
<tr>
<td>Triple-C+A</td>
<td>53.6%</td>
<td>54.3%</td>
<td>42.7%</td>
<td>28.2%</td>
<td>30.5%</td>
<td>34.0%</td>
<td>40.6%</td>
<td>34.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Length</th>
<th>7 days</th>
<th>31.3%</th>
<th>28.1%</th>
<th>24.7%</th>
<th>16.7%</th>
<th>7.8%</th>
<th>1.8%</th>
<th>2.3%</th>
<th>10.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days</td>
<td>48.3%</td>
<td>52.7%</td>
<td>55.9%</td>
<td>46.4%</td>
<td>46.9%</td>
<td>43.9%</td>
<td>30.8%</td>
<td>31.7%</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>20.5%</td>
<td>19.2%</td>
<td>19.4%</td>
<td>36.8%</td>
<td>45.3%</td>
<td>54.2%</td>
<td>66.8%</td>
<td>58.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PPI acid inhibition*</th>
<th>Low</th>
<th>62.0%</th>
<th>56.7%</th>
<th>47.1%</th>
<th>36.2%</th>
<th>39.2%</th>
<th>28.5%</th>
<th>25.3%</th>
<th>33.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>18.7%</td>
<td>25.5%</td>
<td>26.5%</td>
<td>24.5%</td>
<td>23.7%</td>
<td>28.8%</td>
<td>34.6%</td>
<td>24.4%</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>19.3%</td>
<td>17.8%</td>
<td>26.4%</td>
<td>39.4%</td>
<td>37.1%</td>
<td>42.8%</td>
<td>41.4%</td>
<td>42.2%</td>
</tr>
</tbody>
</table>

| Eradication rate (mITT) | 85.3% | 85.1% | 85.9% | 87.4% | 88.2% | 90.9% | 92.2% | 91.3% |

PPI, proton pomp inhibitor; mITT, modified intention-to-treat; A, amoxicillin; C, clarithromycin; M, metronidazole; T, tinidazole; L, levofloxacin; B, bismuth salts; Tc, tetracycline. *Low dose PPI – 4.5 to 27 mg omeprazole equivalents, b.i.d., Standard dose PPI – 32 to 40 mg omeprazole equivalents, b.i.d., High dose PPI – 54 to 128 mg omeprazole equivalents, b.i.d.

Background: After a failed eradication attempt, approximately 10-20% of patients will fail to obtain H. pylori eradication.

Aims: To evaluate the effectiveness of second-line empirical treatments.

Methods: A systematic prospective registry of the clinical practice of European gastroenterologists on H. pylori management was established. All infected adult patients were systematically registered at AEG-REDCap e-CRF until April 2020. Modified intention-to-treat (mITT) and per-protocol (PP) analyses were performed.

Results: Overall, 4,862 patients from 29 European countries were given an empirical second-line therapy. Overall effectiveness was 83.7% (by mITT) and 84% (by PP). Over 97% of patients were compliant. AEs were reported in 28% of the cases. Most frequent second-line prescriptions and effectiveness per antibiotic combination is shown in table 1. After failure of first-line clarithromycin-containing treatment, optimal eradication (>90%) was obtained with moxifloxacin-containing triple therapy, Pylera® or quadruple therapy with levofloxacin and bismuth. In patients receiving triple regimens containing levofloxacin or the standard bismuth quadruple regimen, cure rates were optimized with 14-day regimens using high doses of proton pump inhibitors (PPIs). However, Pylera® or quadruple therapy with levofloxacin and bismuth achieved reliable eradication rates regardless of the PPI dose, duration of therapy, or previous first-line treatment.

Conclusion: Empirical second-line triple therapies generally provided low eradication rates except when prescribing 14 days of levofloxacin or moxifloxacin. However, high effectiveness was obtained with second-line bismuth-containing quadruple therapies.
**TABLE 1.** Frequency of second-line empirical treatment prescriptions and effectiveness by modified intention-to-treat and per-protocol analyses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>% Use</th>
<th>mITT, N (%)</th>
<th>95% CI</th>
<th>PP, N (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triple-A+L</td>
<td>1,522</td>
<td>33.2</td>
<td>1,341 (81)</td>
<td>79-83</td>
<td>1,320 (81)</td>
<td>79-83</td>
</tr>
<tr>
<td>Pylera® (single capsule)</td>
<td>692</td>
<td>15.1</td>
<td>622 (90)</td>
<td>87-92</td>
<td>607 (90)</td>
<td>88-93</td>
</tr>
<tr>
<td>Quadruple-A+L+B</td>
<td>529</td>
<td>11.5</td>
<td>478 (89)</td>
<td>86-92</td>
<td>462 (89)</td>
<td>86-92</td>
</tr>
<tr>
<td>Triple-C+A</td>
<td>477</td>
<td>10.4</td>
<td>231 (81)</td>
<td>76-86</td>
<td>226 (81)</td>
<td>76-86</td>
</tr>
<tr>
<td>Quadruple-M+Tc+B</td>
<td>204</td>
<td>4.4</td>
<td>183 (83)</td>
<td>77-89</td>
<td>177 (84)</td>
<td>78-90</td>
</tr>
<tr>
<td>Quadruple-C+A+M</td>
<td>179</td>
<td>3.9</td>
<td>169 (85)</td>
<td>79-90</td>
<td>167 (84)</td>
<td>79-90</td>
</tr>
<tr>
<td>Triple-A+Mx</td>
<td>143</td>
<td>3.1</td>
<td>135 (91)</td>
<td>86-96</td>
<td>135 (91)</td>
<td>86-96</td>
</tr>
<tr>
<td>Triple-A+M</td>
<td>93</td>
<td>2.0</td>
<td>76 (60.5)</td>
<td>49-72</td>
<td>76 (60.5)</td>
<td>49-72</td>
</tr>
<tr>
<td>Other</td>
<td>1,023</td>
<td>21.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>4,862</td>
<td>100%</td>
<td>3,966 (84)</td>
<td>82-85</td>
<td>3,966 (84)</td>
<td>81-83</td>
</tr>
</tbody>
</table>

mITT, modified intention-to-treat; PP, per-protocol; 95% CI, 95% confidence interval; C, clarithromycin; M, metronidazole; T, tinidazole; A, amoxicillin; L, levofloxacin; B, bismuth salts; Tc, tetracycline; Mx, moxifloxacin; N, Total of patients receiving an empirical treatment; Other, Other second-line empirical treatments with less than 100 patients treated in each category.


**EP2.14 | A deadly hug: Chitosan microspheres functionalized with MSI-78A antimicrobial peptide kill Helicobacter pylori**

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With the end of antibiotic era, newer therapeutic options, as antimicrobial peptides (AMPs), are a demand. Helicobacter pylori (Hp) was classified as 1 of the 16 antibiotic-resistant bacteria¹. MSI-78A is one of the fewer AMPs that demonstrated bactericidal effects against Hp². However, in vivo AMPs undergo protease degradation and proteins aggregation, reducing their effectiveness. AMPs immobilization onto a biomaterial surface is an advocated strategy to overcome these drawbacks³. This work intended the development of biocompatible chitosan microspheres decorated with MSI-78A (AMP-ChMic) capable of crossing the gastric mucosa and kill Hp in situ, after oral administration.

Chitosan microspheres (ChMic), average diameter of 5 μm, were produced by spray drying. A heterobifunctional spacer (NHS-PEG-MAL) was bonded to the ChMic, allowed immobilizing the MSI-78A modified with a terminal cysteine on the C-terminal (MSI-78A-SH) in a controlled orientation.

The resulting AMP-ChMic were able to retain their integrity in a wide range of acidic pH, proving their pH-resistance and validating this approach for gastric settings. The microparticles were tested in vitro against Hp J99 strain (highly pathogenic human strain), at different timepoints and in a microspheres’ range of concentrations (10⁵-10⁷ mics/mL). Antimicrobial effect was seen after 2 hours in higher concentrations and it was maintained up to 6 hours. The fast-bactericidal effect indicates that MSI-78A can retain its activity when immobilized onto ChMic. AMP-ChMic demonstrated high potential for Hp infection management, being an innovative strategy to non-antibiotic therapeutics.


**Acknowledgements:** PTDC/CTM-BIO/4043/2014 & SFRH/BD/146890/2019

Background: Bismuth quadruple with the quadruple single capsule Pylera® (PPI, bismuth, tetracycline and metronidazole) includes the intake of 3 capsules four times a day (3c/6h), according to the technical sheet. This scheme may not be suitable for Spanish eating habits; therefore, some physicians prescribe the treatment in the form of 4 capsules three times a day (4c/8h).

Aim: To assess the effectiveness and safety of Pylera® administered three times a day (4c/8h).

Methods: Systematic prospective registry of the clinical practice of European gastroenterologists on the management of H. pylori infection (Hp-EuReg). All infected adult patients treated with Pylera® were systematically collected at AEG-REDCap e-CRF until June 2019. Modified intention-to-treat (mITT) and per-protocol (PP) analyses were performed.

Results: Of the 2,326 patients, 1,140 (74%) were treated with 3c/6h and 403 (17%) with the 4c/8h. Most of the cases (72%) were naïve to treatment. The PPI dose did not influence the eradication rate. Both treatment schedules showed equivalent compliance, tolerance, and effectiveness (table 1). One patient suffered a serious adverse event (C. difficile infection), in the group 3c/6h.

Conclusions: The prescription of quadruple therapy with single capsule bismuth (Pylera®) given as four capsules three times a day seems to have the same compliance, tolerance and effectiveness as the scheme included in the data sheet (three capsules four times a day).
TABLE 1. Effectiveness (by modified intention-to-treat and per-protocol analyses), compliance and safety of treatment with Pylera® in first-, second-, and third-line

<table>
<thead>
<tr>
<th>Pylera®</th>
<th>Compliance</th>
<th>AEs</th>
<th>Modified intention-to-treat</th>
<th>Per-protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>1st line</td>
</tr>
<tr>
<td>4c/8h</td>
<td>97%</td>
<td>22%</td>
<td>91%</td>
<td>95%</td>
</tr>
<tr>
<td>3c/6h</td>
<td>98%</td>
<td>24%</td>
<td>86%</td>
<td>93%</td>
</tr>
</tbody>
</table>

AEs: adverse events. 4c/8h: four capsules three times a day (every 8 hours); 3c/6h: three capsules four times a day (every 6 hours).

Background: Bismuth-quadruple therapy (PPI, bismuth, tetracycline and metronidazole) has resurfaced in Europe thanks to a three-in-one single-capsule formulation (Pylera®).

Aim: To evaluate the effectiveness and safety of Pylera®.

Methods: International prospective registry of the clinical practice of European gastroenterologists on the management of H. pylori infection (Hp-EuReg). All infected adult patients treated with Pylera® according to data sheet (3 capsules/6h) were systematically collected at AEG-REDCap e-CRF until December 2019. Modified intention-to-treat (mITT) analyses were performed.

Results: Overall, 2,100 patients were prescribed single-capsule bismuth-quadruple therapy (10 days, 3 capsules q.i.d.). The majority of these patients were naïve (63%) and 16% had peptic ulcer. Pylera® achieved a high eradication rate based on the mITT (91.9%). Effectiveness was higher when using Pylera® as a first-line treatment.
(94.6%) but it had also high effectiveness as a rescue therapy, both in second-line (89.3%) or subsequent lines of therapy (3rd-6th line: 91.9%) (Table 1). Compliance was the factor most closely associated with the effectiveness of treatment. Adverse events (AEs) were generally mild-to-moderate and transient, only 3% of patients reporting a severe AE, leading to discontinuation of treatment in 1.7% of patients. Conclusions 

The 10-day treatment with single-capsule bismuth-quadruple therapy (Pylera®) achieves H. pylori eradication in approximately 90% of patients by mITT in real-world clinical practice, both as a first-line and rescue treatment, with a favourable safety profile.

**TABLE 1.** Pylera® effectiveness (by modified intention-to-treat) in first-, and consecutive rescue treatment lines

<table>
<thead>
<tr>
<th>Use, N (%)</th>
<th>mITT, N (%)</th>
<th>95% CI</th>
<th>PP, N (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>2,100 (6*)</td>
<td>1,777 (92)</td>
<td>91-93</td>
<td>1,761 (93)</td>
</tr>
<tr>
<td>Naïve</td>
<td>1,335 (63)</td>
<td>1,166 (95)</td>
<td>93-96</td>
<td>1,158 (95.5)</td>
</tr>
<tr>
<td>2nd line</td>
<td>465 (22)</td>
<td>375 (89)</td>
<td>86-92</td>
<td>370 (90)</td>
</tr>
<tr>
<td>3rd line</td>
<td>212 (10)</td>
<td>174 (89)</td>
<td>84-93</td>
<td>177 (88)</td>
</tr>
</tbody>
</table>

*Of the total of treatments included in the Hp-EuReg up to December 2019 (i.e. N = 34,460); mITT: modified intention-to-treat; PP: per-protocol, N: total number of patients analysed.
Introduction: The safety of Helicobacter pylori eradication treatments and to what extent adverse events (AEs) may influence therapeutic compliance is unknown.

Objective: To assess the frequency, type, intensity and duration of AEs, and their impact on compliance.

Methods: Systematic prospective non-interventional registry of the clinical practice of European gastroenterologists on the management of H. pylori infection. All prescribed treatments and their corresponding safety profile were recorded in an e-CRF in AEG-REDCap until June 2019. AEs were classified depending on the intensity of symptoms as mild/moderate/severe, and as serious AEs (death, hospitalisation, disability, congenial anomaly and/or requires intervention to prevent permanent damage).

Results: The different treatments prescribed to a total of 22,492 naïve and non-naïve patients caused at least one AE in 22% of the cases (Table 1), the classic bismuth-based quadruple therapy being the worst tolerated (37% of AEs). Taste disturbance (7%), diarrhoea (7%), nausea (6%) and abdominal pain (3%) were the most frequent AEs. The majority of AEs were mild (57%), 6% were severe, and only 0.08% were serious, with an average duration of 7 days. The treatment compliance rate was 97%. Only 1.3% of the patients discontinued treatment due to AEs.

Conclusions: H. pylori eradication treatment frequently induces AEs, although they are usually mild and of limited duration. Its appearance does not interfere significantly with the compliance of treatment.

TABLE 1. Safety in naïve and non-naïve patients

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Yes</th>
<th>%</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triple-C+A</td>
<td>1,037</td>
<td>15</td>
<td>14-16</td>
</tr>
<tr>
<td>Quadruple-C+A+M</td>
<td>926</td>
<td>25</td>
<td>23-26</td>
</tr>
<tr>
<td>Pylera®</td>
<td>642</td>
<td>28</td>
<td>26-30</td>
</tr>
<tr>
<td>Quadruple-C+A+B</td>
<td>627</td>
<td>34</td>
<td>34-37</td>
</tr>
<tr>
<td>Triple-A+L</td>
<td>339</td>
<td>21</td>
<td>19-23</td>
</tr>
<tr>
<td>Triple-C+M</td>
<td>184</td>
<td>20</td>
<td>18-23</td>
</tr>
<tr>
<td>Quadruple-A+L+B</td>
<td>180</td>
<td>32</td>
<td>28-36</td>
</tr>
<tr>
<td>Quadruple-A+M</td>
<td>79</td>
<td>22</td>
<td>17-26</td>
</tr>
<tr>
<td>Sequential-C+A+M</td>
<td>50</td>
<td>19</td>
<td>14-24</td>
</tr>
<tr>
<td>Quadruple-M+Tc+B</td>
<td>84</td>
<td>37</td>
<td>30-43</td>
</tr>
<tr>
<td>Quadruple-A+B+</td>
<td>67</td>
<td>32</td>
<td>26-39</td>
</tr>
<tr>
<td>Quadruple-M+D+B</td>
<td>62</td>
<td>33</td>
<td>26-40</td>
</tr>
<tr>
<td>Sequential-C+A+T</td>
<td>5</td>
<td>6.8</td>
<td>2.2-15</td>
</tr>
<tr>
<td>Quadruple-C+A+T</td>
<td>16</td>
<td>17</td>
<td>9.1-26</td>
</tr>
<tr>
<td>Total</td>
<td>4,298</td>
<td>22</td>
<td>22-23</td>
</tr>
</tbody>
</table>

A, amoxicillin; B, bismuth; C, clarithromycin; CI, confidence interval; D, doxycycline; L, levofloxacin; M, metronidazole; T, tinidazole; Tc, tetracycline.

EP2.18 | Bismuth quadruple regimen with tetracycline or doxycycline versus Pylera® as third-line rescue therapy for \textit{H. pylori} infection: A prospective multicenter analysis of the European Registry on \textit{Helicobacter pylori} Management (Hp-EuReg)

O. P. Nyssen; A. Perez-Aisa; L. Rodrigo-Sáez; M. Castro-Fernández; P. Mata Romero; J. Ortúñovoa; J. Barrio; J. Huguet; I. Modollel; N. Alcaide; A. Lucendo; X. Calvet; M. Perona; B. Gomez; B. Gomez Rodriguez; P. Varela; M. Jimenez-Moreno; M. Dominguez-Cajal; L. Pozzati; D. Burgos; B. Lujanda; J. Hinojosa; J. Molina-Infante; T. Di Maia; L. Ferrer; L. Fernández-Salazar; A. Figuerola; L. Tito; C. de la Coba; J. Gomez-Camarero; N. Fernandez; M. Espada; I. Puig; F. Megraud; C. O’Morain; J. P. Gisbert; On behalf of the Hp-EuReg Investigators

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\textbf{Background:} Different bismuth-quadruple therapies containing proton pump inhibitors, bismuth, metronidazole, and a tetracycline have been recommended as third-line \textit{Helicobacter pylori} eradication treatment after failure with clarithromycin and levofloxacin.

\textbf{Aim:} To evaluate the effectiveness and safety of third-line treatments with bismuth, metronidazole and either tetracycline or doxycycline.

\textbf{Methods:} Sub-study of the European Registry on \textit{H. pylori} Management (Hp-EuReg), an international multicenter prospective non-interventional registry of the routine clinical practice of European gastroenterologists. After previous failure with clarithromycin- and levofloxacin-containing therapies, patients receiving a third-line regimen with 10/14-day of PPI, bismuth, metronidazole and either tetracycline (T) or doxycycline (D), or 10-day Pylera® (P) were registered at AEG-REDCap.

\textbf{Results:} Overall, 454 patients were treated: 85 with T, 94 with D, and 275 with P. Overall modified intention-to-treat and per-protocol eradication rates were 81% (D: 65%, T: 76%, P: 88%) and 82% (D: 66%, T: 77%, P: 88%), respectively (Table 1). Higher eradication rates were associated with compliance (OR = 2.96; 95% CI = 1.01-8.84) and no prior metronidazole use (OR = 1.96; 95% CI = 1.15-3.33); P was superior to D (OR = 4.46; 95% CI = 2.51-8.27), and T marginally superior to D (OR = 1.67; 95% CI = 0.85-3.29).

\textbf{Conclusion:} Third-line (after failure with clarithromycin and levofloxacin) \textit{H. pylori} eradication with bismuth quadruple treatment offers acceptable effectiveness and safety. Highest effectiveness was found in compliant patients and in those taking 10-day Pylera® or 14-day tetracycline. Doxycycline appears less effective and therefore should not be recommended.
TABLE 1. Effectiveness (by modified intention-to-treat and per-protocol) and compliance according to the treatment regimen and length

<table>
<thead>
<tr>
<th>Group</th>
<th>Effectiveness, N (%)</th>
<th>Compliance</th>
<th>mITT, N</th>
<th>mITT</th>
<th>95% CI</th>
<th>PP, N</th>
<th>PP</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group T</td>
<td>All</td>
<td>82 (97%)</td>
<td>64</td>
<td>76%</td>
<td>66-86</td>
<td>63</td>
<td>77%</td>
<td>67-86</td>
</tr>
<tr>
<td></td>
<td>10 days*</td>
<td>29 (97%)</td>
<td>19</td>
<td>66%</td>
<td>47-85</td>
<td>19</td>
<td>66%</td>
<td>47-85</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>45 (96%)</td>
<td>45</td>
<td>82%</td>
<td>71-93</td>
<td>44</td>
<td>83%</td>
<td>72-94</td>
</tr>
<tr>
<td>Group D</td>
<td>All</td>
<td>85 (93%)</td>
<td>58</td>
<td>65%</td>
<td>55-76</td>
<td>56</td>
<td>66%</td>
<td>55-77</td>
</tr>
<tr>
<td></td>
<td>10 days*</td>
<td>37 (90%)</td>
<td>25</td>
<td>63%</td>
<td>46-79</td>
<td>23</td>
<td>63%</td>
<td>45-79</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>53 (96%)</td>
<td>32</td>
<td>70%</td>
<td>55-84</td>
<td>32</td>
<td>71%</td>
<td>57-85</td>
</tr>
<tr>
<td>Group P</td>
<td>10 days*</td>
<td>249 (96%)</td>
<td>222</td>
<td>88%</td>
<td>83-92</td>
<td>216</td>
<td>88%</td>
<td>84-92</td>
</tr>
</tbody>
</table>

Group T – tetracycline containing bismuth quadruple therapy, Group D – doxycycline containing bismuth quadruple therapy, Group P – single capsule bismuth quadruple therapy (Pylera®), 95% CI – 95% confidence interval. ITT: intention-to-treat, mITT: modified intention-to-treat; PP: per-protocol. The Chi² test showed statistically significant differences of treatment length in the mITT set between treatment groups (T, D and P) as reported in the table: *P < 0.001.

EP2.19 | European Registry on H. pylori Management (Hp-EuReg): Clinical phenotypes through machine learning of first-line treated patients in Spain during the period 2013-2018

O. P. Nyssen1; A. Sanz-García2; G. Ortega2; J. P. Gisbert1; On behalf of the Hp-EuReg Investigators

1Hospital Universitario de La Princesa, IIS-IP, UAM, CIBEREHD, Madrid, Spain; 2Unidad de análisis de datos, Hospital Universitario de la Princesa, Madrid, Spain

Background: Patients’ segmentation in homogeneous groups could help to improve the effectiveness of current Helicobacter pylori eradication therapy.

Objectives: 1) To group patients from the European Registry on Helicobacter pylori management (Hp-EuReg) according to their demographic and clinical characteristics and to the treatment types through multivariate categorical analysis and subsequent cluster decomposition. 2) To evaluate treatments’ effectiveness.

Methods: Categorical variables used: sex, ethnicity, diagnosis, symptoms, therapeutic indication, treatment and its duration, proton-pump inhibitor (PPI) dose, compliance, adverse events, and region of the prescribing center.

Results: Overall, 8,322 patients were analysed from 2013 to 2018. Table 1 shows the increase of effectiveness, ranging from 78.4% in 2013 to 92.2% in 2018. The lowest effectiveness, for clusters with over 100 patients, was obtained in cluster 1 (2015), with an eradication rate of 82.8%. This cluster comprised: triple therapy with PPI-clarithromycin-amoxicillin and concomitant therapy with PPI-clarithromycin-amoxicillin-metronidazole/tinidazole, lasting both 10 days in most of cases. High PPI dose, 10-day Pylera® treatment obtained over 95% effectiveness in cluster 3 (2018), uniformly distributed among Malaga, Valencia, Ciudad Real, Sevilla, Madrid, and Valladolid. High PPI dose, 14-day concomitant therapy achieved 90.7% effectiveness in cluster 1 (2018), among Malaga, Ciudad Real and Madrid.

Conclusion: The cluster analysis allows both identifying homogeneous groups of patients as well as assessing the effectiveness of the different first-line treatments evaluated.

TABLE 1. Trends in the overall effectiveness (by modified intention-to-treat, per cluster) between 2013 and 2018 in Spain

<table>
<thead>
<tr>
<th>year</th>
<th>Number of clusters</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>3</td>
<td>89.6 (280)</td>
<td>83.2 (619)</td>
<td>80.0 (80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>3</td>
<td>88.2 (1685)</td>
<td>69.6 (46)</td>
<td>83.3 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>5</td>
<td>91.6 (83)</td>
<td>89.4 (839)</td>
<td>82.9 (615)</td>
<td>70.8 (24)</td>
<td>88.2 (17)</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>94.7 (359)</td>
<td>86.1 (853)</td>
<td>92.6 (296)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>3</td>
<td>94.3 (630)</td>
<td>90.6 (636)</td>
<td>91.6 (153)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>3</td>
<td>91.8 (122)</td>
<td>90.7 (161)</td>
<td>96.5 (338)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Simple underlining highlights the lowest effectiveness for groups of more than 100 patients and double underlining the highest effectiveness.
EP2.20 | Empirical versus susceptibility-guided treatment of *Helicobacter pylori* infection: A meta-analysis

M. Espada; O. P. Nyssen; J. P. Gisbert

**Gastroenterology Unit, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), Universidad Autónoma de Madrid, and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain**

**Background:** Treating patients according to antibiotic resistances has frequently been recommended. However, the information on its real effectiveness is scarce.

**Aim:** To perform a meta-analysis comparing empirical versus susceptibility-guided treatment of *H. pylori*.


**Results:** Overall, 38 studies were included (6,525 patients in the empirical and 5,124 in the susceptibility-guided group). *H. pylori* eradication was achieved in 87% versus 77% respectively (RR: 1.13; 95% CI: 1.09-1.17; i²: 72%). Similar results were found when only RCTs were evaluated (22 studies; RR: 1.16; 95% CI: 1.10-1.22; i²: 72%). Similar results were also observed when susceptibility testing was assessed by culture (RR: 1.13; 95% CI: 1.07-1.19) or PCR (RR: 1.14; 95% CI: 1.05-1.23). When assessing first-line treatments (naïve patients) only (28 studies), better efficacy results were obtained with the susceptibility-guided strategy (RR: 1.16; 95% CI: 1.11-1.21; i²: 72%). However, when prescribing empirical first-line quadruple regimens only (both with and without bismuth, excluding the suboptimal triple therapies), no differences in efficacy were found versus the susceptibility-guided group (RR: 1.02; 95% CI: 0.95-1.09); this lack of difference was confirmed in RCTs not based on CYP2C19 gene polymorphism (RR: 1.07; 95% CI: 0.98-1.17). For rescue-therapies (13 studies, most as 2nd-line), similar results were demonstrated with both strategies, both including all studies (RR: 1.09; 95% CI: 0.97-1.22; i²: 82%) and also when only RCTs were considered (RR: 1.15; 95% CI: 0.97-1.36).

**Conclusions:** The benefit of susceptibility-guided treatment over empirical treatment of *H. pylori* infection could not be demonstrated, either in first-line (if the most updated quadruple regimens are prescribed) or in rescue therapies.

M. Espada: None. O.P. Nyssen: None. J.P. Gisbert: None.

**EP2.21 | Real-world comparative effects of three-in-one single capsule bismuth quadruple therapy vs non-bismuth quadruple concomitant therapy: Interim analysis of the European Registry on *H. pylori* Management (Hp-EuReg)**

I. Puig; M. Serra; O. P. Nyssen; G. Fiorini; D. Vaira; I. Saracino; A. Perez-Aisa; N. Fernandez-Moreno; I. Santaeullia; M. Castro-Fernandez; L. Bujanda; A. Lucendó; M. Areia; A. Gasbarrini; M. Romano; A. Gravina; R. Marcos-Pinto; F. Mégraud; C. O’Morain; J. P. Gisbert; O. P. Nyssen; G. Fiorini; D. Vaira; I. Saracino; A. Perez-Aisa; N. Fernandez-Moreno; I. Santaeullia; M. Castro-Fernandez; L. Bujanda; A. Lucendó; M. Areia; A. Gasbarrini; M. Romano; A. Gravina; R. Marcos-Pinto; F. Mégraud; C. O’Morain; J. P. Gisbert; On behalf of the Hp-EuReg Investigators

**1Digestive Diseases Department, Althaia Xarxa Asistencial Universitària de Manresa and Universitat de Vic-Universitat Central de Catalunya (UVicUCC), Manresa, Spain; 2Center for Research in Health and Economics (CRES), Pompeu Fabra University (UPF), Barcelona, Spain; 3Gastroenterology Unit, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), Universidad Autónoma de Madrid, and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain; 4Department of Surgical and Medical Sciences, University of Bologna, Bologna, Italy; 5Servicio de Gastroenterología, Agencia Sanitaria Costa del Sol, Red de Investigación en Servicios de Salud en Enfermedades Crónicas (REDISSEC), Marbella, Spain; 6Hospital de Valme, Sevilla, Spain; 7Department of Gastroenterology, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Hospital Donostia/Instituto Biodonostia, Universidad del País Vasco (UPV/EHU), Donostia, Spain; 8Department of Gastroenterology, Hospital General de Tomelloso, Ciudad Real, Spain; 9Portuguese Oncology Institute, Coimbra, Portugal; 10Gastroenterology Area, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy; 11Università degli Studi della Campania “Luigi Vanvitelli”, Napoli, Italy; 12Department of Gastroenterology, Porto Centre Hospital, Porto, Portugal; 13Laboratoire de Bactériologie, Hôpital Pellegrin, Bordeaux, France; 14Department of Clinical Medicine, Trinity College, Dublin, Ireland**

**Background:** RCTs have strict selection criteria that render results not fully transferable to the clinical practice.

**Objective:** To assess the comparative effects of first-line bismuth-single-capsule (PPI-bismuth-tetracycline-metronidazole) vs non-bismuth quadruple concomitant therapy (PPI-amoxicillin-clarithromycin-nitroimidazole).

**Methods:** The European Registry on *H. pylori* management (Hp-EuReg) data from Spain, Italy and Portugal was used to emulate a target trial with prospective observational data, comparing the relative effectiveness (modified intention-to-treat, mITT; and per-protocol, PP) and safety (adverse events, AEs) of first-line bismuth-single-capsule [10 days, 3 PPI dosages] and concomitant therapy [10/14 days; 3 PPI dosages], rendering 9 prescription strategies. Regression analysis controlling for confounders was used to estimate the relative effects of each strategy.

**Results:** Overall, 2,340 individuals were included. Compared to 10-day concomitant therapy at low PPI doses (n = 484), all bismuth-single-capsule combinations presented an eradication incremental
benefit by mITT ranging from 7.3% (95% CI: 1.1-13%; P = 0.024) with low dose PPI to 12.1% (95% CI: 5.1-19%; P < 0.001) with standard PPI dose. High PPI dosages in the concomitant therapy resulted in an eradication incremental benefit by mITT ranging from 7.7% (95% CI: 2.5-12.8; P = 0.003) to 8.8% (95% CI: 1.1-16.5; P = 0.025) when administered for 14 and 10 days, respectively (Table 1). No differences were found with respect to AEs or severe AEs in any of the assessed strategies.

**Conclusions:** Single-capsule bismuth quadruple therapy and non-bismuth quadruple concomitant therapy appear to have similar risk-benefit ratios when prescribed with high PPI doses.

### TABLE 1. Adjusted estimates* for eradication with respect to non-bismuth quadruple concomitant therapy during 10 days and low dose PPI

<table>
<thead>
<tr>
<th>Strategies</th>
<th>mITT absolute risk difference (95% CI)</th>
<th>P value</th>
<th>PP absolute risk difference (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bismuth-single-capsule, 10 days, low dose PPI</td>
<td>7.4 (1.8-13.1)</td>
<td>0.010</td>
<td>8.9 (3.4-13.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Bismuth-single-capsule, 10 days, standard dose PPI</td>
<td>12.1 (5.1-19.0)</td>
<td>&lt;0.001</td>
<td>12.6 (5.8-9.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bismuth-single-capsule, 10 days, high dose PPI</td>
<td>7.3 (0.9-13.6)</td>
<td>0.025</td>
<td>8.3 (2.0-14.5)</td>
<td>0.009</td>
</tr>
<tr>
<td>Non-bismuth quadruple concomitant therapy, 10 days, standard dose PPI</td>
<td>8.2 (2.1-14.2)</td>
<td>0.008</td>
<td>8.0 (2.2-13.9)</td>
<td>0.007</td>
</tr>
<tr>
<td>Non-bismuth quadruple concomitant therapy, 10 days, high dose PPI</td>
<td>8.8 (1.1-16.5)</td>
<td>0.025</td>
<td>10.0 (2.5-17.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>Non-bismuth quadruple concomitant therapy, 14 days, low dose PPI</td>
<td>4.5 (-1.8-10.8)</td>
<td>0.166</td>
<td>4.6 (-1.6-10.8)</td>
<td>0.145</td>
</tr>
<tr>
<td>Non-bismuth quadruple concomitant therapy, 14 days, standard dose PPI</td>
<td>7.5 (-0.6-15.5)</td>
<td>0.069</td>
<td>6.8 (-1.0-14.6)</td>
<td>0.088</td>
</tr>
<tr>
<td>Non-bismuth quadruple concomitant therapy, 14 days, high dose PPI</td>
<td>7.7 (2.5-12.8)</td>
<td>0.003</td>
<td>7.5 (2.4-12.5)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Low dose PPI: ranging from 4.5 to 27 mg omeprazole equivalents, b.i.d. Standard dose PPI: ranging from 32 to 40 mg omeprazole equivalents, b.i.d. High dose PPI: ranging from 54 to 128 mg omeprazole equivalents, b.i.d. Bismuth-single-capsule (PPI-bismuth-tetracycline-metronidazole). Non-bismuth quadruple concomitant therapy (PPI-amoxicillin-clarithromycin-nitroimidazole). *Estimates controlling for: age, sex, ethnicity, indication, concomitant allergy drug, hospital fixed effects and year fixed effects.


**EP2.22 | Antibiotic resistance trends of *Helicobacter pylori* naive patients in the period 2013-2019: Analysis of the European Registry on *H. pylori* Management (Hp-Eu-Reg)**

**L. Bujanda**; O. P. Nyssen; D. S. Bordin; A. Cosme; B. Tepes; A. Perez-Aisa; D. Vaira; M. Caldas; M. Castro-Fernandez; F. Lerang; M. Leja; L. Rodrigo; T. Rokkas; L. Kupcinskas; T. Rokkas; G. Fadeenko; I. Ariño; G. Fiorini; E. Resina; R. Muñoz; I. Puig; F. Megraud; C. O’Morain; J. P. Gisbert; On behalf of the Hp-EuReg Investigators.

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ABSTRACTS

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Background: Bacterial antibiotic resistance changes over time based on multiple factors. It is essential to study these trends to apply preventive strategies to help reducing such resistances.

Objective: To conduct a time-trend analysis of the antibiotic resistance to *H. pylori* infection in the European Registry on *H. pylori* (Hp-EuReg).

Patients and Methods: International multicenter prospective non-interventional European Registry on *H. pylori* Management (Hp-EuReg) aiming to evaluate the decisions and outcomes of *H. pylori* infection by European gastroenterologists. All infected adult patients diagnosed with culture and with a result of the antibiotic resistance test were registered at AEG-REDCap e-CRF from 2013 to 2019.

Results: A total of 32,447 patients were included, and culture was performed in 3,474 (11%), where 2,483 naïve patients were included for analysis. Resistance to at least one antibiotic was described in 57% of the patients. Resistance to metronidazole (27%) was most frequent, whereas resistance to tetracycline and amoxicillin was below 1%. Clarithromycin resistance remained above 15% throughout the studied years (Table 1). A significant decrease in the metronidazole resistance rate was observed between 2013 (38%) and 2018 (21%).

Conclusion: In naïve patients, resistance to clarithromycin remained above 15% in the period 2013-2019. A progressive decrease in metronidazole resistance was observed. No increasing or decreasing trend was observed in the bacterial resistance to other antibiotics.

### TABLE 1. Antibiotic resistance trends (2013-2019) of *Helicobacter pylori* naïve patients in Europe

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N° Cultures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>282-522</td>
</tr>
<tr>
<td>No resistance</td>
<td>210 (48)</td>
<td>259 (50)</td>
<td>197 (42)</td>
<td>93 (33)</td>
<td>162 (46)</td>
<td>106 (38)</td>
<td>104 (33.5)</td>
<td>33-50</td>
</tr>
<tr>
<td>Clarithromycin (C)</td>
<td>86 (20)</td>
<td>120 (23)</td>
<td>117 (25)</td>
<td>59 (21)</td>
<td>68 (19)</td>
<td>65 (23)</td>
<td>57 (18)</td>
<td>18-25</td>
</tr>
<tr>
<td>Metronidazole (M)</td>
<td>165 (38)</td>
<td>156 (30)</td>
<td>140 (30)</td>
<td>72 (25)</td>
<td>64 (18)</td>
<td>60 (21)</td>
<td>66 (21)</td>
<td>18-38</td>
</tr>
<tr>
<td>Levofloxacin (L)</td>
<td>58 (13)</td>
<td>100 (19)</td>
<td>103 (22)</td>
<td>46 (16)</td>
<td>59 (17)</td>
<td>55 (20)</td>
<td>41 (13)</td>
<td>13-22</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>6 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>10 (3)</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3 (0.7)</td>
<td>1 (0.2)</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
<td>5 (1.4)</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
<td>&lt;1.4</td>
</tr>
<tr>
<td>Dual (C+M)</td>
<td>56 (13)</td>
<td>65 (13)</td>
<td>62 (13)</td>
<td>33 (12)</td>
<td>30 (9)</td>
<td>28 (10)</td>
<td>29 (9)</td>
<td>9-13</td>
</tr>
<tr>
<td>Triple (C+M+L)</td>
<td>22 (5)</td>
<td>31 (6)</td>
<td>32 (7)</td>
<td>16 (6)</td>
<td>12 (3)</td>
<td>12 (4)</td>
<td>8 (3)</td>
<td>3-7</td>
</tr>
</tbody>
</table>

C, clarithromycin; L, levofloxacin; M, metronidazole.
EP2.23 | Tailored therapy based on clarithromycin resistance is effective for the first-line therapy of Helicobacter pylori

S. Moon; H. Kang; C. Lim; J. OH
The Catholic University of Korea, Seoul, Republic of Korea

Background: Standard triple therapy (STT, PPI-clarithromycin-amoxicillin) for Helicobacter pylori (H. pylori) eradication regimen shows a lower treatment success rate recently. Dual Priming Oligonucleotide -based multiplex polymerase chain reaction (DPO-PCR) can be used to detect A2142G and/or A2143G point mutations of H. pylori causing clarithromycin resistance (CAM-R). We compared the eradication rate of H. pylori between empirical STT and tailored therapy by DPO-PCR.

Methods: A total of 575 H. pylori-infected patients were retrospectively evaluated in Eunpyeong St. Mary's Hospital, Korea. Empirical therapy by traditional method (Warthin-Starry silver stain) group was treated with STT. DPO-PCR was performed in the tailored therapy group. The CAM-R positive patients (who had A2142G and/or A2143G point mutations) were treated with bismuth-containing quadruple therapy (PPI-bismuth-metronidazole-tetracycline) for 7-14 days, while the CAM-R negative patients were treated with STT for 7-14 days. Eradication success was defined as a negative 13C-urea breath test at least 4 weeks after the treatment ended.

Results: We included 536 patients, 39 patients were lost to follow-up: 284 patients were treated by empirical STT as first-line and 242 patients belonged to the tailored therapy group. The tailored therapy group was superior to the empirical therapy group in terms of first-line therapy both in the intention-to-treat analysis (70.4% vs 83.7%, P = 0.0002) and in the per-protocol analysis (76.9% vs 89.4%, P = 0.0003). Clarithromycin resistance ratio by DPO-PCR was 33.1% (80/242).

Conclusion: The tailored therapy by DPO-PCR is more effective than the empirical therapy for the first-line therapy of H. pylori in a country with a high clarithromycin resistance.


EP2.24 | Helicobacter pylori antibiotic resistance: Data from the European Registry on H. pylori Management (Hp-EuReg)

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Background: Antibiotic resistance is the major factor affecting our ability to cure Helicobacter pylori infection. Understanding the different H. pylori antibiotic resistances could be the key to improve treatment effectiveness.

Objective: To evaluate the H. pylori antibiotic resistance both prior and after one or several eradication treatments, in order to provide the most appropriate recommendations for the eradication of H. pylori.

Methods: International multicenter prospective non-interventional European Registry on H. pylori Management (Hp-EuReg) aiming to evaluate the decisions and outcomes of H. pylori infection by European gastroenterologists. Infected adult patients diagnosed with culture and with a result of the antibiotic resistance test registered at AEG-REDCap e-CRF from 2013 to 2019. Per-protocol (PP) analysis was performed. The antibiotic bacterial resistances were described by treatment line.

Results: A total of 32,447 patients were included, and culture was performed in 3,474 (11%). In naïve patients, 21% reported single clarithromycin resistance, and 11% dual (clarithromycin and metronidazole) resistance. Antibiotic resistance increased markedly from the first treatment, reaching over 37% dual resistance in second-line treatment (Table 1).

Conclusion: In Europe, culture testing to determine antibiotic resistance against H. pylori is scarce. H. pylori single clarithromycin resistance remains high (>15%) in all treatment lines, and greater than 20% in naïve patients. Dual or triple resistances are frequent and increase remarkably after the first treatment failure. Resistance to amoxicillin or tetracycline is exceptional.

<table>
<thead>
<tr>
<th>Treatment line</th>
<th>Naïve (%)</th>
<th>Second (%)</th>
<th>Third (%)</th>
<th>Fourth (%)</th>
<th>Fifth (%)</th>
<th>Sixth (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>2,485</td>
<td>521</td>
<td>311</td>
<td>97</td>
<td>31</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No resistance</td>
<td>1,054 (42)</td>
<td>74 (14)</td>
<td>26 (8)</td>
<td>7 (7)</td>
<td>4 (13)</td>
<td>1 (9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clarithromycin (C)</td>
<td>531 (21)</td>
<td>298 (57)</td>
<td>217 (70)</td>
<td>72 (74)</td>
<td>23 (74)</td>
<td>5 (45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metronidazole (M)</td>
<td>674 (27)</td>
<td>251 (48)</td>
<td>192 (62)</td>
<td>59 (61)</td>
<td>19 (61)</td>
<td>7 (64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Levofloxacin (L)</td>
<td>438 (18)</td>
<td>134 (26)</td>
<td>130 (42)</td>
<td>44 (45)</td>
<td>12 (39)</td>
<td>3 (27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>17 (1)</td>
<td>4 (1)</td>
<td>5 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>11 (0.4)</td>
<td>3 (1)</td>
<td>2 (0.6)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dual (C+M)</td>
<td>279 (11)</td>
<td>195 (37)</td>
<td>165 (53)</td>
<td>52 (54)</td>
<td>17 (55)</td>
<td>5 (46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triple (C+M+L)</td>
<td>128 (5)</td>
<td>91 (18)</td>
<td>99 (32)</td>
<td>34 (35)</td>
<td>9 (29)</td>
<td>3 (27)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

C, clarithromycin; L, levofloxacin; M, metronidazole.
Background: Antibiotic resistance is the major factor affecting our ability to cure Helicobacter pylori infection. Quadruple therapy is currently recommended; however, triple therapy with two antibiotics may be sufficient in those patients without clarithromycin resistance.

Objective: To evaluate the effectiveness of the treatments according to the clarithromycin H. pylori resistance in Europe.

Methods: International multicenter prospective non-interventional European Registry on H. pylori Management (Hp-EuReg) aiming to evaluate the decisions and outcomes of H. pylori infection. Infected adult patients diagnosed with culture registered at AEG-REDCap e-CRF from 2013 to 2019. Per-protocol (PP) analysis was performed based on the presence or absence of clarithromycin bacterial resistance.

Results: Overall, 5,036 patients were included: 1,747 (35%) were resistant and 3,289 (65%) sensitive to clarithromycin. The overall eradication rate was higher in clarithromycin-susceptible patients (91% vs 84%; \(P < 0.001\)). Triple therapy with a PPI, clarithromycin and amoxicillin achieved over 90% eradication rates in clarithromycin-susceptible patients. However, in those with clarithromycin-resistance, optimal effectiveness was only achieved when treated with quadruple therapy with a PPI, clarithromycin, amoxicillin and bismuth (Table 1).

Conclusions: Classic triple therapy with a PPI, clarithromycin and amoxicillin achieves optimal results (>90%) in patients susceptible to clarithromycin. However, when clarithromycin resistance is unknown, quadruple therapy with a PPI, clarithromycin, amoxicillin and bismuth may be a better treatment option.
### TABLE 1. Effect of the clarithromycin *Helicobacter pylori* on the effectiveness in Europe

<table>
<thead>
<tr>
<th>Treatment schemes</th>
<th>Clarithromycin resistant</th>
<th>Clarithromycin susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>N</td>
</tr>
<tr>
<td>Overall</td>
<td>754</td>
<td>897</td>
</tr>
<tr>
<td>Triple-C+A</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Triple-A+L</td>
<td>165</td>
<td>191</td>
</tr>
<tr>
<td>Triple-A+M</td>
<td>46</td>
<td>55</td>
</tr>
<tr>
<td>Triple-A+R</td>
<td>91</td>
<td>102</td>
</tr>
<tr>
<td>Triple-C+M</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Quadruple-C+A+M/T</td>
<td>43</td>
<td>54</td>
</tr>
<tr>
<td>Quadruple-C+A+B</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Sequential-C+A+T</td>
<td>242</td>
<td>286</td>
</tr>
<tr>
<td>Sequential-C+A+M</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Hybrid-C+A+M</td>
<td>27</td>
<td>34</td>
</tr>
<tr>
<td>Single capsule</td>
<td>66</td>
<td>80</td>
</tr>
</tbody>
</table>

A. amoxicillin; B, bismuth; C, clarithromycin; E, number of eradicated patients; M, metronidazole; N, total number of patients analysed; T, tinidazole; Single capsule: three-in-one single capsule containing bismuth, tetracycline and metronidazole (marketed as Pylera®).

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**EP2.26 | Trends in the effectiveness of eradication therapy in Russia**

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\(^1\)A.S. Loginov Moscow Clinical Scientific Center, Moscow, Russian Federation; \(^2\)A.I. Yevdokimov Moscow State University of Medicine and Dentistry, Moscow, Russian Federation; \(^3\)Tver State Medical University, Tver, Russian Federation; \(^4\)Peoples’ Friendship University of Russia, Moscow, Russian Federation; \(^5\)Central Research Institute of Epidemiology, Moscow, Russian Federation; \(^6\)Laboratory HELIX, Moscow, Russian Federation

The eradication therapy schemes with the effectiveness of at least 90% are recommended for *H. pylori* (HP) eradication. Local monitoring of eradication effectiveness is to be done to find out the current effectiveness rate in each separate region. 13C-UBT is the most recommended modality for the effectiveness control.

**Aim:** Assessment of trends in eradication effectiveness in Russia using the 13C urea breath test (13C-UBT).

**Material and Methods:** The results of 13C-UBT in patients after HP eradication. The treatment was considered as effective, if control test results came negative (DOB < 4‰). We analyzed the data on 1217 patients (age 14-83 years, M – 467, F – 750) and 279 patients (age 14-83 years, M – 112, F – 167) collected in 2017 and in 2019, respectively. Pearson’s chi-square test was used for statistical analysis.

**Results:** The average eradication rate increased insignificantly from 74.3% in 2017 to 78.9% in 2019 (\(\chi^2 = 2,081; p > 0.05\)). In 2017, the rate reached 76.4% in women and 70.9% in men (\(\chi^2 = 4.594; P > 0.05\)) with no difference in the rate between age groups (\(\chi^2 = 0.563; P > 0.05\)). In 2019 it reached 79.6% in women and 77.7% in men (\(\chi^2 = 0.155; P > 0.05\)) with no difference between age groups (\(\chi^2 = 1.799; P > 0.05\)).

**Conclusion:** The HP eradication effectiveness rate in Russia is low. Thorough studies data on treatment schemes and patients’ compliance is necessary for the eradication schemes selection.

EP2.27 | The comparison of side effects and effectiveness of standard triple therapy with clarithromycin and alternative high-dose amoxicillin/bismuth therapy in eradication of H. pylori


The Institute of Clinical and preventive medicine of the University of Latvia, Riga, Latvia; Faculty of Medicine, University of Latvia, Riga, Latvia; Latvian Biomedical Research and Study Centre, Riga, Latvia; Digestive Disease Centre GASTRO, Riga, Latvia

Background: Antibiotics are standard treatment for Helicobacter pylori infection. A standard triple therapy tends to cause wide spectrum of side effects, which may lead to therapy discontinuations, decrease of eradication rate and increase the risk of developing resistance.

Aim: Compare the effectiveness and the spectrum of side effects of standard triple H. pylori eradication therapy and alternative high dose amoxicillin/bismuth therapy.

Methods: Participants were healthy individuals aged 40-64. Eradication subgroup underwent UBT and serology; 483 positive patients were randomly divided into eradication subgroups – standard triple therapy (Amoxicillin 1 g ×2, Clarithromycin 0.5 g ×2 and Esomeprazole 0.04 g ×2 – 14 days) 248 and alternative therapy (Amoxicillin 1 g ×3, Esomeprazole 0.04 g ×2 and Bismuth subcitrate 0.24 g ×2 – 14 days) 235. After 21-28 days side effects and compliance were registered.

Results: During standard treatment of H. pylori with clarithromycin (TER1) 56.9% (n = 141) and in alternative therapy with Bismuth Subcitrate (TER2) 40.0% (n = 94) patients experience side effects (P < 0.01). The most frequent adverse reactions in the TER1 group were bitter taste (P < 0.01), diarrhoea (P < 0.01), fatigue (P = 0.02) and vomiting (P = 0.04), and in the TER2 group – discoloration of the stool (P < 0.01). The higher efficacy of eradication was demonstrated in the TER1 group – 88.1% (n = 140) and relatively the eradication efficacy of TER2 reached 77.9% (n = 116) (P = 0.01).

Conclusions: Clarithromycin-containing therapy more often causes side effects: a bitter taste, diarrhea, fatigue and vomiting. Alternative therapy include discoloration of the stool. Standard triple therapy achieves a higher eradication rate than alternative bismuth therapy.


EP2.28 | The effects of Eupatilin of NSAID-gastropathy associated with H. pylori

I. Skrypnyk, V. Parkhomenko, O. Gopko

Ukrainian Medical Stomatological Academy, Poltava, Ukraine

A topical issue is the study of the effects of Eupatilin on the mucous barrier of the gastroduodenal zone (GDZ) and oxidative stress in patients with H. pylori-related nonsteroidal anti-inflammatory drugs (NSAIDs)-gastropathy. The study involved 43 patients, the mean age of the patients was 63.8 ± 9.1 years. The patients were divided into 2 groups: I (n = 23) – treated with antihelicobacter therapy (AHBT) according to Maastricht-V (2016) recommendations; group II (n = 20) – AHBT and Eupatilin 60 mg 3 times a day for 28 days. The presence of H. pylori was diagnosed by determining the H. pylori antigen in faeces (stool-test). The state of mucous-forming function of GDZ mucous membrane was assessed by the content of N-acetyleneuraminic acid (NANA) and fucoproteins in serum, the activity of free-radical oxidation – by the concentration of TBA-reactive products and catalase activity in the blood serum. Positive effect of Eupatilin after 1.5 months of complex treatment was noticed by lower 1.2-fold (P < 0.05) serum NANA concentration compared with patients of group I, a significantly higher in 1.4 times raise of fucoproteins levels after 14 days of treatment and in 1.5 times (P < 0.02) after 45 days. The antioxidant effect of Eupatilin was confirmed by 1.5-fold lower (P < 0.02) concentrations of TBA-reactive products in serum and 1.2-fold higher (P < 0.05) of serum catalase activity in patients of group II compared to group I. Complex therapy with the inclusion of Eupatilin, in comparison with traditional AHBT, significantly improves the clinical course of H. pylori-associated NSAID-gastropathy due to the cytoprotective and antioxidant effects.

I. Skrypnyk: None. V. Parkhomenko: None. O. Gopko: None.
Aims: To evaluate eradication rate (ER) of antibiotic susceptibility tailored triple therapy (TTT) according to the updated ESPGHAN and NASPGHAN Guidelines (JPGN 2017;64:991-1003).

Methods: Since 2017, 30 centers from 17 European countries reported prospectively demographic, clinical and follow up data of H. pylori infected pediatric patients. For this analysis, we included all treatment naive children with biopsy proven infection and antibiotic susceptibility results for clarithromycin (CLA) and metronidazole (MET), who received TTT with PPI + Amoxicillin + CLA (PAC) or MET (PAM), ER was accessed 4 to 8 weeks after completed treatment.

Results: Of 756 completed cases, 419 met inclusion criteria (52% female, median age: 13 years). Strains susceptible to CLA and MET were detected in 60% (n = 252), double-resistance in 2% (n = 10). Primary resistance to CLA and MET was high (24%, 18%, respectively). PAC was given to 59%, PAM to 41% of cases; 96% were treated for 14 days. Antibiotics were dosed according to guidelines in >80%, while PPI dose was too low in 52%. The ER in relation to country of living, antibiotic susceptibility, treatment regimen and compliance is shown in Table 1. In fully susceptible subgroup, PAC tended to have higher risk for treatment failure than PAM (92% vs 95%, ORadj = 1.6, 95% CI: 0.5-5.0, P = 0.4).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Eradication success</th>
<th>Eradication failed</th>
<th>ORadj (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n (ER%)</td>
<td>376 (90%)</td>
<td>43 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Country of living</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North and West Europe</td>
<td>100 (93%)</td>
<td>7 (7%)</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>South Europe</td>
<td>173 (91%)</td>
<td>18 (9%)</td>
<td>1.4 (0.6-3.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>East Europe</td>
<td>84 (85%)</td>
<td>15 (15%)</td>
<td>2.5 (1.0-6.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Israel &amp; Turkey</td>
<td>19 (86%)</td>
<td>3 (14%)</td>
<td>2.2 (0.5-9.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>Susceptibility groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET-S/CLA-S</td>
<td>233 (93%)</td>
<td>19 (7%)</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>MET-S/CLA-R</td>
<td>80 (87%)</td>
<td>12 (13%)</td>
<td>1.8 (0.8-3.9)</td>
<td>0.1</td>
</tr>
<tr>
<td>MET-R/CLA-S</td>
<td>53 (82%)</td>
<td>12 (18%)</td>
<td>2.8 (1.3-6.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>MET-R/CLA-R</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Treatment regimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPI + AMO + MET (PAM)</td>
<td>159 (92%)</td>
<td>14 (8%)</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>PPI + AMO + CLA (PAC)</td>
<td>217 (88%)</td>
<td>29 (12%)</td>
<td>1.5 (0.8-3.0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Drug dosing according to guidelines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>165 (91%)</td>
<td>17 (9%)</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>202 (89%)</td>
<td>25 (11%)</td>
<td>1.3 (0.7-2.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>Compliance to therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent 90-100%</td>
<td>346 (93%)</td>
<td>27 (7%)</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>Lower than 90%</td>
<td>15 (63%)</td>
<td>9 (37%)</td>
<td>7.7 (3.0-19.2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

ORadj obtained from multivariate logistic analysis adjusted for gender and age. Significant results are given in bold characters.
Conclusions: TTT for 2 weeks reached 90% ER. Increasing drug dose and compliance may further improve treatment success.

T. Le Thi: None. K. Werkstetter: None. J. Cabral: None. K. Kotilea: None. P. Bontems: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); modest; Abbvie, Ferring and Nutricia. F. Consultant/Advisory Board; modest; Biocodex. J. Barrio: None. M. Cilleruelo Pascual: None. M. Homan: None. M. Kori: None. P. Urruzuno: None. N. Kalach: None. Z. Misak: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); modest; GlaxoSmithKline, Abbvie, Pharmas, and Wurth. R. Lima: None. M. Tavares: None. E. Miele: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); modest; BioGaia and Abbvie. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); modest; Nestle, Nutricia, Vian, Friesland, Abbvie, Aventis. F. Consultant/Advisory Board; modest; Adare Pharmaceuticals and Adacete Therapeutics.

J. Sykora: None. A. Krahl: None. K. Matusiewics: None. M. Korkut Ugras: None. F. Rea: None. E. Roma: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); modest; Abbvie. T. Casswall: None. M. Klemenak: None. A. Cseh: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); modest; Abbvie, Biogaia, Ferring and Danone. J. de Laffolie: None. M. Rogalidou: None. A. Lopes: None. H. Banoub: None. S. Koletzkio: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); modest; Mead Johnson, Nestec Nutrition, BioGaia. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); modest; Nestle, Danone, Biocodex, Shire, Abbvie, R-Biopharm, Vifor, Pharmacosmos, Celgene, Thermo Fisher, Janssen.

Methods: The study was performed on two reference strains, H. pylori Tx30a and J99. The activity of myricetin alone and in combination with antibiotics with the highest (clarithromycin and metronidazole) and the lowest resistance prevalence (amoxicillin – a control antibiotic) was performed using the microtiter and checkerboard method. Bacterial morphology and viability were determined by microscopy and flow cytometry.

Results: It was noticed that incubation of H. pylori with 1/16 to 1/2 minimal inhibitory concentrations of myricetin (0.128-1.024 mM) significantly reduced the transition of this bacterium into a coccolid form and increased the average ratio of green/red fluorescence of cells. A synergistic interaction of myricetin was demonstrated with all antibiotics tested, allowing for a 4-16-fold reduction in their concentrations.

Summary: The obtained results suggest that myricetin most probably sensitizes H. pylori to the antibiotics used by keeping this bacterium in a spiral, metabolically active form.

P. Krzyżek: None. E. Paluch: None. G. Gościniak: None.

EP2.31 | Counteracting Helicobacter pylori using drug-free nanostructured lipid carriers (NLC)

R. Chitas1,2,3; C. L. Seabra4; C. Nunes4; P. Parreira1,2; M. C. L. Martins1,2,3

1 I3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; 2 INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; 3 ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; 4 LAQV-REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

Background: Current treatments for Helicobacter pylori (Hp) infection are failing, due to the increase of antibiotic resistance. Drug-free nanostructured lipid carriers (NLC) have been studied for Hp eradication, showing both in vitro/vivo effect against Hp. This work aims to optimize NLC efficiency by fine-tuning physicochemical characteristics, in order to achieve Hp eradication.

Methods: In the preparation of NLC two surfactants were tested, Tween60® and Tween80®. NLC were prepared by hot homogenization followed by ultrasonication. Additionally, half of each formulation was dialyzed, to wash eventual surfactant debris. They were characterized in terms of size, charge and concentration. Dialyzed and non-dialyzed NLC were tested in vitro against Hp strain J99.

Results: NLC with Tween60® had sizes around 250 nm and a surface charge of ~27 mV. The same characteristics were only obtained in NLC with Tween80® after optimization of the ultrasonication parameters. All NLC stocks had a final concentration in the range of 10^14 particles/mL. After 24 hours of incubation, both NLC formulations had similar outcomes, achieving a decrease of 3 logs from 10^7 to 10^5 CFU/mL (bactericidal effect). Non-dialyzed and dialyzed NLC showed also the same bactericidal activity, which indicates that NLC have an effect per se.

Conclusions: TTT for 2 weeks reached 90% ER. Increasing drug dose and compliance may further improve treatment success.

T. Le Thi: None. K. Werkstetter: None. J. Cabral: None. K. Kotilea: None. P. Bontems: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); modest; Abbvie, Ferring and Nutricia. F. Consultant/Advisory Board; modest; Biocodex. J. Barrio: None. M. Cilleruelo Pascual: None. M. Homan: None. M. Kori: None. P. Urruzuno: None. N. Kalach: None. Z. Misak: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); modest; GlaxoSmithKline, Abbvie, Pharmas, and Wurth. R. Lima: None. M. Tavares: None. E. Miele: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); modest; BioGaia and Abbvie. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); modest; Nestle, Nutricia, Vian, Friesland, Abbvie, Aventis. F. Consultant/Advisory Board; modest; Adare Pharmaceuticals and Adacete Therapeutics.

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R. Chitas1,2,3; C. L. Seabra4; C. Nunes4; P. Parreira1,2; M. C. L. Martins1,2,3

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Results: NLC with Tween60® had sizes around 250 nm and a surface charge of ~27 mV. The same characteristics were only obtained in NLC with Tween80® after optimization of the ultrasonication parameters. All NLC stocks had a final concentration in the range of 10^14 particles/mL. After 24 hours of incubation, both NLC formulations had similar outcomes, achieving a decrease of 3 logs from 10^7 to 10^5 CFU/mL (bactericidal effect). Non-dialyzed and dialyzed NLC showed also the same bactericidal activity, which indicates that NLC have an effect per se.
Conclusions: Both formulations (Tween60® and Tween80®) were effective against Hp, and the dialysis step didn’t affect the NLC bactericidal activity. These results support the therapeutic potential of these nanoparticles.


Acknowledgments: PTDC/CTM-BIO/4043/201 and BiotechHealthDoctoralProgram


EP2.32 | Effectiveness of first-line H. pylori eradication therapy according to the daily statin-use: Analysis of the European Registry on H. pylori Management (Hp-EuReg)

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Introduction: The use of statins with antibiotics and proton pump inhibitors has been suggested as a strategy to increase the effectiveness of Helicobacter pylori treatments, mainly based on their anti-inflammatory characteristics. However, evidence published so far still remains scarce.

Aim: To analyse the impact of the daily use of statins in the effectiveness of H. pylori first-line therapies.

Methods: Multicentre prospective non-interventional registry of the clinical practice of European gastroenterologists of the European Registry on H. pylori Management (Hp-EuReg). Patients were collected at AEG-REDCap e-CRF from 2013 to December 2019. Records of naïve patients containing information about the statins’ use were included for current analysis. Modified intention-to-treat (mITT) analysis was performed to evaluate the treatment effectiveness.

Results: Overall, 7,687 patients received an empirical first-line therapy: 60.5% were women and median age was 56 years. From those, 1,895 (25%) were daily statins-users: 45% used simvastatin, 35% atorvastatin, 11% rosuvastatin and 9% other statins. Univariate analysis showed no differences in the treatment effectiveness of the statin-users group versus no statin-users (Table). Modified intention-to-treat (mITT) analysis was performed to evaluate the treatment effectiveness.

Conclusions: The daily use of statins does not seem to increase the effectiveness of H. pylori eradication treatment.

<table>
<thead>
<tr>
<th>TABLE 1. Impact of the statins’ use on the effectiveness of most frequently used first-line empirical treatments in Europe.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily use of statins</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>PPI+C+A</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>PPI+C+M</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>PPI+Bi+Tc+M</td>
</tr>
<tr>
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</table>
### Daily use of statins

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mITT, N (%)</th>
<th>Differences (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI+C+A+M (Sequential)</td>
<td>No 53 (81)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Yes 27 (93)</td>
<td></td>
</tr>
<tr>
<td>PPI+C+A +M (Concomitant)</td>
<td>No 1,189 (88)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Yes 527 (91)</td>
<td></td>
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<tr>
<td>PPI+Bi+C+A</td>
<td>No 612 (88)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Yes 186 (92.5)</td>
<td></td>
</tr>
</tbody>
</table>


**Introduction**: Updated data in Spain is needed to design the best strategy to treat *Helicobacter pylori* infection.
Aim: To analyse the effectiveness of *H. pylori* first-line eradication therapies in Spain.

Methods: Systematic multicentre prospective registry of the clinical practice of gastroenterologists on the management of *H. pylori* infection (Hp-EuReg). All infected adult patients were registered at AEG-REDCap e-CRF from February 2013 to June 2019. Data were subject to quality control. Effectiveness (by modified intention-to-treat) and multivariate analysis were performed. Independent factors evaluated: age, gender, presence of ulcer, proton pump inhibitor (PPI) dose, therapy duration and compliance.

Results: Overall, 10,633 naïve patients from 53 Spanish hospitals were included: median age was 51 years and 61% were women. From those, 10,267 patients received an empirical prescription. Over 90% mITT eradication rate was obtained with first-line 14-day quadruple therapies or 10-day bismuth three-in-one single-capsule (Table). Adverse events occurred in 25% of the cases, 0.2% being serious adverse events (Table). Multivariate analysis reported that 10-14 days therapies (OR = 4), good compliance (>90% drug intake; OR = 4) and high PPI doses (OR = 2) were associated with higher mITT eradication rates.

Conclusions: In Spain, optimal effectiveness (>90%) in first-line treatment was obtained with the non-bismuth concomitant therapy, the bismuth quadruple therapy with amoxicillin and clarithromycin (both for 14 days), and the 10-day bismuth quadruple (single capsule).

### TABLE 1. Effectiveness and safety of the most-frequently prescribed first-line therapies in Spain

<table>
<thead>
<tr>
<th></th>
<th>Effectiveness</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mITT</td>
<td>PP</td>
<td>Adverse events</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>C.I. 95%</td>
<td>N (%)</td>
</tr>
<tr>
<td>Overall first line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>therapies</td>
<td>9,726 (88)</td>
<td>88-89</td>
<td>9,497 (89)</td>
</tr>
<tr>
<td>7 days</td>
<td>159 (60)</td>
<td>52-68</td>
<td>158 (61)</td>
</tr>
<tr>
<td>10 days</td>
<td>6,011 (88)</td>
<td>87-89</td>
<td>5,858 (89)</td>
</tr>
<tr>
<td>14 days</td>
<td>3,522 (90)</td>
<td>89-91</td>
<td>3,449 (90)</td>
</tr>
<tr>
<td>PPI + C + A + M</td>
<td>3,880 (90)</td>
<td>89-91</td>
<td>3,781 (90)</td>
</tr>
<tr>
<td>(Concomitant)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 days</td>
<td>2,232 (88)</td>
<td>87-90</td>
<td>2,175 (89)</td>
</tr>
<tr>
<td>14 days</td>
<td>1,629 (92)</td>
<td>91-93</td>
<td>1,588 (92)</td>
</tr>
<tr>
<td>PPI + C + A</td>
<td>2,544 (83)</td>
<td>82-85</td>
<td>2,498 (84)</td>
</tr>
<tr>
<td>7 days</td>
<td>146 (59)</td>
<td>51-67</td>
<td>145 (59)</td>
</tr>
<tr>
<td>10 days</td>
<td>1,686 (84)</td>
<td>82-86</td>
<td>1,657 (84.5)</td>
</tr>
<tr>
<td>14 days</td>
<td>699 (86)</td>
<td>84-89</td>
<td>683 (87)</td>
</tr>
<tr>
<td>PPI + Single capsule</td>
<td>1,540 (95)</td>
<td>94-96</td>
<td>1,514 (96)</td>
</tr>
<tr>
<td>10 days</td>
<td>1,533 (95)</td>
<td>94-96</td>
<td>1,507 (96)</td>
</tr>
<tr>
<td>PPI + Bi + C + A</td>
<td>1,015 (91)</td>
<td>89-93</td>
<td>1,002 (91)</td>
</tr>
<tr>
<td>14 days</td>
<td>1,004 (91)</td>
<td>89-93</td>
<td>992 (91)</td>
</tr>
<tr>
<td>PPI + C + A + M</td>
<td>222 (81.5)</td>
<td>76-86</td>
<td>192 (84)</td>
</tr>
<tr>
<td>(Sequential)</td>
<td>10 days</td>
<td>221 (81)</td>
<td>191 (84)</td>
</tr>
</tbody>
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EP2.34 | Effectiveness of second-line *H. pylori* eradication treatments in Spain: Results from the European Registry on *H. pylori* management (Hp-EuReg)

M. Caldas1; Á. Pérez-Aisa2; M. Castro-Fernández3; L. Bujanda4; L. Rodrigo5; J. Pérez-Lasala6; J. Barrio7; Á. Lanas8; M. Perona9; B. Gómez-Rodríguez10; I. Moló11; O. Núñez12; R. Ruiz-Zorrilla13; A. Huerta14; E. Iyo15; R. Antón16; A. Campillo17; R. Pajares-Villaroya18; F. Bermejo19; L. Titó20; T. Angueira21; J. Huguet22; P. González-Cordero23; N. Alcaide24; A. Garre25; I. Puig26; O. P. Nyssen27; F. Megraud28; C. O’Morain29; J. P. Gisbert1; On behalf of the Hp-EuReg Investigators

1Gastroenterology Unit of Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), Universidad Autónoma de Madrid, and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain; 2Gastroenterology Unit of Hospital Costa del Sol and Red de Investigación en Servicios de Salud en Enfermedades Crónicas (REDISSEC), Marbella, Spain; 3Gastroenterology Unit of Hospital de Valme, Sevilla, Spain; 4Gastroenterology Unit of Hospital Donostia/Instituto Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Universidad del País Vasco (UPV/EHU), San Sebastián, Spain; 5Gastroenterology Unit of Hospital Universitario Central de Asturias, Oviedo, Spain; 6Gastroenterology Unit of Hospital Basurto, Vitoria-Gasteiz, Spain; 7Gastroenterology Unit of Hospital Universitario Virgen Macarena, Sevilla, Spain; 8Gastroenterology Unit of Hospital Quirón Marbella, Málaga, Spain; 9Gastroenterology Unit of Hospital Universitario Virgen Macarena, Sevilla, Spain; 10Gastroenterology Unit of Hospital Comarcal de Inca, Mallorca, Spain; 11Gastroenterology Unit of Hospital Clínic Universitari de València, Valencia, Spain; 12Gastroenterology Unit of Hospital Universitario Sanitas La Moraleja, Madrid, Spain; 13Gastroenterology Unit of Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 14Gastroenterology Unit of Hospital de Galdakao-Uxusano, Vizcaya, Spain; 15Gastroenterology Unit of Hospital de Galdakao-Usansolo, Vizcaya, Spain; 16Gastroenterology Unit of Hospital Clínic Universitari de València, Valencia, Spain; 17Gastroenterology Unit of Hospital Reina Sofía, Tudela, Navarra, Spain; 18Gastroenterology Unit of Hospital Infanta Sofía, Madrid, Spain; 19Gastroenterology Unit of Hospital Universitario de Fuenlabrada, idipAZ, Madrid, Spain; 20Gastroenterology Unit of Hospital de Matarrá, Barcelona, Spain; 21Gastroenterology Unit of Hospital General de Tomelloso, Tomelloso, Spain; 22Gastroenterology Unit of Consorcio Hospital General Universitario de Valencia, Valencia, Spain; 23Gastroenterology Unit of Hospital San Pedro de Alcántara and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Cáceres, Spain; 24Gastroenterology Unit of Hospital Clínico Universitario de Valladolid, Valladolid, Spain; 25Digestive Diseases Department of Althaia Xarxa Assistencial Universitària de Manresa, Universitat de Vic-Universitat Central de Catalunya (UVic/UCC), Manresa, Spain; 26Laboratoire de Bactériologie, Hôpital Pellegrin, Bordeaux, France; 27Department of Clinical Medicine, Trinity College Dublin, Dublin, Ireland.

**Introduction:** Optimal second-line regimens in *Helicobacter pylori* infection are required based on local previous results.

**Aim:** To analyse the effectiveness of second-line therapies in Spain.

**Methods:** Systematic multicentre prospective registry of clinical practice of gastroenterologists on the management of *Helicobacter pylori* infection (Hp-EuReg). All infected adult patients were registered at AEG-REDCap e-CRF from February 2013 to June 2019. Data were subject to quality control. Effectiveness (by modified intention-to-treat) and multivariate analysis were performed. Independent factors evaluated: age, gender, presence of ulcer, proton pump inhibitor (PPI) dose, therapy duration, use of clarithromycin in the previous line, and compliance.

**Results:** Overall, 2,481 patients received a second-line therapy: median age was 50 years and 66% were women. From those, 2,448 patients received an empirical prescription. Nearly 90% mITT eradication rate was obtained with either moxifloxacin- or levofloxacin containing triple therapy, or with quadruple therapy with levofloxacin and bismuth (all given for 14 days) or with 10-day bismuth three-in-one single capsule (Table). Only 1 patient (0.2%) showed a serious adverse event. Multivariate analysis showed that compliance (>90% drug intake; OR = 3), high PPI dose (OR = 2) and 14-day therapy (OR = 1.5) were associated with higher mITT eradication rates.

**Conclusions:** In Spain, optimal effectiveness (approximately 90%) in second-line treatment was obtained with triple quinolone or quadruple bismuth-quinolone regimens (both for 14 days) or with the 10-day bismuth quadruple (single capsules).
TABLE 1. Effectiveness and safety of the most frequently prescribed second-line therapies in Spain.

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>mITT</th>
<th>PP</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>C.I. 95%</td>
<td>N (%)</td>
</tr>
<tr>
<td>Overall 2nd line therapies</td>
<td>2,295 (84)</td>
<td>82-85</td>
<td>2,247 (84)</td>
</tr>
<tr>
<td>10 days</td>
<td>1,265 (79)</td>
<td>77-81</td>
<td>1,241 (80)</td>
</tr>
<tr>
<td>14 days</td>
<td>1,007 (89)</td>
<td>87-91</td>
<td>986 (90)</td>
</tr>
<tr>
<td>PPI + L + A</td>
<td>893 (78.5)</td>
<td>76-81</td>
<td>881 (79)</td>
</tr>
<tr>
<td>10 days</td>
<td>647 (74)</td>
<td>70-77</td>
<td>636 (74)</td>
</tr>
<tr>
<td>14 days</td>
<td>241 (92)</td>
<td>88-95</td>
<td>240 (92.5)</td>
</tr>
<tr>
<td>PPI + Bi + L + A</td>
<td>451 (89)</td>
<td>86-92</td>
<td>435 (90)</td>
</tr>
<tr>
<td>14 days</td>
<td>444 (90)</td>
<td>86-92</td>
<td>428 (90)</td>
</tr>
<tr>
<td>PPI + Single capsule</td>
<td>409 (88)</td>
<td>85-91</td>
<td>398 (89)</td>
</tr>
<tr>
<td>10 days</td>
<td>399 (88.5)</td>
<td>85-91</td>
<td>390 (89)</td>
</tr>
<tr>
<td>PPI + Mx + A</td>
<td>129 (91)</td>
<td>84-95</td>
<td>129 (91)</td>
</tr>
<tr>
<td>10 days</td>
<td>20 (100)</td>
<td>–</td>
<td>20 (100)</td>
</tr>
<tr>
<td>14 days</td>
<td>109 (89)</td>
<td>82-94</td>
<td>109 (89)</td>
</tr>
<tr>
<td>PPI + C + A + M (Concomitant)</td>
<td>110 (82)</td>
<td>73-89</td>
<td>109 (82)</td>
</tr>
<tr>
<td>10 days</td>
<td>47 (81)</td>
<td>67-91</td>
<td>46 (80)</td>
</tr>
<tr>
<td>14 days</td>
<td>62 (84)</td>
<td>72-92</td>
<td>62 (84)</td>
</tr>
</tbody>
</table>


EP2.35 | Population study on the prevalence and antimicrobial resistance of Helicobacter pylori in West Flanders – Belgium

A. Vanden Bulcke1; B. Waked2; L. Haems2; G. Lambrechts2; A. Hervent3; S. Vervaeke4; F. Baert4
1UZ Leuven, Leuven, Belgium; 2UZ Gent, Gent, Belgium; 3AZ Diamant, Oostende, Belgium; 4AZ Delta, Roeselare, Belgium

Background: Data on Helicobacter pylori (HP) primary resistance in Belgium are largely based on the population of Brussels and Wallonia. Notably Brussels has a substantial proportion of patients with an immigration background, which is not representative for other parts of Belgium.

Methods: A multicenter prospective investigation was performed in West Flanders, Belgium for collecting gastric biopsies for histopathology, Rapid Urease Test (RUT) and if the RUT is positive, a culture was started. The principal investigation question was the prevalence and the primary resistance rate of HP in first line testing. Other investigation topics are the performance of histopathology, RUT and culture.

Results: From October 2017 to February 2019, 512 patients participated. 438 were eligible for analysis in first line testing, of whom 89.7% are native Belgians. 89.8% (n = 88/98) of the cultures showed successful growth of HP but an antibiogram was achieved in only 52.3% (n = 46/88), including 37 in first line testing. The prevalence of HP in first line testing is 17.8% for native Belgians and 75.6% for immigrants. The primary resistance rate is 13.5% for clarithromycin, 29.7% for metronidazole and 29.7% for levofloxacin.

Conclusions: The primary clarithromycin resistance rate of HP in the province West Flanders in Belgium is below 15% which implicates a clarithromycin based triple therapy is still an option for empiric eradication. Conclusions of this study must be interpreted with caution due to the low number of successful antibiograms. Further surveillance in West Flanders is recommended as the resistance rate can differ from the population of Brussels.
EP2.36 | European registry on H. pylori management (Hp-EuReg): Analysis of 1,782 empirical rescue therapies on third and subsequent lines

**Aims:** To evaluate the use and effectiveness of empirical rescue therapies on third and subsequent lines in Europe.

**Methods:** Sub-study of the European Registry on H. pylori Management, an international multicenter prospective non-interventional registry aimed to evaluate H. pylori management in Europe. All cases with ≥3 eradication attempts were extracted until June 2019. Only the empirically prescribed therapies were analyzed. Data were subject to quality review.

**Results:** In total, 1,782 rescue treatments were included: 1,264, 359, 125 and 34 third-, fourth-, fifth- and sixth-line treatments, respectively. Mean age was 51, 69% of patients were women and 5% were allergic to penicillin. Sixty-three different therapies were used, being Pylera® the most commonly prescribed. The most frequent regimens in classical bismuth quadruple therapies (P<0.05).

**Conclusions:** Empirical rescue treatments in third and subsequent lines obtain suboptimal eradication rates in Europe. Only Pylera® and the optimised versions of triple PPI-amoxicillin-levofloxacin and quadruple PPI-bismuth-tetracycline-metronidazole- achieve acceptable outcomes.
Overall eradication rates of the most prescribed empirical therapies on third and subsequent lines.

<table>
<thead>
<tr>
<th>Rescue therapy</th>
<th>Use, N (%)</th>
<th>n</th>
<th>Modified intention-to-treat Effectiveness (95% CI)</th>
<th>Per-protocol n</th>
<th>Effectiveness (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pylera®</td>
<td>416 (23%)</td>
<td>363</td>
<td>84 (80-87)</td>
<td>350</td>
<td>85 (81-88)</td>
</tr>
<tr>
<td>Triple PPI-A-L</td>
<td>277 (15%)</td>
<td>213</td>
<td>78 (72-83)</td>
<td>206</td>
<td>78 (72-84)</td>
</tr>
<tr>
<td>Triple PPI-A-R</td>
<td>231 (13%)</td>
<td>205</td>
<td>66 (59-72)</td>
<td>198</td>
<td>67 (60-74)</td>
</tr>
<tr>
<td>Quadruple PPI-B-Tc-M</td>
<td>171 (9.6%)</td>
<td>162</td>
<td>73 (65-80)</td>
<td>157</td>
<td>73 (66-80)</td>
</tr>
<tr>
<td>Quadruple PPI-B-D-M</td>
<td>115 (6.5%)</td>
<td>109</td>
<td>63 (54-72)</td>
<td>105</td>
<td>64 (54-73)</td>
</tr>
<tr>
<td>Quadruple PPI-A-L-B</td>
<td>95 (5.3%)</td>
<td>81</td>
<td>78 (67-86)</td>
<td>79</td>
<td>80 (69-88)</td>
</tr>
<tr>
<td>Quadruple PPI-C-A-M</td>
<td>62 (3.5%)</td>
<td>57</td>
<td>58 (44-71)</td>
<td>54</td>
<td>59 (45-72)</td>
</tr>
<tr>
<td>Triple PPI-A-M</td>
<td>54 (3.0%)</td>
<td>47</td>
<td>68 (5381)</td>
<td>45</td>
<td>69 (53-82)</td>
</tr>
<tr>
<td>Triple PPI-C-A</td>
<td>43 (2.4%)</td>
<td>33</td>
<td>67 (48-82)</td>
<td>30</td>
<td>67 (47-83)</td>
</tr>
<tr>
<td>Quadruple PPI-C-A-B</td>
<td>28 (1.6%)</td>
<td>24</td>
<td>75 (53-90)</td>
<td>21</td>
<td>86 (64-97)</td>
</tr>
<tr>
<td>Triple PPI-A-Mx</td>
<td>27 (1.5%)</td>
<td>26</td>
<td>69 (48-86)</td>
<td>26</td>
<td>69 (48-86)</td>
</tr>
<tr>
<td>Quadruple PPI-A-R-B</td>
<td>24 (1.3%)</td>
<td>22</td>
<td>59 (36-79)</td>
<td>21</td>
<td>57 (34-78)</td>
</tr>
</tbody>
</table>

A, amoxicillin; B, bismuth; C, clarithromycin; D, doxycycline; L, levofloxacin; M, metronidazole; Mx, moxifloxacin; Tc, tetracycline; R, rifabutin; 95% CI, 95% confidence interval.

Probiotics are recommended in accordance with Maastricht V in treatment of *Helicobacter pylori* gastritis. Probiotic therapy isn't always effective. Autoprophilics — beneficial and indigenous bacteria are more promising. This study aimed to investigate the effectiveness of eradication therapy of *Helicobacter pylori* gastritis (HPG) using autoprophilic enterococci. Methods: The study was conducted on patients with HPG (8 men, 8 women, aged 27-63). HPG was confirmed by studying biopsy samples of the stomach and feces using bacteriological, biochemical (AMA RUT Reader, Russia) immunochromatographic method, ELISA (ArcDIA, Finland) and PCR. Autoprophilics were prepared using indigenous non-pathogenic Enterococcus faecalis isolated from feces or stomach samples and tested for pathogenicity genes. Autoprophilics were administered per os twice daily at 50 mL (8.0 lg CFU/mL) for 20 days. Monitoring of treatment effectiveness was carried out using an immunochromatographic test and ELISA. The composition of microbiota of the stomach and feces was analyzed before and after the treatment by qPCR (Colonoflor OOO «ALFA LAB» and metagenome analysis 16S rRNA). Results: Gastrointestinal dysfunctions (belching, heartburn, flatulence, nausea, epigastric pain, poor appetite) disappeared after therapy. No *Helicobacter pylori* antigens were detected using immunochromatographic method and ELISA. Results of control study were negative. Signs of intestinal dysbiosis such as a decrease in Firmicutes (Lactobacillus, Enterococcus), an increase in gamma-Proteobacteria (opportunistic representatives of the family Enterobacteriaceae) were eliminated. Conclusion: Autoprophilic enterococci can be considered as candidates for an alternative method or important addition to eradication of H.p. infection. The study was supported by Russian Scientific Foundation grant 16-15-10085. E. Ermolenko: None. A.S. Molostova: None. N.S. Gladyshev: None. A.V. Svarval: None. Y.S. Dubosarskiy: None. A.N. Tsapieva: None. K. Ermolenko: None. M.A. Dmitrienko: None. A.N. Suvorov: None.
EP2.38 | Antibiotics susceptibility testing increases *Helicobacter pylori* eradication rates in dyspeptic patients from Egypt: A randomized controlled trial

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**Background:** Eradication rates for *Helicobacter pylori* has declined over the past years. To overcome treatment failure, antibiotics susceptibility-guided therapy was suggested as an effective option for eradication of *H. pylori* infection.

**Aims:** To test the effectiveness of culture-based antibiotics susceptibility-guided therapy versus clarithromycin or levofloxacin triple therapy as first-line therapy for *H. pylori* infection.

**Methods:** Dyspeptic patients performing esophagogastroduodenoscopy were prospectively enrolled. Patients with positive *H. pylori* results by rapid urease test were randomized to receive antibiotics susceptibility-guided therapy or empirical clarithromycin or levofloxacin based triple therapy for 14 days.

**Results:** In total, 82 patients were enrolled (52 in culture and sensitivity-based therapy group and 30 in empirical therapy group) and 50 (60.1%) patients completed the study according to the protocols. Cultures were possible in 28 patients of the culture and sensitivity-based therapy group (53.8%). The overall resistance rates to clarithromycin, amoxicillin, metronidazole, levofloxacin, tetracycline, nitrofurantoin and rifampicin were 32.1%, 32.1%, 78.6%, 7.1%, 17.9%, 17.9% and 21.4% respectively. Empirical triple and antibiotics susceptibility-guided eradication rates were, respectively, 53.3% and 85.7% by intention-to-treat (*P* = 0.035) and 64% and 96% (*P* = 0.005) by per-protocol analysis. Adverse events were reported in 16.6% of patients in empirical triple therapy and 21.4% of in antibiotics susceptibility-guided therapy (*P* = 0.527 NS).

**Conclusions:** Culture-based eradication therapy demonstrated superior eradication rates than empirical therapy as a first-line therapy for *H. pylori* in a region with high rates of antimicrobial resistance.


EP2.39 | Nifuratel in the eradication of *Helicobacter pylori* infection in adults: Results of a randomized comparative clinical trial

N. Dekhnich; A. Triapyshko; R. Kozlov; I. Trushin; A. Kuzmenkov
Smolensk State Medical University, Smolensk, Russian Federation

**Background:** Eradication rates for clarithromycin triple therapy dramatically decrease whatever the reason is, in cases when patients have previous medical history of macrolide exposure. In this regard, there is a search for alternative antibacterial agents with high anti-*H. pylori* activity and a favorable safety profile for eradication of *H. pylori* infection.

**Aim:** Compare 14-day nifuratel-based triple therapy as first-line *H. pylori* treatment with 14-day clarithromycin-based triple therapy.

**Materials/Methods:** In total, 70 *H. pylori*-infected subjects participated in the randomized clinical trial. 35 patients of the first group received a 14-day nifuratel-based triple therapy: esomeprazole (20 mg BID), nifuratel (400 mg BID), and amoxicillin (1000 mg BID). 35 patients of the second group – 14-day clarithromycin-based triple therapy: esomeprazole (20 mg BID), clarithromycin (500 mg BID), and amoxicillin (1000 mg BID). *H. pylori* stool antigen was taken to be checked for eradication.

**Results:** Intention-to-treat (ITT) eradication rates of 14-day nifuratel-based triple and 14-day clarithromycin-based triple therapy were 82.9% and 74.3% (*P* = 0.561), respectively, and the per-protocol (PP) rates were 90.6% and 89.7% (*P* = 1.000), respectively. The adverse events were reported in 17.1% of the first group and 34.3% of patients in the second group. There were no statistically significant differences of eradication rates and adverse events in both groups (*P* > 0.05).

**Conclusions:** 14-day triple nifuratel-based therapy has demonstrated the excellent eradication rates that exceed 90%. Nifuratel triple therapy may be considered as an alternative to clarithromycin triple therapy for patients with previous history of macrolide exposure or macrolide intolerance.

N. Dekhnich: None. A. Triapyshko: None. R. Kozlov: None. I. Trushin: None. A. Kuzmenkov: None.

EP2.40 | Toxic effects of bismuth on *Helicobacter pylori* associated with the intra-gastric acidity: A transmission electron microscopy study

Y. Lee; T. Chiang; C. Shun
National Taiwan University Hospital, Taipei, Taiwan

**Objective:** Whether the toxic effect of bismuth on *Helicobacter pylori* is pH dependent or not is unclear.

**Design:** Patients with positive results of *13C*-urea breath test (UBT) were randomized into 3 groups: (1) no treatment; (2) colloidal bismuth subcitrate (CBS, 120 mg/tab); and (3) proton pump inhibitor (esomeprazole, 40 mg/tab) plus CBS. For the CBS group, patients were separated to: CBS 1 dose 1 hour before endoscopy, CBS 1 dose 4 hours before endoscopy, and CBS 4 times a day 24 hours before endoscopy. For the esomeprazole plus CBS group, patients were separated to: esomeprazole four times a day for 3 days, followed by CBS 1 dose 1 hour before endoscopy, CBS 1 dose 4 hours before endoscopy, and CBS 4 times a day 24 hours before endoscopy. For the esomeprazole plus CBS group, patients were separated to: esomeprazole four times a day for 3 days, followed by CBS 1 dose 4 hours before endoscopy; and esomeprazole four times a day for the first 3 days, followed by CBS four times a day 24 hours before endoscopy. Biopsy samples was examined by transmission electron microscopy (TEM).

**Results:** A total of 21 subjects with positive *13C*-UBT were enrolled. Active budding processes of replication were observed by TEM in...
participants without prior treatment. By contrast, morphological damages of *H. pylori*, including swelling, intra-bacterial vacuolization, and structural degradation with wall eruption, were noted starting from 1 hour after CBS. The phenomena were similarly noted when the CBS was administered in different timing, dosage, and whether the PPI was used or not.

**Conclusions**: An immediate toxic effect of CBS is noted for *H. pylori* infection, which is independent from the intra-gastric acidity.

Y. Lee: None. T. Chiang: None. C. Shun: None.

**EP2.41** | **A real-life study assessing the efficacy of single capsule Bismuth quadruple therapy supplemented with probiotics for *H pylori* eradication in patients naïve to treatment in a high clarithromycin resistance area**

K. Priadko¹ ²; A. G. Gravina³; L. Granata³; R. Cerbone³; P. Ciamarra³; A. Montanaro³; A. Facchiano³; A. Miranda³; M. Romano¹

¹Università degli Studi della Campania “L. Vanvitelli”, Naples, Italy; ²Dnipro State Medical Academy, Dnipro, Ukraine

**Background**: International guidelines suggest the use of bismuth quadruple therapy (BQT) as first-line regimen for *H. pylori* eradication in areas of high clarithromycin (C) resistance. In Italy, an area with high C resistance rate, BQT is only possible through the 3-in-1 capsule formulation. Whether probiotics counteract antibiotic-induced microbiome disturbance, thus decreasing treatment-related adverse events (TRAEs) and increasing compliance to therapy, is still debated.

**Objective**: To assess the efficacy of single capsule BQT supplemented with probiotics in *H. pylori*-infected subjects naïve to treatment.

**Method**: Real-life study performed on 250 *H pylori*-infected dyspeptic patients naïve to treatment. *H pylori* diagnosis was through ¹³C Urea Breath Test (UBT), HpSA or histology. Single capsule BQT (i.e. Pylera®), approved by the Italian National Health System for 10 days, was as follows: Esomeprazole 40 mg bid + Pylera® qid + *Lactobacillus paracasei* formulation (Enterolactis plus ®) bid. Efficacy of treatment was assessed at least 45 days after the end of treatment by UBT. Side effects were evaluated via a questionnaire at the end of therapy.

**Results**: Of total 66 patients, the *H. pylori* eradication rate of modified BQT-2 regimen was 77.3%, and compliance rate was 100%. Metronidazole and Clarithromycin resistance rates were 30.0% and 22.0%, respectively. Eradication rate showed no significant difference between metronidazole susceptible and resistant strains (susceptible 74.3%, 26/35, and resistant 73.3%, 11/15) (P = 1.000). Most of the adverse events were mild and 20 patients (30.3%) presented nausea, epigastric soreness, loose stool, asthenia, skin rash, dizziness, taste perversion, headache, or dyspepsia.

**Conclusions**: Twice a day modified BQT-2 therapy with low dose metronidazole may be suboptimal as an alternative first-line therapy against *H. pylori*, despite of its high compliance.


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**EP2.42** | **Modified bismuth quadruple therapy with low dose metronidazole as first-line therapy for *Helicobacter pylori* infection**

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**Background**: Bismuth-containing quadruple therapy is as an effective alternative first-line therapy for *Helicobacter pylori* infection. We evaluated an efficacy and safety of modified bismuth quadruple regimen twice a daily with low dose metronidazole (BQT-2) as a first-line therapy for *H. pylori* eradication.

**Materials and Methods**: A prospective pilot study was conducted with patients diagnosed *H. pylori*-infection and naïve to eradication. 14 days of modified BQT-2 therapy consisted of rabeprazole 20 mg, amoxicillin 1 g, metronidazole 500 mg, and tripotassium dicitrato bismuthate 600 mg (elemental bismuth 240 mg) twice a daily, given 30 minutes before morning and evening meals. *H. pylori* eradication was assessed by ¹³C-urea breath test conducted at least 4 weeks after completion of therapy.

**Results**: Of total 66 patients, the *H. pylori* eradication rate of modified BQT-2 regimen was 77.3%, and compliance rate was 100%. Metronidazole and Clarithromycin resistance rates were 30.0% and 22.0%, respectively. Eradication rate showed no significant difference between metronidazole susceptible and resistant strains (susceptible 74.3%, 26/35, and resistant 73.3%, 11/15) (P = 1.000). Most of the adverse events were mild and 20 patients (30.3%) presented nausea, epigastric soreness, loose stool, asthenia, skin rash, dizziness, taste perversion, headache, or dyspepsia.

**Conclusions**: Twice a day modified BQT-2 therapy with low dose metronidazole may be suboptimal as an alternative first-line therapy against *H. pylori*, despite of its high compliance.

Background & Aim: A survey on H. pylori resistance performed in Europe in 2018 reported resistance rates of 21% for clarithromycin, 16% for levofloxacin and 39% for metronidazole with significantly higher rates in Southern/Central vs Northern countries. We aimed to study the link between H. pylori resistance and antibiotic consumption.

Methods: Data on antibiotics for systemic use in the community (period 2008-2017) were expressed in Defined Daily Doses per 1,000 inhabitants per day (DID). The model fit and degree of ecological association between antibiotic consumption and resistance data were assessed using generalised linear mixed models based on yearly and cumulative consumption. The model with the best fit was selected by means of the Akaike information criterion.

Results: Model fit improved with cumulated years of usage, but the best fit was obtained using 2013 consumption data. Large variations in consumption were observed for macrolides, from 1.22 DID in the period 2008-2017) were expressed in Defined Daily Doses per 1,000 inhabitants per day (DID). The model fit and degree of ecological association between antibiotic consumption and resistance data were assessed using generalised linear mixed models based on yearly and cumulative consumption. The model with the best fit was selected by means of the Akaike information criterion.

Conclusion: The antibiotic consumption in the community in Europe varies widely and correlates positively with H. pylori resistance levels. Historical knowledge of macrolide and quinolone consumption in previous years provides a simple and useful tool to predict the susceptibility of H. pylori to clarithromycin and to levofloxacin and to optimise treatment strategies.

EP2.43 | Helicobacter pylori resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community

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EP2.44 | Antibiotic resistance breakers to counteract the multidrug-resistance in Helicobacter pylori

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Multidrug-resistance in Helicobacter pylori strongly stimulates the identification of new strategies improving eradication rate. Novel approaches for tackling the global antimicrobial-resistance phenomenon involve natural compounds so-called Antibiotic Resistance Breakers-ARBs expressing antibacterial/anti-virulence activities restoring the efficacy of conventional drugs. This study analysed two ARBs, Pistacia vera oleoresin-ORS and Bovine Lactoferrin-BLF, for their antimicrobial, anti-virulence action and synergistic effect when combined with Levofloxacin-LVX against resistant H. pylori strains. ORS and BLF antimicrobial/anti-virulence effect was in vitro analyzed by MIC/MBC determination, biomass quantification and twitching motility. The synergism with LVX was evaluated by checkerboard assay. In vivo studies were performed using Galleria mellonella that is a recognized experimental model for H. pylori infection, for ORS. A prospective therapeutic trial on two patient groups (treated with esomeprazole, amoxicillin, LVX plus BLF in one group), for BLF. Treatment outcome was determined by 13C Urea Breath test. In vitro ORS was able to synergize with LVX, restoring its effectiveness in LVX resistant strains. ORS, LVX and their synergistic combinations displayed significant biofilm reduction. BLF combined with LVX displayed synergistic effect for all H. pylori strains with MIC reduction until 16 and 32-fold. BLF at ¼ MIC reduced, significantly, the microbial motility. In vivo, ORS and LVX, showed protective effect against H. pylori infection on G. mellonella (62%-63% survival, respectively). In vivo, BLF added to triple therapy, achieved a therapeutic gain of 20%. ORS and BLF can be considered promising potentiators for restoring the LVX susceptibility in resistant H. pylori strains. BLF added to a triple therapy potentiates the therapeutic effect.

The effectiveness of various anti-Helicobacter therapy regimens in Ukraine

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The goal is to evaluate the effectiveness of various anti-Helicobacter therapy (AHBT) regimens in Ukraine in order to select the most optimal treatment tactics.

Object of Study and Methods: 610 patients were examined, with an average age of (50.36 ± 15.13) years. Female (330, 54.1%), Male (280, 45.9%). The most common nosology was chronic atrophic gastritis associated with Helicobacter pylori (HP) (479, 78.5%). Non Investigated Dyspepsia (0, 0.0%), Functional Dyspepsia (2, 0.3%), Duodenal Ulcer (117, 19.2%), Gastric Ulcer (14, 2.3%). Most patients (449, 73.6%) did not receive primary therapy. Patients received different anti-Helicobacter therapy regimens: Dual (2, 0.3%), Triple (325, 53.3%), Quadruple (273, 44.8%), Sequential (0, 0.0%), Hybrid (0, 0.0%), Pylera (single capsule bismuth) (0, 0.0%), Other (10, 1.6%).

Results and Discussion: The most effective eradication scheme is Quadruple with the addition of Bismuth salts of tripotassium dicitrate (BTD) lasting 14 days (92.0%), as well as schemes with the inclusion of probiotics (the most effective addition of Saccharomyces Boulardii) during AHBT with prolonged administration up to 4 weeks after the end of therapy (eradication efficiency is 90.6%). The effectiveness of Quadruple depending on the proton pump inhibitor (PPI) used was as follows: Pantoprazole (92.0%), Esomeprazole (90.0%), Rabeprazole (89.9%), Omeprazole (78.6%).

Conclusions: Ways to increase the effectiveness of AHBT in Ukraine are the addition of BTD to therapy and the duration of therapy is 14 days, the use of high doses of PPI - Pantoprazole, the addition of probiotics to treatment regimens.

Y.V. Nikiforova: None. G.D. Fadieienko: None.

Potential role of new resveratrol derivatives for the management of drug resistant Helicobacter pylori infection

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The emerging spread of multidrug-resistance Helicobacter pylori strains underlines the stringent need of novel treatments to improve the eradication. Nowadays, there is a great attention in alternative strategies based on combined synergistic effect between antibiotics and non-antibiotic compounds resulting in a potentiated effect. Resveratrol arouses great interest for its multifaceted biological and antimicrobial activities, although the in vivo application is limited for its poor bioavailability. In this study, we evaluated the antibacterial and anti-virulence effects of resveratrol and new synthesized phenol-derivatives, alone and combined with Levofloxacin used in H. pylori therapy, in areas where Clarithromycin resistance is major to 15%, against resistant clinical isolates, in vitro and in vivo studies. The antibacterial activity was determined by MIC/MBC evaluation and the synergism with Levofloxacin through the checkerboard tests. The anti-virulence action was assessed by the motility inhibition, biofilm biomass reduction and anti-quorum sensing effect. In vivo Galleria mellonella-model was used to confirm in vitro data. The results showed an increased antibacterial action of the new derivatives with lower MIC (6.25-100 μg/mL) and MBC (12.5-400 μg/mL) values in respect to the resveratrol (MIC = 400 μg/mL, MBC = 800 μg/mL) and a more interesting anti-virulence action of the detected new derivatives in respect to the lead compound, together with a synergistic effect in combination with Levofloxacin against H. pylori strains. Resveratrol and new derivatives showed protective effect against H. pylori infection on G. mellonella. Overall, the results underline the anti-H. pylori effect of resveratrol-phenol derivatives, representing interesting candidates for innovative therapeutic schemes to tackle the H. pylori antibiotic resistance.


Evaluation of antimicrobial activity against Helicobacter pylori and phytochemical analysis of extracts from Passiflora and Ilex species

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Helicobacter pylori is associated with gastroduodenal diseases and gastric cancer. Moreover, bacterial resistance to antimicrobial therapy is a major challenge in the treatment of this infection and find new therapy alternatives and new molecules are important to improve H. pylori eradication. One of these alternatives is the natural products from plant species. This study aimed to evaluate the antimicrobial activity of hydroethanolic and aqueous leaf extracts from some Passiflora and Ilex species against H. pylori. Antimicrobial activity was evaluated with agar dilution technique, following the CLSI recommendations. Reference strains of H. pylori NCTC11637 and NCTC11638 were employed, with DMSO as negative control and amoxicillin as positive control. Phytochemical analysis of the extracts was performed by HPTLC and UPLC-DAD. Our results showed a minimum inhibitory concentration (MIC) of 1000 μg/mL for P. tripartita f. mollissima and P. tarminiana hydroethanolic extracts and for both hydroethanolic and aqueous extracts from I. guayusa. The phytochemical analysis of these active extracts showed distinct chromatographic profiles for each species, although no qualitative difference was observed between hydroethanolic and aqueous extracts. C-glycosylflavonoids were observed as the main
compounds for Passiflora species. For I. guayusa, caffeine was the mayor compound, along with several phenolic acids, among them chlorogenic and caffeic acid. These results suggest an interesting activity for these species. Purification processes for active compounds are ongoing.


EP2.48 | Empiric versus clarithromycin-resistance-guided therapy for Helicobacter pylori based on polymerase chain reaction results in patients with gastric neoplasms or gastric MALT lymphoma: A randomized controlled trial

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We investigated to compare the effect of empirical therapy versus clarithromycin resistance-guided tailored therapy (tailored therapy) for eradication of Helicobacter pylori. In this prospective, single center, open-label randomized controlled trial, we enrolled 72 patients with H. pylori infection from January 2019 through June 2019 in Korea. The patients were randomly assigned to both groups received empirical \( n = 36 \) or tailored therapy \( n = 36 \). Empirical therapy was defined as triple therapy with esomeprazole, amoxicillin, and clarithromycin for 10 days irrespective of clarithromycin resistance. Tailored therapy was triple or quadruple therapy with esomeprazole, metronidazole, tetracycline, and bismuth for 10 days based on genotype markers of resistance determined by polymerase chain reaction.

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Background: Helicobacter pylori (Hp) could be implicated in the pathogenesis of gastritis, peptic ulcer disease, gastric carcinoma, and gastric lymphoma. There is increasing evidence that Hp infection induce oxidative stress in host cells and an inflammatory process that conditions an immunological response both local and systemic. These events may represent an important mechanism leading to epithelial injury in Hp infection.

Objective: The present study was aimed to evaluate the effect of a grape seed extract (GSE) and its fractions (F1 and F2) on the Hp-induced oxidative stress and inflammatory response in epithelial gastric AGS human cells.

Methods: AGS cells were pre-treated with GSE and fractions (2 mg/mL) for 2 hours before infection with seven Hp strains. Intracellular reactive oxygen species (ROS) levels were detected using a redox-sensitive fluorescent probe and pro-inflammatory IL-8 cytokine production was measured by ELISA assay.

Results: Infection of AGS cells with all Hp strains resulted in a significantly \( P < 0.05 \) increase in intracellular ROS generation and IL-8 cytokine production respect to the uninfected controls. However, when AGS cells were pre-treated with GSE and fractions, it was achieved an inhibition percentage of intracellular ROS production in a range from 29.1% to 86.7%. Similarly, the cells pre-treatments with GSE and fractions also reached a significant reduction of IL-8 secretion ranging from 37.2% to 89.6%.

Conclusions: These results suggest that GSE and fractions in the present study may prevent and/or modulate the oxidative stress and inflammatory response induced by Hp infection.

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tested via the Epsilometer test (E-test). RNA-seq was performed for the transcriptome analyses. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were used to investigate the biological functions and signal pathways.

**Results:** Growth of *H. pylori* was significantly inhibited after incubation at 41°C. At inoculation concentration of 0.002 McFarland, the bacterial colony count of the control group (37°C) was 4288.00 ± 184.16 cfu/mL. The count decreased to 2254.67 ± 574.75 cfu/mL after 3 days of 41°C treatment (*P* = 0.0043, compared with control group). After the bacteria were cultured at 41°C, the MIC value dropped from 256 to 8 µg/mL which is the breakpoint of metronidazole resistance. Transcriptome analyses revealed 583 differentially expressed genes which were enriched in substance metabolism, structural constituent of ribosome, oxidoreductase activity and oxyradical scavenging.

**Conclusions:** Culturing in vitro in 41°C can inhibit *H. pylori* proliferation by the possible effects on substance metabolism and ribosome’s structural constituent. Meanwhile, the decreased resistance of *H. pylori* to metronidazole was probably related to the changes of oxidoreductase activity and oxyradical scavenging.

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The chimeric protein CTB-multiHp: Immunological features and the ability to inhibit *Helicobacter pylori* activities

**EP2.51** | Amoxicillin secretion by gastric mucosa in patients with atrophic and nonatrophic gastritis undergoing *H. pylori* eradication therapy

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**Background:** Lower efficacy of *H. pylori* eradication in patients with gastric mucosa (GM) atrophy can be conditioned by lower intensity of antibiotics delivery to gastric lumen because of decreased number of parietal cells taking part in amoxicillin active transport.

**Methods:** 27 patients with normal GM, 12 with atrophic fundal gastritis (AFG) and 26 with atrophic antral gastritis (AAG) were included in study. Endoscopy, histological examination, rapid urease test and GastroPanel® (“Biohit”, Finland) were done in all the patients. On the first day of eradication therapy gastric secretion samples were taken via nasogastric probe 30, 60, 120, 180 and 240 minutes after oral administration of two 500 mg amoxicillin capsules. Amoxicillin concentration in samples was evaluated via liquid chromatography-mass spectrometry.

**Results:** Mean values of amoxicillin concentration in gastric secretion samples were lower (*P* < 0.01, hereinafter - Mann-Whitney U test) in AAG group (1.8 µg/mL) than in patients with normal GM (30.4 µg/mL) and AFG (17.3 µg/mL). Antibiotic concentration at the 30th and 60th minute was lower (*P* = 0.02) in patients with AFG than in patients with normal GM. Concentration at the 120th (*P* < 0.01) and 180th (*P* = 0.02) minute in AFG group was higher than in AAG group, and at the 240th (*P* < 0.01) minute than in both other groups.

Patients with AAG had lower (*P* < 0.01) concentration at the 30th, 60th and 120th minute than patients with normal GM.

**Conclusion:** AFG and AAG are characterized by decreased amoxicillin transport to gastric lumen.

A. Sabлина: None. O. Sablin: None. S. Aleksanin: None. G. Rodionov: None. I. Shantyr': None. I. Ushal: None.

**EP2.52** | The chimeric protein CTB-multiHp: Immunological features and the ability to inhibit *Helicobacter pylori* activities

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**Helicobacter pylori** is a bacterium that infects half of the population worldwide, is causes gastroduodenal diseases, and it is classified as a type I carcinogen by the World Health Organization. The most suitable strategy against *H. pylori* infection is vaccine development. CTB-multiHp is a chimeric protein that is composed of multiple B and T cell epitopes of the seven main antigens involved in the different stages of *H. pylori* pathogenesis. CTB-multiHp was designed by bioinformatics as a vaccine against *H. pylori*, however, its immune response still needs to be experimentally evaluated. In this study were assessed immunological features and the ability of CTB-multiHp antibodies to inhibit in vitro *H. pylori* activities. The experimental results indicated that CTB-multiHp raise specific antibodies that could recognize Urease B, CagA, NapA, VacA, HpaA, HspA and GGT of *H. pylori*. Also, these antibodies showed effective inhibition of urease activity, inhibition of haemagglutination, and neutralization of vacuolating cytotoxic activity of *H. pylori*. Furthermore, THP-1 cells reacted to the presence of CTB-multiHp vaccine by inducing the production of proinflammatory cytokines, in vitro analyses suggest that CTB-multiHp could induce a humoral and cellular immune response, suggesting it is a promising *H. pylori* vaccine candidate to be investigated.

B. Meza: None. F. Ascencio: None. J. Torres: None.
**EP3.01 | Application of gold nanoparticles and metal oxide sensors for non-invasive detection of gastric cancer**

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**Introduction:** Many other cancers with a high burden of the disease, including gastric cancer (GC) would benefit of a reliable non-invasive screening tool but are still lacking one. The analysis of volatile organic compound from breath samples using sensors is a potentially new way to perform GC screening method.

**Aim:** To evaluate the ability of gold nanoparticles (GNP) and metal oxide sensors (MOX) to detect GC patients.

**Methods:** Healthy individuals and GC patients were recruited for study using a POC modular breath analyser prototype, which included three modules with a different type of sensors: 8 GNP, 14 MOX digital, 8 MOX analog sensors. The ability of the sensors to differentiate gastric cancer patients from healthy individuals was compared using ANOVA test for mean values of sensor readings. The level of statistical significance was set at $P < 0.05$.

**Results:** Data from altogether 61 study subjects (41%-females and 51%-males; mean age 51) were included in the analysis, of these 36 were cancer patients. GNP sensors show the best ability to differentiate GC patients from healthy subjects: out of eight sensors, seven GNP sensors (GNP2, GNP3, GNP4, GNP5, GNP6, GNP7, GNP8) can detect GC patients ($P < 0.001$). Digital MOX sensors also are capable of detecting GC patients: out of 15 sensors, six sensors (ZMOD44101A, ZMOD44102A, ZMOD44103A, ZMOD44101B, ZMOD44102B, ZMOD44103B) showed positive result ($P < 0.05$). Meanwhile, analogue MOX sensors show that out of eight sensors, only one sensor (TGS8100) can detect disease ($P = 0.020$).

**Conclusion:** GNP and MOX sensors have shown potential to detect VOC profiles in GC patients, however, standardization of the device readings is required.


**EP3.02 | Identification of volatile organic compounds emitted by gastric juice**

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**Introduction:** Volatile organic compounds (VOCs) released by the human organism mirror normal physiological processes as well as pathological disorders. However, the main unresolved issue is the poor understanding of the sources and metabolic fate of VOCs in the human organism. This problem can be addressed via comparing volatile patterns obtained from different sources (fluids, tissues).

**Aim:** To identify volatiles being emitted by gastric juice (GJ) as an alternative source of information on potential gastric cancer (GC) markers.

**Methods:** A cohort of 10 healthy volunteers were enrolled. For VOCs analysis GJ were collected during gastroscopy. After collection, all samples were frozen. Gas chromatography with mass spectrometric detection coupled with head-space needle trap extraction as the pre-concentration technique was used to identify and quantify VOCs emitted by GJ.

**Results:** A total number of 62 compounds have been identified in the headspace of the GJ samples. Only 33 species exhibited occurrence above 20%. Amongst, them there were several hospital environment-related species (propofol, 1-propanol and 2-propanol). The predominant chemical classes were aldehydes and alcohols with 14 and 9 species, respectively. Apart from these, there were 6 ketones, 7 esters, 6 organic acids, 6 hydrocarbons, 3 aromatics and 3 heterocyclics. Only one compound (acetone) was found in all samples and further 3 (2-methyl-propanal, 2-butanal, cyclohexanone) in all samples but one.

**Conclusions:** Analysis of VOCs emitted by GJ samples provides an opportunity to identify compounds associated with a GC state. These components in turn could be targeted in breath of GC patients and thereby assist the non-invasive diagnosis of GC based on simply in use, portable breath analysers.

Host cell migration and invasion are important processes in gastric cancerogenesis that involve the concerted regulation of cytoskeletal rearrangements by signal transduction proteins such as the actin-binding protein cortactin. Various microbial pathogens subvert cortactin to promote their own uptake, proliferation and spread during infection. Here, we demonstrate that the gastric pathogen and type-I carcinogen *H. pylori* targets cortactin by inducing its tyrosine phosphorylation at Y-466 to trigger cell migration and elongation. The phosphorylation status of cortactin commands its subcellular localization and subsequently induces the recruitment of signaling partners. During infection, cortactin was discovered to undergo tyrosine dephosphorylation at residues Y-421 and Y-486 triggered by inactivation of Src kinase through the T4SS effector protein CagA. However, *H. pylori* infection activates another tyrosine kinase, Abl, which phosphorylates cortactin at Y-466. Phosphorylated cortactin then interacts with the guanine exchange factor Vav2 to stimulate its GEF activity. This specific interaction required the SH2 domain of Vav2 and phosphorylation of cortactin at Y-466. The cortactin/ Vav2 complex then stimulates the small Rho GTPase member Rac1 to trigger actin rearrangements and cell motility. Using *H. pylori* as a model system, this study unravels a previously unrecognized Rac1 activation pathway. We propose that *H. pylori* targets cortactin to locally open the gastric epithelium in order to get access to certain nutrients and thereby disturbs cellular barrier functions that could play a role in gastric disease development.

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EP3.04 | **The repeat regions of Helicobacter pylori VirB10-ortholog CagY can bind and activate toll-like receptor 5**

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*Helicobacter pylori* is an important human pathogen associated with gastric ulceration and neoplasia. It is commonly believed that the bacterium avoids immune recognition by Toll-like receptors (TLRs) because of low intrinsic activity of its flagellin and LPS, important to establish a chronic infection. In particular, TLR5 specifically detects flagellins in various bacterial pathogens, while *H. pylori* evolved mutations in flagellin to evade detection through TLR5. Cancerogenic *H. pylori* strains encode a type IV secretion system (T4SS). The T4SS core component and pilus-associated protein CagY, a large VirB10 ortholog, drives effector molecule delivery into host cells. However, CagY exhibits an additional large N-terminal domain comprising two large repeat sections with an extraordinary structural variability produced by in frame deletion or duplication events. Interestingly, the rearrangements in CagY are immune-driven by the host, sufficient to trigger gain or loss of T4SS function. However, the exact function of the repeats, and whether they are employed in direct host cell interactions is yet unknown. Here, we identified the CagY repeats as a novel flagellin-independent TLR5 agonist. We detected five TLR5 interaction sites, promoting binding of CagY-positive *H. pylori* to TLR5-expressing cells, TLR5 stimulation and intracellular signal transduction. TLR5 expression is also symptomatic for severe gastric diseases in patients. Consequently, CagY constitutes a remarkable VirB10 member detected by TLR5, driving crucial innate immune responses by this eminent human pathogen.


EP3.05 | **Overexpression of toll-like receptor 5 in H. pylori-infected patients with or without neoplasia**

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Toll-like receptors (TLRs) represent a crucial family of pathogen recognition receptors, which sense evolutionarily conserved structures in microbes. A prototypic member is TLR5, which recognizes the conserved D1-domain in flagellins produced by *Salmonella*, *Vibrio* and other pathogens. In contrast, the flagellins of *Campylobacter*, *Bartonella* and *H. pylori* are not recognized by TLR5. These data led to the hypothesis that TLR5 evasion is essential for these bacteria such as *H. pylori* to survive at mucosal surfaces in mammals. However, we examined immunohistochemically the expression of TLR5 in patients with severe gastric diseases. Remarkably, while antrum biopsies from healthy non-infected individuals showed very little or no TLR5 staining, TLR5 signals appeared upon infection and gradually increased with the grade of *H. pylori* colonization and severity of inflammation in the gastric mucosa as classified by the updated Sydney System. Staining for TLR5 was strongly upregulated in patients with *H. pylori*-associated lymphocytic gastritis, with low grade B-cell lymphoma of the MALT-type, autoimmune gastritis and, according to Laurén, the intestinal type of gastric adenocarcinoma, while the signals were significantly reduced in *H. pylori*-negative patients with diffuse type of gastric carcinoma. TLR5-staining was mainly detected in epithelial cells, granulocytes and plasma cells within the lamina propria, while goblet cells (intestinal metaplasia) were devoid of TLR5. Immunoreactive scoring according to Remmele and Stenger confirmed that TLR5 expression is significantly induced by infection and coincides with the grade of inflammation and gastric malignant
progression in patients. The importance of TLR5 overexpression during *H. pylori* pathogenesis is discussed.

S. Backert: None. C. Falkeis-Veits: None. N. Tegtmeyer: None. M. Vieth: None.

### EP3.06 | *H. pylori* chronic gastritis: OLGA and OLGIM evaluation and serum biomarkers for risk assessment of gastric cancer development in Brazilian patients

M. Coelho; H. G. Ribeiro; J. S. Alves; C. G. Gomes; A. P. Ramos; F. P. Marinho; K. S. Lima; R. I. Passos; A. J. A. Barbosa; L. G. Coelho

**UFMG, Belo Horizonte, Brazil**

**Aim:** Prospective study to evaluate the concordance rate between the OLGA and OLGIM systems, as well as to study serum biomarkers performance in patients with precancerous lesions secondary to *H. pylori* chronic gastritis.

**Patients and Methods:** Patients with histologically proven *H. pylori* chronic gastritis with precancerous lesions were recruited and endoscopic biopsies were reported by OLGA and OLGIM systems according to updated Sydney system. Blood samples were collected for biomarkers serological analysis of PGI, PGII, G17 and anti-*H. pylori* antibodies (GastroPanel®, Helsinki, Finland). The cut off values used to define high risk patients were those recommended by the manufacturer: PGI ≤30 µm/L and PGI/PGII ≤3.

**Results:** Forty-one patients, 28 women, mean age 67.3 years were recruited. See Table. The concordance rate found between the classifications was 85.4%. Considering a high risk patient as classified in at least one of the histological staging systems, the final distribution of our sample considers 24 low-risk and 17 high-risk patients for the development of gastric cancer. PGI showed a sensitivity, specificity and accuracy of 0.47, 0.67, and 0.58, respectively, while PGI/PGII showed sensitivity, specificity and accuracy of 0.06, 0.83, and 0.51, respectively.

**Conclusion:** OLGA and OLGIM systems presented a good concordance rate. Simultaneous use of both systems increased the identification of high-risk patients. Biomarker analysis was not effective in differentiating low to high risk patients.

### Distribution of 41 pts as low- and high-risk to cancer development according OLGA and OLGIM systems

<table>
<thead>
<tr>
<th>OLG0</th>
<th>OLG1</th>
<th>OLG2</th>
<th>OLG3</th>
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<tr>
<td>Patients (n)</td>
<td></td>
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</tr>
<tr>
<td>OLGA: Low Risk</td>
<td>1</td>
<td>7</td>
<td>17</td>
<td>9</td>
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<tr>
<td>25</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td>7</td>
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<tr>
<td>16</td>
<td></td>
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<td>OLGIM: Low Risk</td>
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<td>5</td>
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<td>2</td>
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</tbody>
</table>

**M. Coelho:** None. **H. G. Ribeiro:** None. **J. S. Alves:** None. **C. G. Gomes:** None. **A. P. Ramos:** None. **F. P. Marinho:** None. **K. S. Lima:** None. **R. I. Passos:** None. **A. J. A. Barbosa:** None. **L. G. Coelho:** None.

### EP3.07 | Evaluation of the nonspecific immunity in patients with gastric cancer with *Helicobacter pylori* infection

O. Smirnova; A. Sinyakov

**Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation**

**Background:** The mechanisms of the pathogenesis of gastric cancer are still poorly understood. The study of precancerous changes in the stomach is one of the most urgent tasks in the clinic of internal diseases. The aim of the study was to evaluate the chemiluminescent activity of neutrophilic granulocytes in patients with gastric cancer with *H. pylori* infection.

**Methods:** Fifty patients with gastric cancer with *Helicobacter pylori* infection were examined. The control group consisted of 50 healthy individuals. As a method for studying the activity of neutrophilic granulocytes, we used a chemiluminescent analysis of spontaneous and induced production of ROS NG in patients with gastric cancer. Statistical data processing was performed using Statistica software packages for Windows 8.0 and Microsoft Excel, 2007. Processing the obtained data included the calculation of nonparametric data. The statistical significance of the differences was determined using the Mann-Whitney test *P* < 0.05.

**Results:** In patients with gastric cancer with spontaneous and induced chemiluminescence of neutrophilic granulocytes, there was an increase in the time to reach the maximum with spontaneous and induced chemiluminescence compared to the control group. In addition, in patients with gastric cancer with spontaneous and induced chemiluminescence of neutrophilic granulocytes, there was a decrease in the area under the glow curve compared to the control group.

**Conclusions:** Patients with gastric cancer with *Helicobacter pylori* infection have been found to have a depletion of metabolic reserves and a decrease in antitumor effects, as well as a decrease in the response rate of NG in this disease.

O. Smirnova: None. A. Sinyakov: None.
EP3.08 | The role of non-specific monocytic phagocytes in the progression of gastric cancer associated with Helicobacter pylori infection

O. Smirnova; V. Tsukanov; A. Sinyakov; O. Moskalenko; N. Elmanova; E. Ovcharenko
Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation

Background: The role of the immune system in the occurrence and progression of gastric cancer associated with Helicobacter pylori infection is undeniable, while the participation of non-specific phagocytes – monocytes and macrophages in the elimination of foreign antigens is unconditional.

Objective: To study the chemiluminescent activity of monocytes in gastric cancer associated with Helicobacter pylori infection.

Methods: Forty patients with gastric cancer and 50 healthy volunteers were examined. Monocyte activity was studied by chemilumininescent methods. Statistical data processing was carried out using Statistica. The statistical significance of the differences was determined using the Mann–Whitney rank test.

Results: In patients with gastric cancer, a decrease in the maximum intensity of luminescence of monocytes in spontaneous and induced states relative to the control group was detected. The time to reach the maximum luminescence of monocytes increased in the induced state, and the area under the curve of chemiluminescence of monocytes increased in spontaneous and induced states relative to the control group.

Conclusion: In patients with gastric cancer associated with Helicobacter pylori infection, a decrease in monocyte activity is detected due to the duration of the disease and the toxic effects of the tumor products. The inefficiency of phagocytosis of monocytes leads to the spread of the tumor. The project “Development and implementation of a program for screening and early diagnosis of gastric cancer by indicators of the immune, prooxidant and antioxidant systems to reduce mortality and disability” was funded by Krasnoyarsk Regional Fund of Science.

O. Smirnova: None. V. Tsukanov: None. A. Sinyakov: None. O. Moskalenko: None. N. Elmanova: None. E. Ovcharenko: None.

EP3.09 | Features of lipid peroxidation and antioxidant protection in gastric cancer associated with Helicobacter pylori infection

O. Smirnova; A. Sinyakov
Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation

Background: Gastric cancer in Russia ranks 2nd in the structure of mortality from malignant diseases. Helicobacter pylori infection induces a mutation of genes that trigger cell apoptosis in genetic disorders associated with malignant transformation, affects the lipid peroxidation and antioxidant defense genes.

Objective: To study the content of malondialdehyde and the activity of superoxide dismutase and catalase enzymes in gastric cancer associated with Helicobacter pylori infection.

Methods: Forty patients with gastric cancer and 50 healthy volunteers were examined. In the blood serum, the content of malondialdehyde, the activity of superoxide dismutase and catalase enzymes were determined by spectrophotometric methods. Statistical data processing was carried out using Statistica. The statistical significance of the differences was determined using the Mann–Whitney rank test.

Results: In patients with gastric cancer, showed 50-fold increase in the content of malondialdehyde relative to the control group, indicating significant oxidative stress in patients. However, there was a decrease in the activity of superoxide dismutase and an increase in catalase activity in patients with gastric cancer relative to the control group.

Conclusion: Patients with gastric cancer associated with Helicobacter pylori infection exhibit severe oxidative stress. An imbalance is detected in the bifunctional system of superoxide dismutase – catalase, the activity of the main enzyme of the antioxidant defense superoxide dismutase is reduced, while the high activity of catalase indicates massive cell decay in gastric cancer, and it can be assumed that in conditions of high catalase activity, cells lining blood vessels are subject to significant oxidative stress.

O. Smirnova: None. A. Sinyakov: None.

EP3.10 | Peculiarities of glutathione antioxidant protection functioning in gastric cancer associated with Helicobacter pylori infection

O. Smirnova; A. Sinyakov
Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation

Background: Up to 800,000 new cases of gastric cancer are diagnosed each year in the world. Gastric adenocarcinoma remains in second place among oncological diseases leading to death, the risk of developing gastric cancer in infected patients is 2-4 times higher than in the rest. The pathogenesis of cancer involves lipoperoxidation and antioxidant defense.

Objective: To study the state of the processes of lipid peroxidation and the glutathione link of antioxidant defense in gastric cancer associated with Helicobacter pylori infection.

Methods: Forty patients with gastric cancer and 50 healthy volunteers were examined. The content of malondialdehyde, reduced glutathione, the activity of glutathione-S-transferase and glutathione peroxidase were determined in the blood serum by spectrophotometric methods. Statistical data processing was carried out using Statistica. The statistical significance of the differences was determined using the Mann–Whitney rank test.
**Results:** In patients with gastric cancer there was an increase in the concentration of malondialdehyde in plasma compared with the control group. The concentration of glutathione-S transferase and glutathione peroxidase in plasma in patients with cancer increased compared with the control group. The level of restored glutathione in patients with gastric cancer was significantly higher than in all other studied groups.

**Conclusion:** In patients with gastric cancer, lipid peroxidation processes are enhanced, proven by an increase in serum malondialdehyde. The high activity of the enzymes of glutathione link and the high content of glutathione prove an increase in the activity of the antioxidant system aimed at combating oxidative stress.

O. Smirnova: None. A. Sinyakov: None.

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Alaska Native (AN) people have higher gastric cancer (GCAn) incidence and mortality rates compared to other U.S. populations. Using an AN-specific cancer registry, we evaluated the trends in GCAn between 1988 and 2017. Data were obtained from the Alaska Native Tumor Registry, a population-based central cancer registry that captures all cancer in American Indian/AN people living in Alaska. Rates were calculated for 3-year time periods and age-adjusted using the 2016 Alaska census as the standard. The trend across time periods was analyzed using Poisson regression. A total of 429 cases of GCAn occurred in the AN/AN population between 1988 and 2017. The age-adjusted rate ranged from a high of 26.9 per 100,000 persons in the 1991-1993 period to a low of 14.2 in 2015-2017 period, however no significant trend was found over the 30 year period. The mean age of people diagnosed with GCAn was 59.6 years, with 11.4% <40 years old; 63.4% of cases were male. The majority of cancers were classified as adenocarcinoma without additional subtyping (53.6%). The most commonly defined subtype was signet-ring cell (SRC) (18.2%). Tumors were more often SRC in people <40 years (35.0%) compared to people ≥40 years (16.1%, P < 0.00001). These data show rates of GCAn in the AN population from 1988 to 2017. Individuals <40 years were more likely to have SRC tumors, a histology that is typically much more aggressive.


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**EP3.12 | Gastric cancer risk factors due to lifestyle**

O. V. Shtygasheva1; E. S. Ageeva2

1Katanov Khakass State University, Abakan, Russian Federation; 2Medical Academy named after S.I. Georgievsky of Vernadsky CFU, Simferopol, Russian Federation

**Aim:** To study frequency and expression of vital risk factors in patients with gastric cancer (GC). Material and Method. A survey of patients of the Republic of Khakassia with a 1st diagnosis of GC (2018-2019) was conducted. We evaluated the smoking index (IS, packs/year), alcohol, nutrition.

**Results:** The smoking has been established in 45% of GC patients. Among man 36% were “heavy smokers,” 23% were “unconditional smokers.” A relationship was established between the male gender and the number of cigarettes smoked (r = 0.51, P = 0.02). 100% of respondents confirmed a history of alcohol consumption, significant alcohol more 50 g/day - 27% of men and 17% of women. Smoking history and IS are interrelated with frequent alcohol consumption (r = 0.72 and r = 0.70, P = 0.05) in men. In women, a directly proportional relationship was found between IS and alcohol (r = 0.83, P = 0.03), long time smoking (r = 0.86, P = 0.05), and number of cigarettes (r = 0.84, P = 0.04). Excessive consumption of salt and red meat (more 30 g/day) and canned foods was confirmed in 85% of patients. The male was interrelated with the frequency of consumption of salted and fried (r = 0.58, P = 0.04) and smoked and fried foods (r = 0.63, P = 0.04). Factor analysis to distinguish two groups. The 1st group of factors includes gender (r = 0.7, P = 0.04), smoking experience (r = −0.92, P = 0.05), the number of cigarettes smoked (r = −0.95, P = 0.04), IS (r = −0.91, P = 0.05), alcohol (r = −0.75, P = 0.03). The 2nd is the frequent use of frying food (r = −0.83, P = 0.04).

**Conclusion:** The premorbid and primary disease prevention system are elimination of cancer-related predictors of lifestyle.

E.S. Ageeva: None. O.V. Shtygasheva: None.

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**EP3.13 | Diffuse type gastric cancer is associated with high titer of anti-Helicobacter pylori antibody**

J. Lim1; S. Cho2; S. Kim2; J. Kim2

1Seoul National University Hospital, Healthcare System Gangnam Center, Healthcare Research Institute, Seoul, Republic of Korea; 2Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea

**Introduction:** The implication of serum anti-Helicobacter pylori antibody titer on the pattern of carcinogenesis is rarely investigated. This study is designed to evaluate the effect of anti-H. pylori IgG titer on the development of each type of gastric cancer.

**Methods:** We retrospectively reviewed prospectively collected cohort of health screening population who underwent regular upper gastrointestinal endoscopy and laboratory tests including serum anti-H. pylori IgG between October 2003 and December 2018.
Results: Among the total of 12,4794 individuals who had screening endoscopy and test for serum anti-*H. pylori* IgG antibody, 63,235 individuals with positive result in anti-*H. pylori* IgG were enrolled. High titer was defined as the IgG greater than the upper limit. Overall, 18,603 showed high titer of IgG. High titer group developed gastric cancer less commonly compared with low titer group (log Rank P-value <0.001). However, among those who developed gastric cancer, high titer group had more patients with diffuse type cancer than low titer group (51.8% vs 38.7%, P = 0.032).

Conclusions: Low titer of anti-*H. pylori* IgG is related with high rate of gastric carcinogenesis. However, those with high titer have a tendency to develop diffuse type gastric cancer more commonly than those with low titer.

J. Lim: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant: Seoul National University Hospital. S. Cho: None. S. Kim: None. J. Kim: None.

EP3.14 | Aberrant MUC13 expression as prognostic marker for gastric cancer

B. Oosterlinck; T. Breugelmans; J. De Man; K. Geboes; J. Kupčinskas; A. Link; B. De Winter; A. Smet

1University of Antwerp, Antwerp, Belgium; 2Ghent University Hospital, Ghent, Belgium; 3Lithuanian University of Health Sciences, Kaunas, Lithuania; 4Otto-von-Guericke University, Magdeburg, Germany

Background: One of the hallmarks of gastric adenocarcinoma is aberrant mucin expression. Gastric- and intestinal-type mucins are widely expressed in gastric tumours, but their clinical importance is still controversial in relation to disease progression and outcome. Here, we investigated mucin expression in human gastric adenocarcinomas and their correlation with disease outcome.

Methods: Gastric biopsies of tumour and adjacent tissue of two independent cohorts of Belgian (n = 45) and Lithuanian (n = 43) patients were analysed for mucin expression. Relative expression of gastric (MUC1, MUC5AC and MUC6) and intestinal mucins (MUC2, MUC4 and MUC13) was determined by RT-qPCR. The adenocarcinomas were classified by expression leading to gastric (predominantly MUC5AC and MUC6), intestinal (predominantly intestinal and MUC1), mixed (all types) and unclassified/null (neither gastric nor intestinal) mucin phenotypes. The overexpression threshold was defined as a 0.2*MNE increase (mean normal tissue expression) in the tumour compared to normal tissue. Correlation with 5-year survival (Kaplan-Meier analysis) and other clinical traits (tumour stadia, histology, age, gender, …) was tested.

Results: Our analysis classified the cancers as gastric (14.5%), intestinal (25.0%), mixed (17.1%) and unclassified (43.4%) mucin phenotypes. MUC13 overexpression was observed in 55.3% of the cases. Interestingly, 30% of the complete cohort -intestinal and mixed type- had a MUC13 expression exceeding a 1.75*MNE threshold. This correlated with decreased survival (P = 0.017, log-rank test). For the other mucins tested, this association could not be established.

Conclusion: Our results highlight a key role for MUC13 in gastric cancer progression and survival. More research is required to understand its exact mechanism.


EP3.15 | Cost-effectiveness of *Helicobacter pylori* screening and eradication to prevent stomach cancer in Spain

J. López-Saavedra; E. Martínez-Junquera; L. Fontan; M. López-Torreblanca; I. Lorenzana-Sánchez; T. Alarcón

1Autonomous University of Madrid, Madrid, Spain; 2Hospital Univ. La Princesa, Madrid, Spain

Introduction: *Helicobacter pylori* is believed to cause up to 69% of stomach cancers (SC). SC is the seventh most deadly in Spain, with its prevalence increasing in population over 50 years. Eradication of *H. pylori* has been proposed as a prevention strategy against SC. The aim of this study is to compare the costs of *H. pylori* screening and treatment with the costs derived from SC in the Spanish National Health System (SNHS).

Methods: We designed a Markov model with cohort simulation to evaluate the cost-effectiveness of *H. pylori* screening and eradication. A probabilistic sensitivity analysis was performed by applying the Monte Carlo simulation with 1000 iterations using Microsoft Excel 365. The protocol consisted of a Stool Antigen Test (SAT), a confirmation C13-Urea Breath Test (UBT), and a quadruple 14 days eradication therapy with rabeprazole, clarithromycin, amoxicillin and metronidazole. The model compared this protocol (Scenario 1) with no screening or treatment for *H. pylori* (Scenario 2).

Results:

<table>
<thead>
<tr>
<th></th>
<th>Scenario 1</th>
<th>Scenario 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average cost</td>
<td>7,234.55€</td>
<td>20,182.66€</td>
</tr>
<tr>
<td>Average of QALYs gained</td>
<td>20.75</td>
<td>14.9</td>
</tr>
</tbody>
</table>

The average Incremental Cost-Effectiveness Ratio (ICER) amounted to ~2,224.67 €/QALY (Quality-Adjusted Life Year) and a consequent probability of cost-effectiveness of 100% at any cost-effectiveness threshold.

Conclusion: This analysis support the hypothesis that, through the screening and treatment for *H. pylori* above 50 years old, SC can be prevented and health costs for the SNHS can be reduced. However, the analysis has some limitations and more studies are needed.

EP3.16 | In vitro profiling of volatile organic compounds released and consumed by CLS-145 and HGC-27 gastric cell lines

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Introduction: Analysis of volatile organic compounds (VOCs) released by human body provides an emerging approach for cancer screening. The metabolism of cancer cells differs from that of normal ones and this difference can be detected in the VOCs profiles. In this context in vitro studies are of considerable importance for the explorations of these changes in exhaled breath and other body emanations.

Aim: The main aim of this study was to pinpoint the volatile metabolic signature of selected gastric cancer cell lines and detect possible differences between these fingerprints.

Methods: Gas chromatography with mass spectrometric detection (GC-MS) and head-space needle trap extraction (HS-NTE) as the pre-concentration method were applied to profile VOCs produced and metabolized by two Human Gastric Carcinoma cell lines (CLS-145, HGC-27) and Human Stomach Epithelial Cells (HSEC), the latter serving as a control.

Results: In total 12 sets of cultures (three cultures containing different lines and medium without cells) were prepared. Twelve species (2-methylpropanal, 2-methyl-2-propanal, 2-methylbutanal, 3-methylbutanal, hexanal, heptanal, nonanal, benzaldehyde, 2-ethyl-furan, 2-pentyl-furan and 2-methyl-furan and dimethyl disulfide) were found to be consumed and ten (ethyl acetate, ethyl propanoate ethyl-α-methylbutyrate, 2-pentanone, 2-heptanone, 2-nonanone, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-ethyl-1-hexanol and 2-methyl-3-(methylthio) furan) were produced by all lines under study. Interestingly, HGC-27 cells emitted a number of unique VOCs (2-undecanone,2-tridecanone, 2-pentadecanone, 2-nonadecanone, cyclopentadecanone and toluene).

Conclusions: The results obtained within this study provide evidence that gastric cancer alters the VOCs profiles of cell lines under study. The components of these fingerprints secreted from human organism via e.g. breath could assist in the non-invasive diagnosis of gastric cancer employing sensitive mobile platforms.

EP3.18 | Endoscopic biopsy sampling during gastroscopy – Discrepancies across Europe

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Background: National and international guidelines give robust recommendations on which biopsies should be taken in the presence of endoscopic signs of gastric inflammation. This study aimed to give an overview on current practice in tertiary referral centres across Europe.

Methods: Data was collected at ten tertiary referral centres across Europe. Demographic data, the indication for each procedure, and endoscopic findings were recorded as well as what biopsies were taken. Findings were compared between centres and factors influencing the decision to take biopsies were explored.

Results: A total of 9425 gastroscopies were analysed. Biopsies were taken in 56.6% of procedures with significant variation between centres (P < 0.001). Interestingly, fewer biopsies were taken in centres routinely applying the updated Sydney classification for gastritis assessment (46.0%) compared to centres where this was done only upon direct request (75.3%, P < 0.001). Similar results were documented for centres stratifying patients according to the OLGA
system (51.8% vs 73.0%, \( P < 0.001 \)). More biopsies were taken in centres following the MAPS guidelines on stomach surveillance (68.1% vs 37.1%, \( P < 0.001 \)). Although the patients’ age had no influence on the decision to take biopsies in the whole cohort (\( P = 0.537 \)), in 8 centres biopsy sampling was significantly more likely in younger patients (\( P < 0.05 \)). Anticoagulatative therapy had no statistical influence. The percentage of procedures with biopsies correlated with additional costs per procedure in case of biopsies at the respective centres (\( r = 0.709, P = 0.022 \)).

**Conclusion:** Adherence to guideline recommendations for biopsy sampling at gastroscopy seems inconsistent across the participating centres. Our data suggest rather centre-specific policies.


### EP3.19 | Impact of *Helicobacter pylori* infection and its virulence factor CagA on DNA damage repair machinery

**E. Kontizas** 1,2, **S. Tastsoglou** 1, **T. Karamitros** 3, **Y. Karayiannis** 1,2, **P. Kollias** 3, **A. Hatzigeorgiou** 3, **A. Mentis** 3, **D. N. Sgouras** 3

1Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece; 2Department of Genetics and Biotechnology, Faculty of Biology, School of Science, National and Kapodistrian University of Athens, Athens, Greece; 3Laboratory of Bioinformatics, Hellenic Pasteur Institute, Athens, Greece

*Helicobacter pylori* (*Hp*) infection promotes chronic inflammation and plethora of DNA damages including strand breaks, base alterations, point mutations, microsatellite instability and epigenetic alterations. The gastric epithelial cells, in order to prevent genomic instability, require an integrous DNA damage repair (DDR) machinery which, however, has been reported to be modulated by the infection. CagA is a major *Hp* virulence factor that deregulates host cell functions such as proliferation, apoptosis and chromosomal integrity. Its pathogenic activity is partly regulated by tyrosine phosphorylation on repeated EPIYA-motifs. Our aim was to identify putative effects of *Hp* infection and CagA on DDR-machinery, investigating the transcriptome of AGS cells, infected with wild-type, ΔCagA and EPIYA-phosphorylation-defective strains. Upon RNA-Sequencing on polyA-selected transcripts we performed Differential-Expression-Analysis, Pathway-Enrichment-Analysis and visualization on KEGG-Pathway-Maps per DDR-mechanism. Key DDR-components that were observed to be downregulated in a CagA-related manner were validated via Western-Blot utilizing AGS and the non-cancerousGES1 cell lines. Transcriptome analysis revealed that a notable number of DDR-genes were downregulated during *Hp* infection resulting to potential modulation of Base-Excision-Repair, Mismatch-Repair and a more intricate deregulation of Nucleotide-Excision-Repair and Homologous-Recombination. CagA contributes to NTHL1, MUTYH, FEN1, APE1, POLD1, LIG1 and RAD51 downregulation and those observations were verified on the protein expression level, with the exception of APE1 that was on contrary observed to be upregulated. Our study accentuates the role of CagA, as a significant contributor of the *Hp* infection-mediated DDR-modulation, disrupting the balance between DNA damage introduction and repair thus favoring genomic instability and potentially contributing to gastric carcinogenesis.

D.N. Sgouras: None. E. Kontizas: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Work was partly supported by the I. Kampouris Donation for Research Program Cancer & EMMPRIN of the Hellenic Pasteur Institute. S. Tastsoglou: None. T. Karamitros: None. Y. Karayiannis: None. P. Kollias: None. A. Hatzigeorgiou: None. A. Mentis: None.

### EP3.20 | The role of lactate in gastric carcinogenesis

**K. Vinasco-Pacheco;** H. Mitchell; N. Kaakoush; N. Castano-Rodriguez

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**Introduction:** As lactate is involved in several hallmarks of cancer, microbially derived lactate could be a pivotal contributing factor in non-cardia gastric cancer (GC). Here, we investigated the impact of important genes involved in lactate metabolism in a high-risk ethnic Chinese population, in which we have previously shown lactic acid-producing bacteria (LAB) enrichment to be associated with GC, and a low-risk Caucasian population.

**Methods:** Functional germline polymorphisms in MCT1 (rs1049434), MCT2 (rs1000708, rs3763980, rs7976956, rs995343) and MCT4 (rs9907757) were detected by MALDI-TOF mass spectrometry in 302 ethnic Chinese (B6 GC/216 controls) and 355 Caucasians (119 GC/236 controls). RNA was extracted from gastric biopsies from ethnic Chinese and reverse transcribed to cDNA for qRT-PCR of MCT1, MCT2, MCT4, and HCAR1.

**Results:** In the Caucasian population, the MCT4 rs9907757 C allele significantly increased the risk of GC by 2-fold (OR: 2.190, 95% CI: 1.14 to 4.22). In the Chinese population, the MCT4 rs9907757 C allele might increase the risk of GC (OR: 1.67, 95% CI: 0.81 to 3.43). qRT-PCR analyses revealed that MCT1, MCT2, MCT4, and HCAR1 were downregulated in GC patients were compared to controls.

**Conclusions:** MCT4 rs9907757 appears to be associated with GC in Caucasian and ethnic Chinese individuals. The expression levels of MCTs and HCAR1 were differentially expressed in GC patients from a high-risk population. These same individuals were previously shown to present with LAB enrichment in their gastric mucosa. Overall, these results indicate an important role for lactate in gastric carcinogenesis, some of which could be microbiobly derived.

EP3.21 | ER stress and inflammation via autophagy influence Helicobacter pylori-related gastric cancer

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Background: Disrupted autophagy has been linked to increased susceptibility to infection and cancer. Polymorphisms in autophagy-related genes, such as ATG16L1 rs2241880, have been shown to deregulate the autophagic pathway, impairing pathogen clearance and increasing intestinal endoplasmic reticulum (ER) stress. Here, we investigate the role of this SNP in H. pylori-mediated gastric cancer (GC) and its molecular pathways, in Dutch, Australian and Colombian populations.

Methods: ATG16L1 rs2241880 was genotyped in subjects presenting with gastric precancerous lesions, GC patients and controls. The functional relevance of ATG16L1 rs2241880 on H. pylori-mediated IL-8 induction and ER stress, was determined using CRISPR/Cas9 modified cell lines and organoids. Expression of important markers of ER stress (GRP78, CHOP1 and spliced XBP1 mRNA) were assessed in gastric tissues.

Results: The ATG16L1 rs2241880 G-allele increased the risk of severe gastric precancerous lesions and cancer in the studied populations. In vitro models demonstrated that H. pylori reduces ER stress via autophagy, which appears to be influenced by the ATG16L1 rs2241880 genotype. Similarly, IL-8 production is dramatically increased in cells homozygous for the ATG16L1 rs2241880 G-allele.

Conclusions: The ATG16L1 rs2241880 G-allele is associated with gastric carcinogenesis in diverse ethnic groups with varying degrees of GC risk. Altered H. pylori-induced ER stress pathways and pro-inflammatory mediators, are some of the mechanisms involved.

EP3.22 | Leukaemia Inhibitory Factor signalling for targeting cancer stem cells in gastric adenocarcinoma

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Gastric cancer (GC) is a bad prognosis disease with high number of relapse cases and 5-year survival rate of less than 20%. Cancer stem cells (CSCs), making the heterogeneous architecture of gastric tumours, present chemo-resistance mechanisms contributing to tumour maintenance and recurrence. Their targeting in cancer therapy thus seems of utmost importance. The Hippo pathway has recently been implicated in gastric CSC properties and its regulation by Leukaemia Inhibitory Factor Receptor (LIFR) and its ligand LIF has been described in breast cancer. Consequently, this study aimed to determine the effect of LIF on CSC phenotype and properties in GC cell lines and patient derived xenograft (PDX) cells, where it has not been investigated yet. LIF treatment decreased tumoursphere forming capacity, an important tumorigenic property, of both GC cell lines and PDX cells. In addition, LIF increased activation of LATS1/2 Hippo kinases thereby decreasing downstream YAP/TAZ nuclear accumulation, TEAD transcriptional activity and target gene expression in our models. LIF anti-CSC effects were reversed by Hippo kinase inhibition but not by STAT3 inhibition, highlighting the role of the Hippo pathway in LIF-induced anti-tumourigic properties. Furthermore, KMplot database analysis show that low LIF and LIFR expression is associated to patients’ survival underlining the interest of LIF as potential therapy. In conclusion, this study displays LIF anti-CSC properties in GC, linked to activation of Hippo kinases LATS1/2. This could in fine lead to the development of targeted strategies against CSCs and help decrease the number of relapse cases and bad prognosis in gastric cancer.

EP3.23 | Cell-free DNA somatic alterations in plasma of gastric cancer patients: Mutational spectra, association with tumor size and survival

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Since the discovery of the circulating plasma cell-free DNA (cfDNA) it was shown that cfDNA could harbour genetic aberrations from malignant tissue. However, there is a lack of studies conducted in gastric cancer (GC) analysing cfDNA mutational spectra. The aim of study was to compare GC tissue and cfDNA mutational profiles and analyse associations with different clinical features, levels of oncogenes. GC tissue and blood were collected from 29 patients who were recruited at the Department of Gastroenterology, Lithuanian University of Health Sciences Hospital. Whole exome sequencing was performed for GC patients’ tissue and paired WBC samples, while targeted NGS consisting of 38 cancer-associated genes was performed for cfDNA only. After WES analysis, in total for 23 of 29 patients (76.7 %) mutations associated with GC were detected. Matching tissue cfDNA alterations were detected for 14 of 23 (60.9 %) GC patients in genes: TP53, ERBB2, FAT1, MLH1, FAT4, SPEN, KMT2C, MUC16, ACVR2A, ERBB4, PREX2, and SYNE1. Matching tumour and plasma alterations were detected significantly more often in samples of the patients with larger tumours (55.6 % and 10.0%, T3-T4 and T1-T2 respectively, $P = 0.018$). Kaplan-Meier survival analysis revealed that detectable alterations in cfDNA or number of mutations could be related to overall survival ($P > 0.05$).

Our results show, that tissue and cfDNA matching mutations were mostly detected for GC patients with larger tumours and may enable cfDNA analysis for monitoring of the GC patients’ disease state. Supported by the grant from Research Council of Lithuania No. LMT-K-712-01-0130_MULTIOMICS.


EP3.24 | Induction of precancerous phenotype of normal gastric epithelial cells after their long-term incubation with supernatant from Helicobacter pylori-infected cancer-associated fibroblasts in vitro. Involvement of transforming growth factor beta and its receptors

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The $H. pylori$-induced inflammatory reaction may lead to a cascade of pathogenic events resulting in development of chronic gastritis, gastroduodenal ulcers and cancer. This is illustrated by deregulation of gastric tissue cells self-renewal process by cancer-associated fibroblasts (CAFs) playing the crucial role in process of epithelial-mesenchymal transition (EMT). We reported that EMT process initiated by $H. pylori$-induced CAFs has changed the phenotype of gastric epithelial RGM-1 cells. Here the long-term (l.t.) incubation of RGM-1 cells with supernatant collected from $H. pylori$-infected CAFs has lead to a cascade of EMT cell transformation. The enhanced proliferation and phenotypical plasticity was observed in these cells compared with long-term $H. pylori$ non-infected supernatant which presented stable epithelial myofibroblast transition EMMyoT of epithelial cells (Lgr+ /Oct4+/Sox2+/c-Myc+/Klf4- pattern). The enhanced proliferation of l.t.EMT +RGM-1 under $TGF\beta$ stimulation can regulate an expansion of pro-invasive RGM-1 cells. The secretome of $H. pylori$-reprogrammed fibroblast prompts the enhanced proliferation of l.t.EMT-MGC-1 under $TGF\beta$ stress, thus potentially facilitating $H. pylori$-induced neoplasia. $TGF\beta$R1/2 activation may regulate an expansion of pro-invasive RGM-1 cells. Residual $TGF\beta$R1 activity along with $TGF\beta$R2 may negatively affect the proliferation of l.t.EMT-MG-1 fibro cells.

The secretome of $H. pylori$-infected fibroblasts prompts the enhanced proliferation of l.t.EMT-MG-1 under $TGF\beta$ stress, thus potentially facilitating $H. pylori$-induced neoplasia. $TGF\beta$R1/2 activation can regulate an expansion of pro-invasive RGM-1 cells. Residual $TGF\beta$R1 activity along with $TGF\beta$R2 activation may negatively affect the proliferation of l.t.EMT-MG-1 fibro cells. Therefore, regulating their "stromal" phenotype. We propose that $H. pylori$ infection activating normal fibroblasts towards CAFs results in induction of normal epithelial cells changes towards precancerous phenotype mediated by $TGF\beta$ influence initially by $TGF\beta$-dependent EMT induction and subsequently by limiting $TGF\beta$ signaling. Our study seems to underline the importance of proper balance of $TGF\beta$ signaling at different stages of tumor development.

EP3.25 | Microbial abundance of *H. pylori* assessed by high-throughput sequencing and prognosis of gastric cancer patients

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**Introduction:** Microbiome is increasingly recognized as a crucial factor in gastric cancer development. Certain bacteria types may impact prognosis of cancer patients, however, little is known on prognostic role of *Helicobacter pylori* (*H. pylori*) Infection in gastric mucosa of gastric cancer patients using high throughput sequencing analysis. In this work, we studied the prognostic role of microbial alteration and in particular *H. pylori* infection in gastric cancer patients.

**Materials and Methods:** DNA from well-characterized paired gastric cancer and adjusted non-tumorous tissue were obtained from 64 gastric cancer patients. Region V1-V2 of the 16S rRNA gene was amplified by PCR and barcoded for high throughput sequencing using Illumina platform. Clinical and pathological characteristics as well as survival data were available for follow up period of up to 7 years.

**Results:** After normalization, roughly 5000 reads per samples were obtained and taxonomically annotated. Overall, cancer and adjacent non-tumorous tissues showed microbial differences among, which was primarily determined by *H. pylori* status. *H. pylori* status was not associated with a distinct tumour characteristics or tumour pathology. Mucosal *H. pylori* positivity had no association with differences in overall survival of patients with gastric cancer.

**Conclusion:** Although *H. pylori* plays a key role in gastric cancer development, *H. pylori* positivity of the tumour tissues is not associated with a distinct prognostic phenotype. Further research is process to unravel the prognostic role of the other microbial communities in gastric tumour patients.

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EP3.26 | *Helicobacter pylori* and Epstein-Barr virus co-infection in Greek patients with gastric malignancies

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**Background/Aim:** Gastric cancer development has been associated with infection of *Helicobacter pylori* (*H. pylori*) and Epstein-Barr virus (EBV). Prevalence of *H. pylori* and EBV associated gastric cancer and *H. pylori*-EBV co-infection rates vary worldwide. We aimed to evaluate the frequency of EBV infection in patients diagnosed with gastric cancer, with and without *H. pylori* infection.

**Methods:** Gastric biopsies (n = 56) from patients endoscopically suspected for presence of gastric neoplasia were included. *H. pylori* infection and clarithromycin resistance status was analyzed by RT-PCR targeting the 23S rRNA gene of *H. pylori*, while the encoded small ribonucleic acid 1 (EBER 1) fragment region of the EBV genome was used to evaluate presence of the virus.

**Results:** Histopathology characterized cases as peptic ulcer disease (n = 11) and gastric cancer (n = 45). Mean age was 72 ± 11.8 y.o., while males predominated 71.4% (40/56) overall. *H. pylori* presence was detected in 35.6% (16/45) gastric cancer cases and 35.7% (20/56) overall, whereas EBV was detected in 20% (9/45) of the patients with gastric cancer and 17.9% (10/56) overall. *H. pylori*-EBV co-infection was detected in 11.1% (5/45) gastric cancer and 9% (1/11) peptic ulcer cases. Among patients positive to *H. pylori*, 5 strains (25%) were clarithromycin-resistant with the most characteristic mutation at position A2146G in the 23S rRNA gene.

**Conclusion:** An overall 20% presence of EBV in gastric biopsies from gastric cancer cases was detected supporting its association with gastric carcinoma development. Further investigation are warranted to comprehend the role of *H. pylori*-EBV co-infection in gastric malignant disease development.


EP3.27 | Increasing trend of gastroesophageal junction in the primary site of endoscopic resected gastric cancers

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**Background & Aims:** The frequency of cardiac cancers seems to increase in Eastern countries as well as Western. The aim of this study
was to investigate the incidence rate, including cardia dysplasia as well as cardia cancer, and to analyze the clinical and pathological characteristics.

**Methods:** We reviewed medical records, endoscopic findings and pathological characteristics of patients who had undergone endoscopic resection at University Hospital from 2005 to 2019. To compare the gastric atrophy, we confirmed the pepsinogen I/II ratio, Kimura-Takemoto classification and Sydney classification. We have defined gastroesophageal (GE) junction as the distal end of palisade vessels. The location of tumor epicenter within 1 cm from GE junction was classified as group A, within 1–2 cm as group B, 2–5 cm as group C.

**Results:** Of the 4,029 patients we identified, 120 (3.0%) had cardia cancers (Group A, B and C), and 276 (6.9%) had cardia neoplasm including dysplasia. The mean age of people with cardiac cancer was 64 ± 11 years old. The proportion of cardiac cancer increased over time (2005-2009, 2.2%; 2010-2014, 2.5%; 2015-2019, 3.6%; P<0.05). This increase was mainly associated with an increase of group A. Age, sex and smoking history did not differ among groups, but obesity was significantly higher in group A. Endoscopic atrophy was more common in group B and C. Closed-type of atrophy was 47% in the group A. Pepsinogen I/II ratio of group A was higher compared to group B and C. The rate of Helicobacter pylori infection was also significantly lower in group A.

**Conclusions:** Recently, the incidence of cardia cancer and dysplasia has increased in Korea. The increase in cardiac cancer and dysplasia was associated with an increase in neoplasm within 1 cm from GE junction. Cardia cancer and dysplasia were associated with obesity and without atrophy (non-Helicobacter pylori related).

Y. Choe: None. M. Choi: None. S. Lee: None.

**EP4.02 | The actin-binding protein cortactin is required for efficient AGS cell elongation by H. pylori CagA, but not for vacuolization and apoptosis by VacA**

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Cortactin is an actin-binding protein and actin-nucleation promoting factor regulating cytoskeletal rearrangements in eukaryotes. Thus, cortactin represents an attractive target for pathogens to manipulate a given host cell to their own benefit. One of the pathogens following this strategy is the gastric pathogen *H. pylori*. During infection of gastric epithelial cells, *H. pylori* hijacks multiple cellular kinase signaling pathways, leading to the disruption of key cell functions, in which the vacuolating cytotoxin VacA and translocated effector protein CagA play important roles. Specifically, by overruling the phosphorylation status of cortactin, *H. pylori* alternates the activity of molecular interaction partners of this important protein, thereby manipulating the performance of actin-cytoskeletal rearrangements and cell movement. Based on siRNA knockdown and other studies, it was recently reported by various groups that VacA utilizes cortactin for its cellular uptake, intracellular travel and induction of apoptosis by a mitochondria-dependent mechanism. To investigate this phenomenon, we produced a complete knockout mutant of cortactin in two cell lines, AGS and Caco-2, by CRISPR-Cas9 technology. These cells were infected with *H. pylori* wild-type or isogenic vacA and cagA mutant strains. Unexpectedly, the functional inactivation of cortactin did not prevent the uptake and formation of VacA-dependent vacuoles, nor the induction of apoptosis by internalized VacA, while the induction of the CagA-dependent AGS cell elongation phenotype was strongly reduced through suppression of a unique mechanism of cortactin to activate focal adhesion kinase. Thus, we provide evidence that cortactin is required for the function of internalized CagA, but not VacA.

N. Tegtmeyer: None. F. Fiedler: None. S. Backert: None. J. Knorr: None.
ABSTRACTS

EP4.03 | Type IV secretion of *Helicobacter pylori* CagA and ADP-heptose into oral epithelial cell lines

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*Helicobacter pylori* typically colonizes the human stomach, but the bacteria can occasionally be detected in the oral cavity. Clinical outcome during gastric colonization depends on presence of the *cag* pathogenicity island (PAI). This *cag*PAI encodes a typical type-IV secretion system (T4SS) for translocation of the effector molecules CagA and pro-inflammatory ADP-heptose. Upon injection into host cells, the CagA protein undergoes tyrosine-phosphorylation as demonstrated by infection of cultured gastric AGS cells, resulting in cell elongation. Here we investigated whether *H. pylori* can exert these responses during interaction with cells from the oral epithelium. To this purpose, three oral epithelial cell lines (HN, CAL-27 and BHY) were infected with various *H. pylori* strains, and CagA delivery and IL-8 induction by ADP-heptose were monitored. Interestingly, all three oral cell lines were resistant to elongation upon infection, despite proper bacterial binding. Moreover, T4SS-dependent CagA delivery was absent, though IL-8 secretion by ADP-heptose was induced. Lack of CagA delivery was due to absence of CEACAM expression in all oral cells, while these surface molecules have recently been recognized as *H. pylori* T4SS receptors. Genetic introduction of either CEACAM-1, CEACAM-5, or CEACAM-6 into oral cells was sufficient to trigger CagA delivery and phosphorylation to levels similar to those in infected gastric AGS cells. These results demonstrate that lack of CEACAM receptors on the surface of the oral epithelial cells was responsible for resistance to CagA-dependent pathogenic activities, and confirms the important role for the T4SS-dependent interaction of these receptors with *H. pylori* in the gastric epithelium.

S. Backert: None. N. Tegtmeier: None. T.D. Ghete: None.

EP4.04 | The role of pro-inflammatory and anti-inflammatory cytokines in the development of chronic atrophic gastritis with *Helicobacter pylori* infection

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*Background:* *Helicobacter pylori* infection is detected in nearly 80% of people with chronic gastritis. The presence of a microorganism triggers a local inflammatory process in the gastric mucosa. Cells of the immune system involved in the immune response generate a cascade of cytokines aimed at eliminating the microorganism. The aim of this investigation was to study the content of pro-inflammatory and anti-inflammatory cytokines in patients with chronic atrophic gastritis associated with *Helicobacter pylori* infection.

Methods: 50 patients with chronic atrophic gastritis without and 50 patients with *Helicobacter pylori* were studied. The control group consisted of 50 healthy individuals. Determination of the content of cytokines (IL-2, IL-4, IL-8, IL-10) in the blood serum was performed by enzyme-linked immunosorbent assay. Statistical data processing was carried out using Statistica software packages for Windows 8.0. The statistical significance of the differences was determined using the Mann-Whitney test \( P < 0.05 \). Results: In patients with chronic atrophic gastritis, there was an increase in IL-2 and IL-8 compared to the control group. In patients with chronic atrophic gastritis with *Helicobacter pylori*, an increase in IL-2, IL-4, IL-8 and IL-10 was detected in comparison with both groups.

Conclusions: In atrophic gastritis with *Helicobacter pylori* infection, immuno-inflammatory changes in the Th-1 and Th-2 types are activated. Activation of pro-inflammatory and anti-inflammatory cytokines helps to limit the inflammatory process in the gastric mucosa. Pathogen elimination does not occur, which contributes to an increase in the infectious load on the patient and the progression of atrophic changes in the gastric mucosa.

O. Smirnova: None. A. Sinyakov: None.

EP4.05 | Immunoglobulins in chronic gastritis and chronic atrophic gastritis with *H. pylori* infection

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*Background:* One of the main pathogenetic factors in the occurrence of chronic gastritis is *Helicobacter pylori* infection, and its severity depends on the virulence of *H. pylori* strains. The aim of the work was to study the characteristics of the response of the humoral immunity in patients with *H. pylori*- associated diseases.

Methods: 50 patients with chronic gastritis in combination with *H. pylori* infection (CG), 50 patients with chronic atrophic gastritis in combination with *H. pylori* infection (CAG), and 50 practically healthy volunteers were examined. The quantitative determination of IgA, IgM, IgG, IgE was carried out by the enzyme immunoassay using reagent kits manufactured by Vector-Best. Statistical data processing was performed using Statistica 7.0. The significance of differences \( P < 0.05 \) between the indices of independent samples was evaluated by the Mann-Whitney criterion.

Results: In patients with CG and CAG, in combination with *H. pylori* infection, unidirectional changes in the humoral immunity were observed, an increase in IgG \( (p_{1-2} = 0.01; p_{1-3} = 0.002) \) and an increase in IgA \( (p_{1-2} = 0.03; p_{1-3} = 0.01) \) compared with the control group.

Conclusions: In chronic gastritis and chronic atrophic gastritis in combination with *H. pylori* infection, immune disorders occur associated with regulatory T-cell imbalance, and the development of the immune response according to Th2—a mechanism with activation of the humoral immunity. First of all, immunoglobulins of the IgG and IgA class begin to be actively produced on bacterial antigens.

O. Smirnova: None. A. Sinyakov: None.
EP4.06 | Features of the functioning of monocytes in chronic gastritis associated with *Helicobacter pylori* infection

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**Background:** Chronic gastritis and chronic atrophic gastritis have a common pathogenesis due to infection with *Helicobacter pylori*. Objective: to study the chemiluminescent activity of monocytes in chronic gastritis associated with *Helicobacter pylori* infection.

**Methods:** 40 patients with chronic gastritis, 25 patients with chronic atrophic gastritis, and 50 practically healthy volunteers were examined. Monocyte activity was studied by chemiluminescent methods. The maximum luminescence intensity, the time to reach the maximum luminescence, and the area under the chemiluminescence curve in the spontaneous and zymosan-induced states were determined. To enhance the glow used luminol. Statistical data processing was carried out using Statistica. The statistical significance of the differences was determined using the Mann–Whitney rank test.

**Results:** In patients with chronic gastritis, there was an increase in all the studied parameters in the induced state. In patients with chronic atrophic gastritis, the indicators of maximum intensity and area under the glow curve in spontaneous and induced states, and the time to reach the maximum glow in the induced state increased.

**Conclusion:** In patients with chronic atrophic gastritis associated with *Helicobacter pylori* infection, an increase in the activity of monocytes in spontaneous and induced conditions is detected, probably due to chronic inflammatory and histodestructive processes in the gastric mucosa, aimed at restoring the patient’s homeostasis. The project “Development and implementation of a program for screening and early diagnosis of gastric cancer by indicators of the immune, prooxidant and antioxidant systems to reduce mortality and disability” was funded by Krasnoyarsk Regional Fund of Science.

O. Smirnova: None. V. Tsukanov: None. A. Sinyakov: None. O. Moskalenko: None. N. Elmanova: None. E. Ovcharenko: None.

EP4.08 | Features of lipid peroxidation and antioxidant protection in chronic atrophic gastritis associated with *Helicobacter pylori* infection

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**Background:** Objective: to evaluate the content of malondialdehyde and the activity of antioxidant enzymes (superoxide dismutase and catalase) in chronic atrophic gastritis associated with *Helicobacter pylori* infection.

**Methods:** 40 patients with chronic gastritis, 25 patients with chronic atrophic gastritis, and 50 practically healthy volunteers were examined. The content of malondialdehyde, the activity of superoxide dismutase and catalase were determined in the blood serum by spectrophotometric methods. Statistical data processing was carried out using Statistica. The statistical significance of the differences was determined using the Mann–Whitney rank test.

**Results:** In patients with chronic gastritis and in patients with chronic atrophic gastritis in the blood serum, an increase in the content of malondialdehyde relative to the control group was revealed, which indicates an increase in lipid peroxidation in these patients. The activity of antioxidant protection depended on the type of chronic gastritis, with superficial gastritis, the activity of the superoxide dismutase enzyme increased relative to the control group, and in patients with chronic atrophic gastritis, the activity of both enzymes: superoxide dismutase and catalase increased.

O. Smirnova: None. V. Tsukanov: None. A. Sinyakov: None. N. Moskalenko: None. N. Elmanova: None. E. Ovcharenko: None.

EP4.07 | Role of monocytes during chronic atrophic gastritis associated with *Helicobacter pylori* infection

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**Background:** Chronic gastritis and chronic atrophic gastritis have a common pathogenesis due to infection with *Helicobacter pylori*.

**Objective:** To study the chemiluminescent activity of monocytes in chronic gastritis associated with *Helicobacter pylori* infection.

**Methods:** 40 patients with chronic gastritis, 25 patients with chronic atrophic gastritis, and 50 practically healthy volunteers were examined. Monocyte activity was studied by chemiluminescent methods. The maximum luminescence intensity, the time to reach the maximum luminescence, and the area under the chemiluminescence curve in the spontaneous and zymosan-induced states were determined. To enhance the glow used luminol. Statistical data processing was carried out using Statistica. The statistical significance of the differences was determined using the Mann–Whitney rank test.

**Results:** In patients with chronic gastritis, there was an increase in all the studied parameters in the induced state. In patients with chronic atrophic gastritis, the indicators of maximum intensity and area under the glow curve in spontaneous and induced states, and the time to reach the maximum glow in the induced state increased.

**Conclusion:** In patients with chronic atrophic gastritis associated with *Helicobacter pylori* infection, an increase in the activity of monocytes in spontaneous and induced conditions is detected, probably due to chronic inflammatory and histodestructive processes in the gastric mucosa, aimed at restoring the patient’s homeostasis. The project “Development and implementation of a program for screening and early diagnosis of gastric cancer by indicators of the immune, prooxidant and antioxidant systems to reduce mortality and disability” was funded by Krasnoyarsk Regional Fund of Science.

O. Smirnova: None. V. Tsukanov: None. A. Sinyakov: None. O. Moskalenko: None. N. Elmanova: None. E. Ovcharenko: None.
Conclusion: In patients with chronic gastritis associated with *Helicobacter pylori* infection, increased lipid peroxidation is offset by activation of superoxide dismutase. In patients with chronic atrophic gastritis associated with *Helicobacter pylori* infection, oxidative stress is opposed by activation of the bifunctional superoxide dismutase-catalase system, and high catalase activity proves the presence of histodestructive changes in the gastric mucosa, accompanied by cell breakdown and the influence of oxidative stress on the endothelium.

O. Smirnova: None. A. Sinyakov: None.

EP4.09  |  The role of the glutathione part of antioxidant protection in the development of atrophy in chronic gastritis associated with *Helicobacter pylori* infection

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_Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russian Federation_

**Background:** The development of atrophy depends on the genetic predisposition and individual response to *H. pylori* infection.

Objective: to evaluate the content of malondialdehyde and components of the glutathione antioxidant defense component in chronic atrophic gastritis associated with *Helicobacter pylori* infection.

**Methods:** 40 patients with chronic gastritis, 25 patients with chronic atrophic gastritis, and 50 practically healthy volunteers were examined. The content of malondialdehyde, reduced glutathione, the activity of glutathione-S-transferase and glutathione peroxidase were determined in the blood serum by spectrophotometric methods. Statistical data processing was carried out using Statistica. The statistical significance of the differences was determined using the Mann-Whitney rank test.

**Results:** The content of malondialdehyde increased 3 times in patients with chronic gastritis and 5 times in patients with chronic atrophic gastritis relative to control group. In patients with chronic gastritis, the activity of only the glutathione peroxidase enzyme increased, in patients with chronic atrophic gastritis, the activity of both enzymes increased, while the content of reduced glutathione did not statistically significantly differ from the control group.

**Conclusion:** In patients with chronic gastritis associated with *Helicobacter pylori* infection, lipid peroxidation is enhanced, which is compensated by the activation of a component of the antioxidant glutathione system. In patients with chronic atrophic gastritis associated with *Helicobacter pylori* infection, pathogenetic changes are caused not only by inflammation, but also by a histodestructive process in the gastric mucosa, which leads to a more pronounced oxidative stress and activation of two components of antioxidant defense, aimed at reducing manifestations.

O. Smirnova: None. A. Sinyakov: None.

EP4.10  |  Indicators of proliferation and apoptosis of gastric epithelial cells in the indigenous inhabitants of the Republic of Tyva with *Helicobacter pylori*-positive duodenal ulcer

V. V. Tsukanov; O. V. Peretyat’ko; E. V. Kasparov; A. S. Pulikov; A. V. Vasyutin; J. L. Tonkikh; T. V. Polivanova; V. A. Vshivkov

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**Introduction:** Studying cell renewal in the gastric mucosa will create new perspectives for understanding the pathogenesis of *Helicobacter pylori*-associated diseases (Blagih J. et al., 2020).

**Methods:** An immunohistochemical study of gastric mucosa epithelial cells with identification of proliferation markers (Ki-67 and PCNA) and apoptosis (bcl-2 and p53) was performed in 32 Tuvinsians (15 men and 17 women, average age 47.7 years) with *Helicobacter pylori*-positive duodenal peptic ulcer (group A) and in 24 *Helicobacter pylori*-positive Tuvinsians (12 men and 12 women, average age 46.4 years) without peptic ulcer (group B) in the Republic of Tyva.

**Results:** Indices apoptosis (bcl-2 and p53) had no significant differences when comparing patients with duodenal ulcer and the control group. In antrum Ki-67 was found in 6.2% of cells in group A and in 5.0% of epithelial cells in group B (P = 0.04). For the gastric body these indicators were, respectively, 5.9% and 4.1% (P = 0.01). The proportion of cells with PCNA in the antrum was 8.3% in group A and 6.0% in group B (P = 0.01). In the gastric body these indicators were, respectively, 8.5% and 7.0% (P = 0.02). As a result, regulatory ratios (proliferation indicators/apoptosis indices) were significantly higher in group A compared to group B.

**Conclusion:** Among the indigenous inhabitants of the Republic of Tyva, there is a shift in the proliferative-apoptotic relationship of gastric epithelial cells towards an increase in proliferation activity in patients with *Helicobacter pylori*-associated duodenal ulcer compared with people without peptic ulcer disease with *Helicobacter pylori* infection.

V.V. Tsukanov: None. O.V. Peretyat’ko: None. A.S. Pulikov: None. A.V. Vasyutin: None. J.L. Tonkikh: None. T.V. Polivanova: None. V.A. Vshivkov: None. Е.V. Kasparov: None.

EP4.11  |  Immunophenotype of cell infiltrate of the gastric mucosa lamina propria in indigenous inhabitants of Khakassia with *Helicobacter pylori*-positive duodenal ulcer

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**Aim:** To study the immunophenotype of the gastric mucosa lamina propria in indigenous inhabitants of Khakassia with duodenal ulcer.
Methods: We examined 22 indigenous inhabitants with *Helicobacter pylori*-positive duodenal ulcer (group A) and 23 indigenous people without peptic ulcer (group B) in Khakassia. The cell infiltrate immunophenotype in the gastric mucosa lamina propria was determined using immunohistochemistry. The percentage of CD4+ (T-helpers), CD8+ (T-killers), CD20+ (B-lymphocytes) and HLA-DR+ (marker of late activation of immune cells) cells was determined in 10 randomly selected fields of view (≥1000 cells) in mononuclear cells of the gastric mucosa lamina propria (inflammatory infiltrate).

Results: In the antrum, there were no significant differences in the content of CD8+ and CD20+ cells when comparing group A and group B patients. The proportion of CD4+ cells in the antrum was 33.6% in group A and 24.1% in group B (P = 0.01). For HLA-DR+ cells, these indices were 18.9% and 11.3%, respectively (P = 0.02). In the gastric body, an increase in the proportion of CD4+ cells (17.2% versus 8.0%, P = 0.01) CD8+ cells (10.2% versus 3.4%; P = 0.008), CD20+ cells (30.0% versus 13.4%, P < 0.001) and a decrease of the immunoregulatory index CD4+/CD8+ (1.7 versus 2.4, P = 0.04) was found in group A compared with group B. The proportion of HLA-DR+ cells in the gastric body was 15.4% in group A and 8.7% in group B (P = 0.07).

Conclusion: We found activation of the immune response in the gastric mucosa lamina propria in group A in comparison with the indicators of group B among Khakassians.

V.V. Tsukanov: None. A.V. Vasyutin: None. J.L. Tonkikh: None. O.V. Shptygasheva: None. N.N. Butorin: None.

EP4.13 | *Helicobacter pylori* infection affects nitric oxide system in patients with chronic antral gastritis and diabetes mellitus type 2

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Background: Gastric pathology in patients with type 2 diabetes mellitus (T2DM) may have pathogenetic peculiarities that are important for treatment optimization. Objective: to assess state of nitric oxide (NO) system in patients with chronic antral gastritis (CAG) and concomitant T2DM depending on *Helicobacter pylori* (HP) status.

Materials and Methods: Ninety-two patients with CAG and T2DM were examined, 71 (77.2%) patients had positive *H. pylori* antigen stool test, 21 (22.8%) were HP-negative. To form 2 equal groups, we randomly selected 40 patients: group I (n = 20) – HP-positive CAG and T2DM; II (n = 20) – HP-negative CAG and T2DM. Also 20 healthy individuals were enrolled for control. Nitrites (NO2), inducible (iNOS) and endothelial nitric oxide synthase (eNOS) were measured in blood serum.

Results: In comparison to healthy individuals, level of NO2 among the patients of the groups I and II increased 4.5 and 3.5 times more respectively (p < 0.05). Activity of iNOS increased 8.2 times more in patients of the group I, and 4.6 times more – in the group II compared to healthy subjects (p < 0.05). On the contrary, activity of eNOS decreased 2.7 and 2.3 times less in patients of the groups I and II respectively in comparison to control. In the group I level of NO2 was 1.4 more, iNOS ~ 1.8 times more, eNOS ~ 1.2 times less in comparison to the group II (p < 0.05).

Conclusion: HP-infection in patients with CAG and T2DM increases NO2 production and induces the activity of iNOS. It also potentiates endothelial dysfunction through eNOS activity reduction.

I. Skrypnyk: None. T. Radionova: None. G. Maslova: None.

EP4.14 | Active forms of oxygen in monocyte culture under high dissemination with *Helicobacter pylori*

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Colonization of the stomach *Helicobacter pylori* leads to the release of reactive oxygen species by phagocytes and the development of inflammation of varying severity depends on the effectiveness of the phagocytosis process. Objective: to determine the intensity of the formation of reactive oxygen species in a monocyte culture with a high titer on the gastric mucosa of bacteria and associated lesions of the stomach and duodenum.

Objects and Methods: Forty-four children with high dissemination and associated ulcers of the gastroduodenal zone (group A) and 34 children – low dissemination but without peptic ulcers (group B) were examined. The concentration of bacteria was taken into account by microscopic tests for biopats of stomach mucosa. Culture of blood monocytes isolated by separation on ficoll and adhesion on plastics. The baseline level and the level after zymosan stimulation of the ROS in the culture of blood monocytes were determined by the chemiluminescent method. Strains *H. pylori* were detected in all patients by breath test (UBT).

Results: Secondary reactive oxygen species in monocyte culture in group 1 were produced 3.5 times more intensively compared to group 2 at the baseline level and upon antigen induction. The activation of the superoxide radical associated with the primary forms of oxygen in group 1 increases 5-fold at the basic level and when antigen-induced comparison with group 2. Conclusions: Marked the increase of the activity in producing active forms of oxygen culture of monocyte in children with high dissemination of *H. pylori* associated gastric and duodenal ulcers.

I. Litvinova: None. O. Kolencukova: None. S. Tereshchenko: None. N. Gorbacheva: None. V. Tsukanov: None.
Results

H. pylori infection led to significant increases in IL-8 and CXCL1 mRNA expression in AGS cells, THP-1 cells and the gastric epithelium. In AGS cells, infection resulted in a significant decrease in expression of the Notch receptors Notch1, 2 and 3, ligands Jagged2 and Delta-like 4, and the transcription factors HES1 and HEY1. Similarly, in biopsies from H. pylori-infected gastritis patients (N = 25) compared to uninfected controls (N = 17) significantly lower median expression of Notch4 (46%; P = 0.02), Jagged2 (39%; P = 0.01), HES1 (38%; P = 0.02) and HEY1 (45%; P = 0.03) was seen. In macrophages, H. pylori infection led to a significant decrease in HES1 expression but significant increases in other Notch components: Jagged1, Notch1, Delta-like 4 and notably RBP-J which is the principle effector of the Notch pathway. Similar expression of Notch pathway components in gastric epithelial cells and macrophages was seen in THP-1 cells treated with E. coli LPS.

Conclusion: H. pylori infection is associated with cell-type specific changes in the expression of Notch pathway components in epithelial cells and macrophages. Further experiments will be necessary to discover the function of Notch signalling during H. pylori pathogenesis.

risk of severe gastrointestinal diseases, such as gastric cancer. The cagA gene encodes a cytotoxin-associated antigen (CagA) involved in the pathogenicity of the bacteria. This study aimed to investigate the prevalence of cagA genes among H. pylori isolates from patients with different esogastroduodenal lesions and for phylogenetic analysis of the isolated bacteria according to gene sequence. A total of 117 gastric biopsies were collected from patients with different esogastroduodenal disease. Polymerase chain reaction (PCR) product of selected samples of positive for cagA was undergone direct sequencing. A total of 77 strains of H. pylori were analyzed. Most strains of H. pylori carried cagA (62/77; 80.5%). The presence of cagA was associated with severity of esogastroduodenal lesions (P < 0.001). Moreover, our study not detect any difference in phylogenetic distribution between severe and not severe disease. This might be explained, at least in part, by phylogenetic analyzes were performed with the partial fragment of the cagA gene. The presence of the cagA gene may be a putting marker of the severity of H. pylori infection. This is the first study to investigate the phylogenetic population structure of the epidemic strains of H. pylori in Brazil. Further study with a larger number of strains is necessary to confirm the proposed associations between the specific EPIYA type and their hosts' clinical status and the identified sequence motifs as genetic markers. 


EP4.18 | Clinical treatment outcomes and prognosis of gastric MALToma: A single center experience

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Introduction: There is association between H. pylori infection and gastric MALToma. Guidelines advise H. pylori eradication as 1st therapy for H. pylori positive MALToma, and also involved-site radiation therapy (ISRT) for H. pylori negative MALToma. We investigated the treatment outcomes and prognosis of gastric MALToma.

Methods: We collected gastric MALToma patients who were histopathologically confirmed from Jan. 2005 till Dec. 2019, and retrospectively reviewed their medical records.

Results: A total of 56 patients were enrolled: mean age was 61 ± 14, 23 were male (41%). The common site was corpus (31, 56%). Endoscopic gross appearance was variable, such as IIC (27, 49%), polyloid or mass (8, 15%), discoloration (6, 11%). According to modified Ann Arbor staging, most of patients were corresponding to IE (50, 89%), 28 (50%) patients showed H. pylori infection. Among them, 26 patients received eradication therapy. 23 patients (88%) achieved successful eradication. As a result of therapy, 19 patients (83%) achieved complete remission (CR). Another 28 patients (50%) showed non-H. pylori infection, 10 patients received empirical H. pylori eradication therapy. Among them, 6 patients (60%) achieved CR. Another 11 patients and 2 patients who failed CR after successful H. pylori eradication received RT. Among them, 12 (92%) achieved CR. Out of 51 patients who were followed up by imaging and endoscopic study, 41 (80%) reached CR after treatment with various modalities. Among them, 25 patients by H. pylori eradication (61%) including empirical eradication (6, 15%), 12 by RT (29%), and 4 (10%) by surgery (2) or ESD (2).

Conclusion: H. pylori eradication is a 1st treatment for the MALToma. Although RT is considered as a 1st treatment for H. pylori negative patients, empirical H. pylori eradication could be applied before RT.


EP4.19 | Helicobacter pylori dupA virulence gene: Risk factor or protector of gastropathies?

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Helicobacter pylori infection is considered the main etiology of chronic gastritis, peptic ulcer and gastric cancer. The duodenal ulcer promoter gene (dupA) located in the plasticity region of the H. pylori genome is homologous to the virB gene, which encodes a type IV secretion protein in Agrobacterium tumefaciens. Some studies have shown associations between gastroduodenal diseases and positive dupA strains, but there is no consensus in the literature. As a result, this study aimed to evaluate the presence of the dupA gene in infectious strains in the Brazilian Midwest; relate to the clinical outcomes presented by dyspeptic patients and evaluate the origin of circulating strains. Gastric biopsies from 117 dyspeptic patients were analyzed using histological and molecular techniques. The screening for H. pylori infection was performed using the hpx gene (16S rRNA) and the positive H. pylori samples were subjected to the detection of the dupA gene. DupA positive samples were sequenced and used for phylogenetic analysis. The results demonstrated a significant relationship (P < 0.0001) between the presence of the dupA gene in the infecting strain and the absence of duodenal ulcer. Furthermore, it was observed that in women the infection by H. pylori dupA positive, increased the chance of developing gastritis by two times. The dupA sequences obtained in this work were grouped in the same clade as sequences from Western countries. It is concluded that the presence of dupA can be considered a protective factor for peptic ulcer, however it can induce the development of gastritis in women.

ABSTRACTS

EP4.20 | Contribution of Helicobacter pylori to foam cell formation in experimental atherosclerosis

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Introduction: The correlation between Helicobacter pylori (H. pylori) infection and atherosclerosis remains controversial, therefore there is need to investigate some mechanisms taking place during atherosclerosis development, especially in the accompaniment of persistent bacterial infection.

Aim: The main objective of the work was to study the contribution of H. pylori surface antigens and LPS in the transformation of macrophages into foam cells, which are crucial for atherosclerosis development.

Materials and Methods: The in vitro model employed in the study included THP-1 cells, which change into macrophages upon PMA treatment. Next, THP-1 macrophages were exposed to H. pylori LPS and glycine extract purified from H. pylori CCUG strain 17874 and containing composition of following antigens: CagA, VacA, Hsp, UreB, UreA. LPS E. coli was used as a reference control for bacterial components and 7KCh as a positive control. Transformation of macrophages into foam cells was visualized microscopically after 24 and 48 hours based on observation of lipid droplets stained red by Oil Red O method.

Results: Our results indicate that both H. pylori LPS and surface antigens (glycine extract), induced transformation of macrophages into foam cells. Among all the stimulators used in the study, the greatest foam-forming potential was shown by H. pylori glycine extract in concentrations of 0.1 and 1 μg/mL. Our results indicated, that only macrophages THP-1 transformed into foam cells. THP-1 monocytes treated with H. pylori components showed no evidence of lipid droplets.

Conclusions: Obtained results indicate that persistent infection with H. pylori may contribute to development and progression of proatherogenic changes.

A. Krupa: None. A. Dziuba: None. M. Mikolajczyk-Chmiela: None.

EP4.21 | Modulation of Nrf2-mediated antioxidant response after infection with Helicobacter pylori may contribute to gastric cancer

O. Martin; S. Bacon; L. Seeneevasen; P. Lecompte-Saint-Jean; E. Sifré; P. Lehours; C. Varon

Chronic infection with Helicobacter pylori is the major risk factor of gastric cancer (GC) through induction of chronic inflammation and oxidative stress. Our team demonstrated that H. pylori infection leads to an epithelial to mesenchymal transition (EMT) favoring GC. The transcription factor Nrf2 is the major driver of cellular antioxidant response activated after a high oxidative stress. Nevertheless, few studies have investigated the role of Nrf2 in H. pylori-induced GC. The aims of the project are: (i) to check the modulation of Nrf2 signaling pathway and (ii) to decipher the role of Nrf2 in EMT after H. pylori infection. Kinetic infection of GC cells with H. pylori induces an early activation of Nrf2 signaling pathway, shown by increase of nuclear translocation of Nrf2, by activation of the antioxidant responsive element sequence and by increased expression of target genes and proteins. However, 24 hours after infection, the Nrf2 signaling pathway is inhibited and level of glutathione, an important antioxidant protein, is dramatically decreased. This inhibition leads to an increase of cellular oxidative stress, characterized by an increase of ROS accumulation. Inhibition of Nrf2 by CRISPR-Cas9 strategy leads to an increase of cells harboring a mesenchymal phenotype and EMT-related gene expression, suggesting that Nrf2 pathway is important to maintain the epithelial integrity. Therefore, the inhibition of Nrf2 pathway, highlighted after H. pylori infection, may participate to EMT, an early event in cancer progression. These preliminary results may indicate that the modulation of Nrf2-antioxidant response after H. pylori infection contributes to gastric cancer.


EP4.22 | Modulation of Helicobacter pylori adhesion to gastric epithelial cells by BCGOnko vaccine mycobacteria

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Introduction: Due to increasing antibiotic resistance of H. pylori clinical isolates new preparations supporting anti-H. pylori therapy are necessary. The BCG-onko mycobacteria having immunomodulatory properties are considered.

Aim: To assess the effect of BCG-onko H. pylori driven mucin (MUC5AC) production by gastric epithelial cells and H. pylori adhesion.

Materials and Methods: Himalayan Cavia porcellus were inoculated (permission58/LB45/2016) per os with Brucella broth or with H. pylori CCUG17874 reference strain (10^{10} CFU/mL) 3× at 2 days intervals or with BCG-onko (1×10^{6} CFU/mL) (Biomed, Lublin, Poland), before or after treatment with H. pylori. The primary gastric epithelial cells from Cavia porcellus were treated with live H. pylori or soluble components of these bacteria: glycine-acid extract (GE) 10 μg/mL, CagA 1 μg/mL, urease A subunit 5 μg/mL or LPS 25 ng/mL, for 24 h in the presence or absence of BCG-onko. MUC5AC was detected using anti-MUC5AC antibody (MyBiosource, USA) FITC conjugate whereas adhesion of H. pylori by staining with anti-H. pylori FITC and BCG-onko with Live/Dead BacLight.
**EP4.23 | Induction of autoantibodies in response to infection with CagA-positive Helicobacter pylori**

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**Introduction**: Molecular mimicry between pathogen and host components may result in an induction of cross-reactive antibodies during infection. It has been suggested that *H. pylori* can contribute to systemic diseases, like coronary heart disease (CHD), due to molecular mimicry. The BLAST algorithm analysis showed the sequence similarity between *H. pylori* CagA protein and human or *Cavia porcellus* tumor necrosis factor receptor (TNFR). The aim is to estimate whether CagA+ *H. pylori* induce the cross-reactive antibodies towards TNFR common sequence.

**Material and Methods**: Sera from: healthy donors – HD, uninfected or asymptptomatically infected with *H. pylori* (48), patients with CHD (54), *Cavia porcellus* uninfected (10) or experimentally infected with *H. pylori*, 7 (10), 28 (20) or 60 (10) days from inoculation, were used for this study. The ELISA assay with P1 peptide (with the CagA and TNFR protein shared sequence) was performed. In order to know whether anti-P1 antibodies were induced during *H. pylori* infection, the sera before and after adsorption with heat inactivated *H. pylori* were used for the ELISA. The biological activity of anti-P1 antibodies was determined in the complement fixation assay.

**Results**: Cross-reactive antibodies were only present in patients with CHD infected with CagA+ *H. pylori* and the levels of such antibodies significantly decreased after adsorption of sera with *H. pylori*. Anti-P1 antibodies were also produced in *Cavia porcellus* infected with *H. pylori*. Immune complexes, P1-anti-P1 Iggs, activated complement.

**Conclusions**: Antibodies induced in CHD patients during CagA+ *H. pylori* infection, potentially cross-reactive in vivo with TNFR, may participate in pathogenesis of CHD.

W. Gonciarz: None. A. Tomaszewska: None. T. Rechcinski: None. M. Chmiela: None.
EP5.02 | Prevalence of *Helicobacter pylori* infection in north-eastern Romania - data from a tertiary care center

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**Background:** Worldwide, *Helicobacter pylori* (*H. pylori*) infection affects about 80% of the population, with significant differences in prevalence among geographical areas, socio-economic resources &gt access to medical services.

**Aim:** In our study, we assessed the prevalence of *H. pylori* infection in a medical center from North-Eastern Romania.

**Methods:** *H. pylori* infection was analyzed in consecutive patients who addressed the Emergency County Hospital (ECH) Bacău Analysis Laboratory during June 1st 2018–May 31st 2019. *H. pylori* infection was diagnosed by fecal antigen assay (antigen test). Demographic data (age, gender, environment of origin) &gt comorbidities were analysed. RESULTS. We analysed 2048 patients presented to the ECH Bacău Analysis Laboratory, from which 819 (40%) patients were detected as having *H. pylori* infection. No significant differences were identified between genders (men 40.29%, 305/757, &gt women 39.81%, 514/1291). Subjects from rural areas had a higher prevalence of *H. pylori* infection than those from urban areas (rural 47.28%, 296/626, urban 36.77%, 523/1422). *H. pylori* infection was significantly associated with age, the highest prevalence is found in subjects aged 31-40 years (54.28%, 89/164) &gt the lowest in patients aged 0-6 years (25.84%, 23/89). The highest prevalence of infection was found in patients admitted in the Internal Medicine section (50.71%, 107/211), the Gastroenterology (41.21%, 237/575), Endocrinology 42.85%, 30/70; Nephrology 48.64%, 18/37).

**Conclusion:** The prevalence of *H. pylori* in the population addressed to Emergency County Hospital-Bacău is lower than that estimated by current literature at national level. Further epidemiological studies are needed to establish incidence &gt prevalence rates nationally, in order to increase awareness among specialists &gt primary care physicians.

E.V. Popovici: None. C. Muzica: None. A. Trifan: None.

EP5.03 | Changes in the prevalence of *Helicobacter pylori* over last 25 years among Lithuanian medical students

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**Introduction:** The prevalence of *Helicobacter pylori* (*HP*) is decreasing in the Western world while remaining high in developing countries. There is limited up-to-date information about the prevalence of *HP* in central and Eastern Europe.

**Aim:** To assess the prevalence of *HP* and its trend over the last 25 years among students of Lithuanian University of Health Sciences (LUHS).

**Methods:** Students from the 1st to the 4th study year were randomly selected in the Medical and Nursing Faculties in year 2020. They were tested for the presence of antibodies against *HP* performing serological test from finger blood. Similar studies on the prevalence of *HP* among LUHS students were performed in 1995 (“Helisal” test), 2012 and 2016 (“SureScreen Diagnostics Ltd” test was used).

**Results:** Overall 148 students tested (120 (81.1%) female). The mean age (MA) was 20.4 ± 1.7 years. *HP* test was positive in 21 (14.2%) students. *HP* detected in 19 (16.1%) female and in 2 (7.1%) male students, P > 0.05. 120 (MA= 21.3 ± 1.0 years) students investigated in 1995; 187 students (MA = 22.4 ± 0.7 years) in the year 2012 and 262 students (MA = 20.4 ± 1.0 years) in 2016. In 1995, HP test was positive in 62 students (51.7%), in 2012 – in 57 students (30.4%), and in 2016 – in 69 students (26.3%). The statistically significant difference (P < 0.05) was found between all study years, except between 2012 and 2016.

**Conclusions:** *Helicobacter pylori* was established in 14.2% of students of LUHS. Over the last 25 years, the prevalence of *HP* among students of LUHS has decreased significantly.


EP5.04 | Evaluation of dyspeptic symptoms in *Helicobacter pylori*-positive and negative students of Lithuanian University of Health Sciences

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**Introduction:** HP-positive people with chronic gastritis are thought to be more likely to complain of dyspeptic symptoms, however, this statement is often not supported by the studies.

**Aim:** To determine the relation between *HP* infection and dyspeptic symptoms among students of Lithuanian University of Health Sciences (LUHS).

**Methods:** 148 students (mean age 20.4 ± 1.7) of LUHS (81% female) participated in the study. HP was tested serologically from capillary blood (SureScreen Diagnostics). Anonymous questionnaire assessed dyspeptic symptoms: epigastric pain or discomfort, heartburn, regurgitation, hunger-like-pain, nausea, borbovrymus, epigastric fullness, belching, abdominal distention, constipation, diarrhea, hard faeces, liquid faeces, urgency to defecate, incomplete feeling of defeation. The intensity of symptoms rated by 7-grade Likert scale.

**Results:** HP test was positive in 21 (14.2%) students, negative – in 125 (84.4%) students and 2 (1.4%) tests were non-informative and they were eliminated from analysis. The most prevalent symptoms...
were borborygmus (73%), hunger-like-pain (66%), and abdominal distention (62%). The prevalence of belching and diarrhea differs significantly between HP-positive and HP-negative students. Belching was present in 6 (29%) HP-positive and in 66 (53%) HP-negative students \( (P = 0.037) \). Diarrhea reported by 2 (9%) HP-positive and 42 (34%) HP-negative students \( (P = 0.025) \). There were no significant differences in the prevalence of other dyspeptic symptoms between the two groups. The intensity of dyspeptic symptoms does not differ significantly between HP-positive and HP-negative students.

**Conclusions:** The symptoms of belching and diarrhea were significantly more prevalent in HP-negative students. No significant association between HP infection and the intensity of dyspeptic symptoms was observed.


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**EP5.06 | The influence of parental education on the prevalence of Helicobacter pylori in rural children**

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**Aim of the Research:** To study the influence of parental education on the prevalence of *H. pylori* among rural children.

**Material and Methods:** 1535 children aged 7-17 years were examined in rural areas of the Central Asian region (southern Siberia). By random sampling, 270 children with gastroenterological complaints underwent gastroscopy with a biopsy fence. *H. pylori* was diagnosed in biopsy sections after Giemsa staining. Data on the education of parents of the examined children were collected. Parents’ education is divided into three clusters: higher, secondary vocational and secondary (including incomplete secondary). The results were processed using logistic regression analysis (odds ratio (OR) and 95% confidence interval (CI)).

**Results:** Father’s education did not affect the prevalence of *H. pylori* in schoolchildren (higher education: 0.36 (0.09-1.37), \( P = 0.184 \); secondary education: 1.39 (0.53-3.64), \( P = 0.625 \)). The presence of higher education in the mother had a positive effect on the prevalence of *H. pylori* in schoolchildren (0.22 (0.05-0.92), \( P = 0.044 \)). In families where the mother had a secondary education, the prevalence of *H. pylori* among school-age children increased (4.35 (1.27-14.93), \( P = 0.017 \)).

**Conclusion:** Mother’s education has a pronounced effect on the prevalence of *H. pylori* among rural children, which is obviously the result of the connection of this factor with the sanitary-hygienic culture in the family. Father’s education does not affect the frequency of *H. pylori* in children.

T.V. Polivanova: None. V.A. Vshivkov: None.

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**EP5.05 | The prevalence of CagA Helicobacter pylori in children of the Mongoloid population of Siberia**

V. A. Vshivkov; T. V. Polivanova

Federal Research Center «Krasnoyarsk Science Center» of the Siberian Branch of the Russian Academy of Sciences – Scientific Research Institute for Medical Problems of the North, Krasnoyarsk, Russian Federation

**Aim:** To study the prevalence of the CagA strain *Helicobacter pylori* in children of the Mongoloid population of Siberia (Russian Federation).

**Material and Methods:** A cross-sectional survey was conducted of children of the Mongoloid population of Siberia (558 schoolchildren of the Republic of Tuva and 622 schoolchildren of the Republic of Buryatia) aged 7-17 years. By random sampling, the prevalence of the CagA strain of *H. pylori* was studied by determining the serum IgG to the pathogen antigen (ELISA) in 212 children (112 Tuvans; 100 Buryats).

**Results:** The presence of IgG in serum to the *H. pylori* CagA antigen was found in 54.5% of the examined Tuvans and in 42.0% of the Buryats \( (P = 0.069) \). Among Tuvinians aged 7-11 years old, CagA-seropositive children accounted for 55.6%, and at the age of 12-17 years, rates were 54.1% \( (P = 0.896) \). In the Buryats of the younger age group, the presence of CagA *H. pylori* was determined in 29.6%, and in the older age group - 46.6% \( (P = 0.127) \). Differences in the prevalence of *H. pylori* CagA among boys (51.7% in Tuvans and 35.1% in Buryats) and girls (55.4% in Tuvans \( P = 0.731 \) and 46.0% in Buryats \( P = 0.286 \)) was not in the surveyed territories.

**Conclusion:** The high frequency of the CagA strain of *H. pylori* was found in children of the Mongoloid population of Siberia. Infection occurs mainly before the age of 12 years.

V.A. Vshivkov: None. T.V. Polivanova: None.
of HP, obtained in 2017 and 2019 in Russia. Materials and methods. Results of 13C-UBT tests of individuals, who had not received eradication therapy. The samples with DOB >4% were considered as positive test result. Pearson's chi-square test was used for statistical analysis. Data of 2926 individuals (M = 1169, F = 1757, average age 42.1 year) were obtained in 2017 and of 8840 individuals (M = 3163, F = 5677, average age 42.8 year) – in 2019. Results. Average HP prevalence was 42.5% in 2017, and 35.3% in 2019, without significant gender differences (P > 0.05). In the Central FD positive results were in 43.1% and 35.4% respectively, in Northwestern FD – 43.0% and 34.7%, in Volga FD – 40.6% and 33%, in Southern FD – 48.4% and 36.5%, in North Caucasus – 50.9% and 44.2%, in Ural FD – 40.4% and 32.7%, in Siberian FD – 42.5% and 41.2%, and in Far Eastern FD – 35% and 37.7%. A significant increase in the prevalence with age was noted. Conclusion: Our data showed, that the average prevalence of HP in Russia was decreasing from 42.5% in 2017 to 35.3% in 2019, without significant varies in federal districts.


EP5.09 | Relationship of Helicobacter pylori infection to lifestyle, sociodemographic and economic profile in dyspeptic patients


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Helicobacter pylori (H. pylori) is a gram-negative, coiled, microaerophilic and mobile bacterium. This microorganism colonizes the gastric mucosa of approximately half of the world's population. H. pylori infection can trigger an inflammatory response causing gastritis, ulcers or progressing to more severe clinical outcomes such as gastric adenocarcinoma. Due to the relationship of infection with the development of gastric neoplasms, the bacterium has been characterized by the World Health Organization (WHO) as a type 1 carcinogen. Given the above, the present study aimed to evaluate the association of H. pylori with possible risk factors for infection. We analyzed 100 questionnaires containing sociodemographic, socioeconomic and lifestyle questions of patients submitted to upper digestive endoscopy, followed by biopsy. H. pylori was diagnosed by histopathological examination. Statistical analyzes using Fisher's exact test and odds ratio were performed to assess the association of bacterial infection with possible risk factors. Half of the study population was infected with H. pylori, 60% (60/100) women. Among the infected patients, 75% were between 22 and 59 years old and most had the diagnosis of gastritis/pangastritis. There was statistical significance.

Patients with AUGIB are older with more comorbidities, their mortality remains unchanged. Main risk factors for AUGIB are NSAID's and antiplatelets use with clear trend of decreasing of peptic ulcer disease (mainly gastric ulcers) and Helicobacter pylori infection.


EP5.08 | Time trends in acute upper gastrointestinal bleeding in Serbian adult population

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Acute upper gastrointestinal bleeding (AUGIB) is a common medical emergency encountered worldwide. Despite medical, endoscopy and technical advances, it remains associated with significant morbidity and mortality. The aim of this study was to evaluate changes in clinicoepidemiologic characteristics of patients who presented with AUGIB during the last 10 years in Serbia. Data from 126 patients admitted with AUGIB in our center during the year 2018. were compared with retrospectively collected data from 124 patients admitted with AUGIB in 2013. year and with 121 patients from 2008. year. Mean age of patients increased from 63.84 ± 14.1 years to 65.71 ± 14.27 and 68.92 ± 12.77, and the number of patient's comorbidity, too. The percentage of NSAID's and antplatelets use remained stable, whereas the use of oral anticoagulants drugs increased significantly during this period (P = 0.041).

Table 1 revealed clinical data and risk factors during 2008, 2013, and 2018. Percentage of Helicobacter pylori positive in bleeding group in 2008. was 60.6%, in 2013. 64.93% and 39% in 2018 (X^2 = 15.33, , P = 0.00047, P < 0.05). During the period of these 10 years duodenal erosions increased significantly (X^2 = 11.84, P = 0.0027, P < 0.05) while gastric ulcer decreased significantly (X^2 = 6.21, P = 0.044, P < 0.05).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric ulcers</td>
<td>26.44%</td>
</tr>
<tr>
<td>Duodenal ulcers</td>
<td>31.4%</td>
</tr>
<tr>
<td>Gastric erosions</td>
<td>12.4%</td>
</tr>
<tr>
<td>Duodenal erosions</td>
<td>8.26%</td>
</tr>
<tr>
<td>Variceal bleeding</td>
<td>15.4%</td>
</tr>
<tr>
<td>Other</td>
<td>13.23%</td>
</tr>
<tr>
<td>H. pylori</td>
<td>60.6%</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>63.63%</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>6.44%</td>
</tr>
<tr>
<td>Mortality</td>
<td>14.05%</td>
</tr>
</tbody>
</table>
between former smokers and bacterial infection (P = 0.030) in addition to the use of well water (P = 0.005) and untreated water (P = 0.015).


EP5.10 | Retrospective analysis of the \textit{H. pylori} eradication rates in Crimean population

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\textbf{Background:} Maastricht V Consensus recommends that, as a first-line therapy clarithromycin or bismuth containing regimes. The different prevalence levels and eradication rates of \textit{H. pylori} in reports from different regions requires permanent local monitoring of these indices.

\textbf{Aim:} We compared the dynamics of eradication rates first-line clarithromycin-based triple and quadruple-based bismuth therapy in the Crimean population from 2011 to 2019.

\textbf{Methods:} We compared of outcome eradication therapy (our dates 2011-2013- first period, 543 patients) with period from 2017-2019 (second period, 689 patients).

\textbf{Results:} There were no significant clinical differences in \textit{H. pylori} prevalence over the compared periods (58.3% vs 49.1%, P > 0.05).

The eradication rates of PPI-clarithromycin-based triple therapy: 7 days – (ITT: 61.67% vs 63.2%, P > 0.05), 10 days – (ITT: 78.7% vs 86.3%, P < 0.05), 14 days (ITT: 79.4% vs 90.1%, P < 0.05). The bismuth containing quadruple regimes became more commonly used (19.3% vs 38.9%, P < 0.05) in \textit{H. pylori}-positive patients. \textit{H. pylori} eradication rates were for 10 days (ITT: 81.2% vs 84.0%), for 14 days (ITT: 87.6% vs 86.9%, P > 0.05).

\textbf{Conclusions:} A comparison of the dynamics of the results of triple and quadruple therapy in the Crimean population from 2014 to 2020 shows the preservation of the effectiveness of 10 day regimes. This allows us to recommend their further use in our region. Over the past observation period, there has been a tendency to a decrease in the prevalence of \textit{H. pylori} infection in our region.

V. Kryvy: None. I. Klyaritskaia: None. T. Tsapyak: None. E. Semenikhina: None.

EP6.01 | Changes in the fluoroquinolone resistance of \textit{Helicobacter pylori} over a 14-year period and discovery of a novel mutation in DNA gyrase: A single center study in Korea

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\textbf{Objective:} Eradication failure of \textit{Helicobacter pylori} is increasing due to antimicrobial resistance. The aim of this study was to investigate the changes in the prevalence and mechanism of fluoroquinolone resistance of \textit{H. pylori} in Korea.

\textbf{Methods:} \textit{H. pylori} strains were isolated from 143 patients and 48 patients at a single tertiary hospital in 2005-2006 and 2017-2018, respectively. The minimum inhibitory concentrations (MICs) of fluoroquinolone were determined by the serial 2-fold agar dilution method. The breakpoint for fluoroquinolone resistance was >1.0 µg/
mL. Sequence analyses of gyrA/gyrB and natural transformation studies were performed to investigate the mechanism of resistance.

**Results:** The resistance rates for levofloxacin and moxifloxacin were equally 16.8% (24/143) in 2005-2006, which increased up to 43.8% (21/48) for both antibiotics in 2017-2018. The range of MIC values for resistant strains increased overall, from 2 to 8 µg/mL in 2005-2006 to 4-16 µg/mL in 2017-2018. Among 24 resistant strains from 2005-2006, mutation of gyrA was observed in 95.8% (23/24). Transformation experiments revealed that the mutation of gyrB, observed in 12.5% (3/24), was not associated with resistance. All of 21 resistant strains from 2017-2018 showed gyrA mutation. Among these, a novel mutation of gyrA (Gly-85) which has never been reported before, was detected in one strain and was confirmed to be associated with fluoroquinolone resistance by natural transformation experiments.

**Conclusion:** The prevalence of fluoroquinolone resistance of *H. pylori* has markedly increased over time in Korea. Fluoroquinolone should be carefully used for *H. pylori* eradication in Korea, considering the high prevalence of fluoroquinolone resistance.

S. Rhie: None. J. Park: None. T. Shin: None. J. Kim: None. B. Kim: None. J.G. Kim: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; the Korean College of Helicobacter and Upper Gastrointestinal Research.

**EP6.02 | Clarithromycin resistance of Helicobacter pylori strains isolated in St. Petersburg, Russia**

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**Introduction:** Currently, the problem of the effectiveness of eradication therapy of *H. pylori* infection remains relevant. One of the main causes of failure during eradication is the resistance of *H. pylori* (Hp) to the antibiotics used.

**Aim:** Detection of point mutations in the 23S rRNA gene of Clar-resistant (Clar) clinical Hp strains isolated from patients with pathology of the stomach or duodenum in St. Petersburg, Russia. Materials and methods. The resistance of 87 *H. pylori* strains to antibiotics was determined using the disk-diffusion method. Sequencing (by Sanger) of an amplification product of 1402 bp the 23S rRNA gene of eight Hp strains was performed using direct (AGTCGGGTCTTAAGCCGAG) and reverse (TTCTCCTGATACGTCTTCCAG) primers. Chromatogram processing, alignment of sequences to the reference sequence GenBank acc. no. U27270 and identification of nucleotide substitutions were performed using the Uniprot UGENE 1.12 program. Results. During the work, 28 (32.18%) strains of clarithromycin-resistant Hp were identified. Sequencing of the 23S rRNA Clar gene region of the Hp isolates identified 3-6 different point mutations in each of them. Point mutations were detected in the following positions: G1513A, A1821G, G1826A, T1830C, A2142G, A2143G, T2182C, T2244C. The most frequent mutations were T2244C and G1513A, found in 8 and 7 sequenced Hp strains, respectively. Mutation A2142G was found in only one strain, T2182C – in three.

**Conclusions:** The high rate of *H. pylori* resistance to clarithromycin was revealed in St. Petersburg, Russia. Analysis of point mutations in the 23S rRNA region revealed genetic heterogeneity of Clar Hp isolates.

A. Svarval: None. D. Starkova: None. R. Ferman: None.

**EP6.03 | The genomic polymorphism of clinical strains of H. pylori isolated in St. Petersburg, Russia**

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Pasteur Institute, Saint Petersburg, Russian Federation

The aim of our work was to study the genomic polymorphism of clinical isolates of *H. pylori* obtained from various manifestations of *H. pylori* infection in St. Petersburg. Materials and methods. We studied 64 *H. pylori* strains isolated from patients with chronic gastritis (CG), duodenal ulcer (DU), gastric cancer (GC). The obtained DNA was used for PCR for the detection of cagA, oipA genes and typing of the vacA gene. Amplification of gene regions was carried out in a Bio-Rad C1000 Thermal Cycler thermal cycler (USA). The results were visualized using the GelDoc gel documentation system (Bio-Rad, USA). Results. 67% of cagA+ Hp pylori strains were detected. The presence of the cagA gene was observed in 82.6% and 100% of *H. pylori* isolates at DU and GC. 95% strains at UD had vacAs1 allele. All *H. pylori* isolates vacAs1/m1 and vacAs2/m2 were carriers of the alleles i1 (vacAs1/m1/i1) and i2 (vacAs2/m2/i2), respectively. A functionally active OipA gene was found only in 64% of *H. pylori* isolates. Most oipA+ isolates (85%) were carrier of the cagA gene and the vacAs1 allele. The dominant combined genotype cagA+/oipA+/s1/m1/i1, which was found in 34% of clinical isolates of *H. pylori*, was revealed. Thus, an analysis of the genomic polymorphism of the *H. pylori* clinical isolates isolated from patients with helicobacteriosis revealed a genetic heterogeneity of the pathogen population in St. Petersburg, Russia.

A. Svarval: None. D. Starkova: None. R. Ferman: None.

**EP6.04 | Helicobacter pylori resistance, treatment outcome and point mutation analysis based on molecular testing in Serbian adult population**

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Increased resistance to antibiotics is the major cause of treatment failure of *H. pylori* infection. The aims of this study were to determine the prevalence of *H. pylori* colonization, resistance rate of *H. pylori* to
clarithromycin and fluoroquinolones and corresponding point mutations and to assess the treatment outcome of the molecular tests-based eradication protocol. In the study enrolled 149 patients, who underwent upper gastroscopy from 2018-2020. Obtained bioplates were sent for histopathological evaluation and molecular diagnosis (GenoType HelicoDR, Hain) for the detection of H. pylori and point mutations confers clarithromycin and fluoroquinolone resistance. Treatment outcomes of H. pylori infection were assessed using urea breath test. H. pylori resistance to clarithromycin and fluoroquinolone was found in 51.9% and 45.5% strains, respectively. Primary resistance to clarithromycin and fluoroquinolone was found in 37% and 14.8%, respectively. Dual resistance to these antibiotics was more common in patients over 60 years old, compared to younger (23.1% vs 11.3%). The A2143G point mutation was found in 85% of clarithromycin-resistant H. pylori, while mutation in gyrA87 was the most common of the fluoroquinolone-resistant strains (31.4%). More than one mutation of the respective gene was found in 10% of clarithromycin- and 19.8% of fluoroquinolone-resistant H. pylori. The success rate of H. pylori eradication therapy was significantly higher in “naive” patients (91.3%) than in non-treatment-naive patients 71% (P < 0.05). High resistance rate of H. pylori to clarithromycin required revision of the standard eradication protocol used in Serbia. Molecular-based treatment lead to high eradication rates of H. pylori.


EP6.05 | Clarithromycin resistance is more common among less virulent Helicobacter pylori strains – Data from an Irish centre

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Trinity College Dublin, Dublin, Ireland

Introduction: Increasing antibiotic resistance is making H. pylori eradication progressively difficult. Virulence factors including the vacuolating cytotoxin A (VacA), cytotoxin-associated genes A and E (CagA and CagE) and the outer inflammatory protein A (OipA) play a role in determining the severity of disease.

Aims/Background: To evaluate correlations between clarithromycin and fluoroquinolone resistance and virulence factor genotype.

Methods: DNA was isolated from stomach biopsies of RUT-positive patients attending Tallaght University Hospital. The GenoType HelicoDR assay (Hain Lifesciences) was used to detect resistance-mediating mutations. Genotyping for virulence factors was done by PCR. SPSS software was used to perform statistical analysis.

Results: 127 patients were included (mean age 48.3 ± 15.7 years; 59% male), regardless of H. pylori treatment history. Overall resistance rates to clarithromycin and fluoroquinolones were 54.3% and 11.8%, respectively, 30.7%, 52.7% and 56.7% of strains were cagA+, cagE- and oipA-positive, respectively. VacA genotyping revealed that 60.6% were S1/M2, 23.6% S1/M1, 15% S2/M2 and 0.8% S2/M1. Clarithromycin resistance was significantly lower in cagA-positive than cagA-negative strains (38.5% vs 61.4%, respectively; χ² = 5.7, P = 0.02). Similarly, fewer cagE-positive strains were clarithromycin resistant than cagE-negative strains (44.8% vs 65%, respectively, χ² = 5.2, P = 0.02). Clarithromycin resistance was also lower among more virulent vacA S1 genotypes compared to S2 (49.5% vs 80%, respectively; χ² = 6.3, P = 0.01). No significant associations between virulence factor genotype and fluoroquinolone resistance were found.

Conclusion: Less virulent vacA genotypes and the absence of cagA and cagE are associated with clarithromycin resistance.


EP6.06 | Pathway of focal adhesion and its relevant circRNAs and IncRNAs are involved with perianal Crohn’s disease

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1Department of Gastroenterology, Guangdong Provincial People’s Hospital and Guangdong Academy of Medical Sciences, South China University of Technology, Guangzhou, China; 2The Second Clinical School, Southern Medical University, Guangzhou, China

Background and Aim: Perianal Crohn’s disease (P-CD) is a subtype of CD suffering from perianal abscess or fistula, the molecular mechanisms of which are not elucidated. Our aim is to screen the signal pathways, circRNAs, and IncRNAs correlated with the pathogenesis of P-CD.

Methods: Colonic mucosal samples were collected from four patients with P-CD, four with non-P-CD (NP-CD), all were newly diagnosed as CD without infliximab or azathioprine usage. The coding RNAs and ncRNAs were remained whereas rRNAs were removed. After getting the total RNAs from mucosal samples, PCRs was performed. HISAT2, Stringtie, CPC, CFCI were applied when the “clean data” were filtered to analyze the correlation of “lncRNA-mRNA”. Anchors reads were compared through “Find_circ” to determine the targeted circRNAs. Through miRNA sponges, the complicated ceRNA regulatory networks of circRNAs, IncRNAs, and miRNAs formed.

Results: 527 genes, 186 IncRNAs and 114 circRNAs differentially expressed between P-CD and NP-CD (all P < 0.05), with 249 up-regulated and 278 down-regulated genes, 97 up-regulated and 89 down-regulated IncRNAs, 50 up-regulated and 64 down-regulated circRNAs in P-CD compared with NP-CD. Among the 47 mostly clustered genes, ERBB2 and ITGA1 were involved with focal adhesion in P-CD. Three circRNAs, including hsa_circ_0043837, hsa_circ_0019225 showed a significant function in regulating the expression of the target genes and leading to dysfunction of focal adhesion, which were significantly associated with the development of P-CD.

Conclusions: Focal adhesion is a notable pathway for P-CD, it could be associated with decreased fibroblast migration in the fistula tissues of P-CD patients.

S. Dai: None. J. Hu: None. Y. Huang: None.
EP6.07 | Comparison in vitro of activity of various macrolides against *Helicobacter pylori*

**N. Dekhnich; N. Ivanichic; R. Kozlov**  
Smolensk State Medical University, Smolensk, Russian Federation

**Background:** Clarithromycin is almost exclusively used worldwide for *H. pylori* eradication therapy among macrolides. However, some authors suggest that other macrolides have potential for this indication. The aim of our study was to compare in vitro activity of clarithromycin, erythromycin, azithromycin and josamycin against *H. pylori* isolates collected during 2010-2017 in Smolensk, Russia.

**Materials/methods:** Antimicrobial susceptibility testing was done by agar dilution method. Interpretation of susceptibility testing results for clarithromycin was performed according EUCAST (v 8.0) breakpoints. Resistance breakpoints for erythromycin, azithromycin, and josamycin were set at ≥1.0 mg/L, as for clarithromycin.

**Results:** A total of 276 *H. pylori* isolates were tested. MIC₅₀ and MIC₉₀ values for clarithromycin, azithromycin, erythromycin, and josamycin were: 0.015 mg/L and 0.125 mg/L, 0.125 mg/L and 0.25 mg/L, 0.125 mg/L and 0.5 mg/L, 0.25 mg/L and 1 mg/L. MIC ranges for clarithromycin, azithromycin, erythromycin, and josamycin were: 0.015-16 mg/L, 0.015-156 mg/L, 0.015-128 mg/L, and 0.015-256 mg/L, respectively. Resistance rates were as follows: clarithromycin ~ 5.1%, azithromycin ~ 7.5%, erythromycin ~ 8%, josamycin ~ 23.2%. Only for 1 of 14 clarithromycin-resistant strains MIC of josamycin was less than 1 mg/L (0.5 mg/L), for other 13 clarithromycin-resistant isolates MICs of josamycin were significantly higher than MICs of clarithromycin (1-16 mg/L vs 2-256 mg/L).

**Conclusions:** Clarithromycin demonstrated the highest in vitro activity against *H. pylori* among tested macrolides.

N. Dekhnich: None. N. Ivanichic: None. R. Kozlov: None.

EP6.08 | East-Asian *Helicobacter pylori* strains synthesize heptan-deficient lipopolysaccharide

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1Department of Life Sciences, Imperial College London, London, United Kingdom; 2Division of Microbiology, Department of Biology, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany

The lipopolysaccharide O-antigen structure expressed by the European *Helicobacter pylori* model strain G27 encompasses a trisaccharide, an intervening glucan-heptan and distal Lewis antigens that promote immune escape. However, several gaps still remain in the corresponding biosynthetic pathway. Here, systematic mutagenesis of glycosyltransferase genes in G27 combined with lipopolysaccharide structural analysis, uncovered HP0102 as the trisaccharide fusosyltransferase, HP1283 as the heptan transferase, and HP1578 as the GlcNAc transferase that initiates the synthesis of Lewis antigens onto the heptan motif. Comparative genomic analysis of G27 lipopolysaccharide biosynthetic genes in strains of different ethnic origin revealed that East-Asian strains lack the HP1283/HP1578 genes but contain an additional copy of HP1105 and JHP0562. Further correlation of different lipopolysaccharide structures with corresponding gene contents led us to propose that the second copy of HP1105 and the JHP0562 may function as the GlcNAc and Gal transferase, respectively, to initiate synthesis of the Lewis antigen onto the Glc-Trio-Core in East-Asian strains lacking the HP1283/HP1578 genes. In view of the high gastric cancer rate in East Asia, the absence of the HP1283/HP1578 genes in East-Asian *H. pylori* strains warrants future studies addressing the role of the lipopolysaccharide heptan in pathogenesis.


**EP6.09 | The role of two PDZ domains in HtrA from *Helicobacter pylori* in the protein quality control system**

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*Helicobacter pylori* is a Gram-negative bacterium that colonizes the mucus layer covering the gastric epithelium of humans. The HtrA protein from *H. pylori* (HtrA₂ₜₚₚ) is an important secreted virulence factor involved in the colonization process by proteolysis of components of the adherens and tight junctions between epithelial cells. As a protease and chaperone, HtrA is involved in the protein quality control system in the periplasm, which is particularly important under stress conditions. The HtrA₂ₜₚₚ protein comprises a protease...
domain at the N-terminus and two PDZ domains (PDZ1 and PDZ2) at the C-terminus. In HtrA homologs from other bacteria, the PDZ domains play important regulatory roles, including modulation of the proteolytic activity and transformations of the oligomeric forms. To expand knowledge about HtrA, we examined by mutagenesis the importance of the PDZ1 and PDZ2 domains to maintain the proteolytic activity of the enzyme and ability of HtrAHp to form the high-order oligomers. In the experiments, we produced a series of HtrA Hp variants lacking either one or both PDZ domains. We found, that the PDZ2 domain is important to form high-order oligomers. The variants lacking the PDZ1 or both domains formed trimers only. The PDZ1 domain was crucial for efficient proteolytic activity. Furthermore, using the H. pylori mutant strains expressing HtrA without one or both PDZ domains, the importance of these domains for the H. pylori survival under stress conditions was demonstrated. The work was supported by Grant no. UMO-2016/21/B/NZ2/01775 from the Polish National Science Center.

U. Zarzecka: None. S. Backert: None. J. Skorko-Glonek: None.

EP6.10 | New Helicobacter pylori prophage phylogenetic population from Colombian strains

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Prophages are present in about 20% of Helicobacter pylori strains, forming four populations. Here, we did a phylogenetic characterization of the prophages from Colombian H. pylori strains, using the prophage sequence typing (PST). Totally 213 Colombian H. pylori strains were screened for holin and integrase genes. Additionally 16 genomes from Colombian strains presenting prophage sequences were retrieved from public databases. Results from STRUCTURE were analyzed with the Structure Harvester tool for easier detection of the number of populations that best fit the data. A phylogenetic tree was constructed using neighbor-joining method and Kimura two-parameter model. We found a lower frequency of holin and integrase genes than previously observed. Only 24 isolates (11.3%) were positive for integrase and 6 (2.8%) were positive for both genes. The PST analysis showed evidence of five populations, including a new fifth population exclusively composed of Colombian strains. The phylogenetic tree clusters were consistent with this new population. To the best of our knowledge this is the first report of H. pylori prophages from the Americas (Colombia), surprisingly revealing a new population. Isolation, admixture and miscegenation that began with the arrival of the Europeans and African slaves to America could explain this phenomenon.

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A.B. Muñoz: None. A.A. Trespalacios: None. F.F. Vale: None.

EP6.11 | Frequency of Helicobacter pylori cagA, dupA and vacA genotypes and their association with severity of gastric pathologies in clinical patients in midwestern Brazil

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Helicobacter pylori (H. pylori) is a Gram negative bacterium associated with the development of severe gastric pathologies such as atrophy, metaplasia and gastric adenocarcinoma. As a result, the microorganism is considered a type 1 carcinogen by the International Agency for Research on Cancer (IARC). The virulence factors present in the infecting strain determine the clinical outcome of the infection and can be used as specific markers for the severity of gastric diseases. The aim of this study was to evaluate the prevalence and association of cagA, vacA and dupA virulence genotypes with gastric pathologies. Antral and gastric body biopsies of 117 dyspeptic patients were analyzed by histological and molecular techniques. Screening for H. pylori infection was performed using the hpx gene (16s rRNA). Positive samples were submitted to detection of virulence genes vacA, cagA, dupA. The prevalence of infection was 64.1%, with a high frequency of positive cagA (80.0%), dupA (70.7%) and vacA (56.0%) strains. The cagA gene was detected in all strains present in patients with severe pathologies, whereas the isolated vacA gene was not detected in this group. In addition, interestingly, patients with severe diseases had fewer virulence genes in the infecting strain. The association of cagA and dupA genotypes was determined in greater number among patients with severe pathologies. The circulating H. pylori strains in the Brazilian Midwest present high heterogeneity of cagA, vacA and dupA virulence frequency. The presence and combination of various virulence factors may influence the clinical outcome.

ABSTRACTS

EP6.12 | Comparative genomic analysis of novel Helicobacter pylori strains isolated from domestic cats with gastritis reveals unique genetic profile that may contribute to colonization and pathogenicity in feline hosts

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While Helicobacter pylori (Hp) is considered a human-specific pathogen, natural Hp infection has been detected in domestic, commercially available cats with chronic gastritis. Cat strains experimentally recapitulated gastritis in specific-pathogen free cats and mice. As the factors that influence Hp host specificity are unknown, genomes from four representative Hp strains isolated from the domestic cats and a mouse-passaged Hp cat strain were compared with 257 Hp genomes from human, macaque, mouse, and gerbil natural or experimental hosts, which are to elucidate the genetic profile for Hp colonization and pathogenicity in cats. Pan-genome phylogenetic analysis showed the Hp cat genomes formed a distinct clade that neighbored strains B38, 29CaP, and G-Mx-2006-583 isolated from human patients with gastritis and gastric cancer and was distant to clades containing strains capable of colonizing nonhuman animal species. Average nucleotide identity showed the Hp cat genomes were nearly identical (>99.7%) to each other and distinct from other strains (~95%). The pan-genome for Hp cat genomes contained 1142 core and 519 accessory genes. 28 genes were unique, but comprised hypothetical annotations. Numerous genes were truncated, elongated, or fragmented due to mutations, especially in polynucleotide repeats regions, which may inactive or modify product function. Disruptions frequently occurred in methyltransferase, outer membrane, transporter and metabolic genes. Vacuolating cytotoxin subtype s1-i1/i2-m2 was present in the Hp cat genomes. The cag pathogenicity island was absent in the Hp cat genomes. The results from this analysis suggest Hp cat strains have a unique genetic profile that enabled cat colonization and resulting gastritis.

A. Mannion: None. Z. Shen: None. J.G. Fox: None.

EP6.13 | Current worldwide population structure of Helicobacter pylori

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The ancient coevolution of Helicobacter pylori with its human host has generated a phylogeographic structure that culminated in the formation of different populations that mirror human migrations (Falush et al. 2003; Linz et al. 2007). Genetic diversity of H. pylori has, so far, been divided into 7 populations and 14 subpopulations (Falush et al. 2003; Linz et al. 2007; Moodley et al. 2009), while new ones continue to be described as more samples from different regions are studied (Yahara et al. 2013; Montano et al. 2015; Thorell et al. 2017; Muñoz-Ramirez et al. 2017; Gutierrez-Escobar et al; 2017; Current genomic studies in some H. pylori populations have shown distinctive patterns of diversity associated with pathogenesis (Shaffer et al. 2011; Berthenet et al. 2018), suggesting a population-based research for quantitative genomic analysis to understand the progression of disease. We want to study the evolution and pathogenicity of several thousand of genomic sequences of H. pylori from Asia, as well as from other parts of the world. For this, we are first analyzing the population structure of ~3000 public samples available from different sources and concentrated in the EnteroBase database (Zhou et al. 2020) using fineSTRUCTURE (Lawson et al. 2012) and other reference-based approaches.

R.C. Torres: None. D. Falush: None.

EP6.14 | Muller’s ratchet-like effect in Helicobacter pylori, a highly recombining bacterial species

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Helicobacter pylori can be subdivided into different subpopulations: Africa, Asia and Europe and Middle East (Falush et al. 2003, Montano et al. 2015). The Asian population has been formed after an out-of-Africa bottleneck while the European and Middle Eastern ones could be the result of an hybridization between the African and Asian populations. The African and Asian populations present similar levels of genetic diversity while the mutation load is higher in the Asian population (higher dN/dS). Moreover, the sites where non-synonymous mutations accumulated in Asian population show a deficit of Asian ancestry in European and Middle Eastern populations. This excess of non-synonymous mutations after a bottleneck is similar to the consequence of Muller’s ratchet, i.e. the accumulation of deleterious mutations in asexual populations (Muller 1964). Although a bottleneck could cause the loss of the fittest bacteria, H. pylori is a highly recombinating species, which should cancel the Muller’s ratchet-like effect. In this poster, we present simulations of the evolution of the African and Asian populations after the bottleneck aimed at finding the values of the parameters to reproduce this observation and explain it.

E. Tourrette: None. D. Falush: None.

EP6.15 | Characterization of Helicobacter pylori Infection in the upper gastrointestinal tract of twins

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Introduction: Helicobacter pylori (H. pylori) is an infectious disease that causes chronic gastritis, may contribute to development of
Helicobacter pylori infection and metabolic syndrome-related nonalcoholic fatty liver disease severity

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Nonalcoholic fatty liver disease (NAFLD) emerges as an important global burden and Helicobacter pylori infection (Hp-I) linked with insulin resistance (IR) and metabolic syndrome (MetS) has been suggested as a risk factor for NAFLD, though controversy exists. This retrospective study aimed to investigate a potential impact of active Hp-I on NAFLD severity in morbidly obese patients underwent bariatric surgery and gastric biopsy for documentation of Hp-I. Of 64 eligible participants, 15 with active Hp-I (23.4%) showed higher rates of nonalcoholic steatohepatitis (NASH) than those without Hp-I (86.7% vs 26.5%, respectively; P < 0.001). Regarding histological lesions, steatosis grade (P = 0.027), ballooning (P < 0.001), lobular inflammation (P = 0.003) and fibrosis stage (P < 0.001) were also more severe in Hp positive patients. Equally, liver function tests, IR, dyslipidemia and arterial hypertension were significantly higher in Hp positive patients. In conclusion, Hp-I linked with MetS was associated with higher rates of NASH severity in morbidly obese patients with active Hp-I, findings offering potential clinical implication.

<table>
<thead>
<tr>
<th></th>
<th>[N (%)]</th>
<th>[N (%)]</th>
<th>P</th>
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<tbody>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>7.8 ± 5.2</td>
<td>12.2 ± 8.3</td>
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<tr>
<td>HbA1c (%)</td>
<td>5.7 ± 0.6</td>
<td>6.0 ± 1.3</td>
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<td>HOMA-IR</td>
<td>4.3 ± 3.9</td>
<td>10.1 ± 6.8</td>
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<td>Prediabetes/Diabetes</td>
<td>24 (49.0)</td>
<td>9 (60.0)</td>
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<td>Arterial hypertension</td>
<td>18 (36.7)</td>
<td>10 (66.7)</td>
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<tr>
<td>Dyslipidemia [%]</td>
<td>21 (42.9)</td>
<td>11 (73.3)</td>
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<td>FLIP [%]</td>
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<tr>
<td>No NAFLD</td>
<td>9 (18.4)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
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<tr>
<td>NAFL</td>
<td>27 (55.1)</td>
<td>2 (13.3)</td>
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<tr>
<td>NASH</td>
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<td>13 (86.7)</td>
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<tr>
<td>Severe NASH [%]</td>
<td>6 (12.2)</td>
<td>7 (46.7)</td>
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<tr>
<td>NAS [%]</td>
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<td>Grade 2</td>
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<td>7 (46.7)</td>
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<tr>
<td>Grade 3</td>
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<td>5 (33.3)</td>
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<tr>
<td>Ballooning [%]</td>
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<td>0</td>
<td>28 (57.1)</td>
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<td>20 (40.8)</td>
<td>10 (66.7)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 (2.0)</td>
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<td>Lobular inflammation</td>
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<tr>
<td>2</td>
<td>6 (12.2)</td>
<td>6 (40.0)</td>
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</tr>
<tr>
<td>Fibrosis stage [%]</td>
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EP7.01 | A potential association between Helicobacter pylori infection and metabolic syndrome-related nonalcoholic fatty liver disease severity
Data are presented as mean ± standard deviation (SD) for continuous, and frequencies (percentage) for categorical variables. Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FLIP, fatty liver inhibition of progression; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model of assessment insulin resistance; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis.


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Samsung Medical Center, Seoul, Republic of Korea

### EP7.02  |  Impact of *Helicobacter pylori* eradication on the risk of incident nonalcoholic fatty liver disease

**Background:** Previous studies reported an association between *Helicobacter pylori* (H. pylori) infection and nonalcoholic fatty liver disease (NAFLD), yet it is now questioned about whether H. pylori eradication reduces the risk for incident NAFLD.

**Methods:** This cohort study included 3,780 adults without NAFLD at baseline, who participated in a repeated health screening examination including an H. pylori-specific immunoglobulin G antibody test. Fatty liver was diagnosed by ultrasonography.

**Results:** During a median follow-up of 7.9 years, NAFLD developed in 1,294 participants. Participants with persistent H. pylori infection (persistent group) had a higher incident rate of NAFLD than those whose infections had previously been successfully eradicated (eradication group) (P < 0.001). In a multivariable model adjusted for age, sex, body mass index (BMI), smoking status, alcohol intake, and metabolic variables, the persistent group exhibited a higher risk of incident NAFLD than the eradication group [hazard ratio (HR), 1.36; 95% confidence interval (CI), 1.18-1.56]. In the multivariable analysis, higher BMI (HR, 1.19; 95% CI, 1.16-1.22), current smoking (HR, 1.27; 95% CI, 1.10-1.45), several metabolic abnormalities (higher glucose level, lower high-density lipoprotein cholesterol level, and higher triglycerides level) were significant risk factors for NAFLD. Subgroup analysis also revealed that persistent H. pylori infection was correlated to an increased risk of NAFLD.

**Conclusions:** Persistent H. pylori infection was associated significantly with the independent development of NAFLD. H. pylori infection may have a pathophysiological role in NAFLD development and, after successful eradication of H. pylori, the risk of incident NAFLD might decrease.

T. Kim; None. H. Lee; None. J. Lee; None. J. Kim; None. Y. Min; None.

### EP7.03  |  Cohort study of *Helicobacter pylori* infection and the risk of Incident osteoporosis in women

**Background:** Previous studies suggested a link between *Helicobacter pylori* (H. pylori) infection and osteoporosis, yet large-scale longitudinal studies are lacking to elucidate this association.

**Methods:** A cohort study of 10,482 women, who participated in a repeated health screening examination including an H. pylori-specific immunoglobulin G antibody test, was conducted to evaluate the association between H. pylori and osteoporosis development. Osteoporosis was diagnosed by dual energy X-ray absorptiometry (DXA).

**Results:** During the 77,515.3 person-years follow-up, women with H. pylori infection had a higher rate of incident osteoporosis than those who were uninfected. In a multivariable model adjusted for age, body mass index, menopausal status, smoking status, regular exercise, and comorbidities including hypertension, diabetes mellitus, dyslipidemia, stroke, or ischemic heart disease, the hazard ratio (HR) for incident osteoporosis in women with H. pylori-infection compared to those without infection was 1.23 (95% confidence interval [CI], 1.04-1.46). The association between H. pylori and the development of osteopenia was also evident. In the multivariable analysis, menopause (HR, 1.66; 95% CI, 1.29-2.13) and increasing age (HR, 1.07; 95% CI, 1.05-1.08) were significant risk factors for osteoporosis, while higher BMI level (HR, 0.84; 95% CI, 0.81-0.87) was a protective factor of the osteoporosis risk.

**Conclusions:** In a cohort study, H. pylori infection was associated with an increased risk of osteoporosis, independent of risk factors and confounding factors. Additional studies are needed to determine whether H. pylori eradication can reduce the risk of osteoporosis in especially women.

T. Kim; None. H. Lee; None. J. Lee; None. J. Kim; None. Y. Min; None.

### EP7.04  |  The prevalence of *Helicobacter pylori* and the structure of the gastric mucosa in elderly patients with GERD

**Background:** Previous studies suggested a link between *Helicobacter pylori* (H. pylori) infection and osteoporosis, yet large-scale longitudinal studies are lacking to elucidate this association.

**Methods:** A cohort study of 10,482 women, who participated in a repeated health screening examination including an H. pylori-specific immunoglobulin G antibody test, was conducted to evaluate the association between H. pylori and osteoporosis development. Osteoporosis was diagnosed by dual energy X-ray absorptiometry (DXA).

**Results:** During the 77,515.3 person-years follow-up, women with H. pylori infection had a higher rate of incident osteoporosis than those who were uninfected. In a multivariable model adjusted for age, body mass index, menopausal status, smoking status, regular exercise, and comorbidities including hypertension, diabetes mellitus, dyslipidemia, stroke, or ischemic heart disease, the hazard ratio (HR) for incident osteoporosis in women with H. pylori-infection compared to those without infection was 1.23 (95% confidence interval [CI], 1.04-1.46). The association between H. pylori and the development of osteopenia was also evident. In the multivariable analysis, menopause (HR, 1.66; 95% CI, 1.29-2.13) and increasing age (HR, 1.07; 95% CI, 1.05-1.08) were significant risk factors for osteoporosis, while higher BMI level (HR, 0.84; 95% CI, 0.81-0.87) was a protective factor of the osteoporosis risk.

**Conclusions:** In a cohort study, H. pylori infection was associated with an increased risk of osteoporosis, independent of risk factors and confounding factors. Additional studies are needed to determine whether H. pylori eradication can reduce the risk of osteoporosis in especially women.

T. Kim; None. H. Lee; None. J. Lee; None. J. Kim; None. Y. Min; None.
The GERD diagnosis was based on the Montreal Consensus (Vakil N. et al., 2006). Morphological evaluation of the gastric mucosa was performed using the modified Sydney system (Dixon M.F. et al., 1996). *Helicobacter pylori* was determined by the morphological method in the biopsies preparations stained by Giemsa.

**Results:** *Helicobacter pylori* was diagnosed in 62.2% of the elderly and in 77.9% of middle age patients with GERD (OR = 0.47; CI 0.32-0.68; \( P < 0.001 \)). The frequency of atrophy in the antrum was 71.0% in elderly patients and 6.9% in middle age persons (\( P < 0.001 \)). In the gastric body these indicators were, respectively, 20.7% and 0% (\( P < 0.001 \)). The frequency of intestinal metaplasia in elderly patients was 23.2% in antrum and 3.4% in gastric body; in middle age patients – 4.1% in antrum (\( P < 0.001 \)) and 0% in gastric body (\( P = 0.01 \)).

**Conclusion:** In elderly GERD patients in comparison with middle age persons with GERD, more often determined atrophy and intestinal metaplasia and less often *Helicobacter pylori* in the gastric mucosa, which suggests differences in the GERD pathogenetic mechanisms in the studied age groups.

V.V. Tsukanov: None. E.V. Onuchina: None. A.V. Vasyutin: None. J.L. Tonkikh: None.

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**EP7.05 | Clinical implication of *Helicobacter pylori* infection for eosinophilic esophagitis: A single center experience**

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*Seoul National University Bundang Hospital, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea*

**Background/Aim:** To investigated the relationship between EoE and *Helicobacter pylori* infection in the Korean population including recent data.

**Methods:** This was a single-center case-control study. We enrolled patients diagnosed with EoE from 2003 to 2020 based on histology with eosinophilic infiltration with \( \geq 15 \) eosinophils per high-power field (HPF). A total of 48 patients were identified to have EoE, and 9 patients who did not know *H. pylori* infection were excluded. Finally, 39 patients were included to the analyses. We also incorporated 78 age- and sex-matched controls (1 case : 2 controls). Patients with rapid urease test-positive or with the evidence of *H. pylori* infection on histology were classified as *H. pylori*-positive.

**Results:** Among the EoE patients, 25 (64.1%) were men and median age at the diagnosis was 18.1 years (range: 10 month-79 years) 16 patients presented epigastric pain, 16 vomiting, 13 dysphagia, 10 failure to thrive. 31 patients (79.5%) had allergic diseases such as food allergy (67%), allergic rhinitis (23.1%), atopic dermatitis (10%), asthma (7.7%), chronic urticaria (5.2%), drug allergy (5.2%), and dermatographism (2.6%). Nineteen patients (49%) had peripheral eosinophilia (eosinophil count in peripheral blood of \( \geq 500/\mu L \)). While 17 of 78 (21.8%) controls had *H. pylori* infection, only 2 (5.1 %) EoE patients had evidence of *H. pylori* infection. EoE was inversely associated with *H. pylori* infection (odds ratio 0.19, 95% confidence interval 0.04-0.89, \( P = 0.0346 \)).

**Conclusion:** Inverse association was noted between *H. pylori* infection and EoE. It may indicate protective effect of *H. pylori* infection for EoE.


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**EP7.06 | The features of *Helicobacter pylori* infection and gastrointestinal manifestations of allergy in children of Eastern Siberia**

**S. V. Smirnova; A. A. Barilo; A. A. Feizer**

*Scientific Research Institute of Medical Problems of the North - a separate division of the Federal, Krasnoyarsk, Russian Federation*

**Background:** The gastrointestinal tract is the often shock organ of allergic reactions. Gastrointestinal manifestations (GI) of allergies can be associated with *Helicobacter pylori* (HP) infection and combined with the damage of the other organs and systems.

**Aim:** To study the features of gastrointestinal manifestations of allergies and frequency of occurrence of *Helicobacter pylori* infection in children with allergic diseases in Eastern Siberia.

**Materials and Methods:** The study included 29 children with gastrointestinal manifestations of allergies from Eastern Siberia. The presence of Helicobacter infection was determined by the enzyme immunoassay (ELISA) (the concentration of total antibodies to the CagA *H. pylori* antigen). The state of the gastrointestinal tract was evaluated by the fibrogastroscopy (FGS).

**Results:** We determined the structure of other allergic manifestations: respiratory (allergic rhinitis, bronchial asthma and their combination) in 51.7% (n = 15), cutaneous (atopic dermatitis, urticaria) – 17.2% (n = 5) and dermarespiratory – 31% (n = 9) cases. The frequency of occurrence of HP infection in our study was 27.5% (n = 8). The analyze of the FGS showed that esophagitis, bulbitis, erosive lesions are more often in the group of non-HP-infected children, but they did not reach statistical significance. The incompetence of cardia were statistically significantly more often in HP-infected children compared to non-HP-infected children: 100.0% (n = 8) and 57.1% (n = 12) of cases, respectively, \( P = 0.025 \).

**Conclusion:** Thus, gastrointestinal manifestations of allergies in children with allergic diseases in Eastern Siberia are often accompanied by *Helicobacter pylori* infection and statistically significantly more often have incompetence of the cardia.

S.V. Smirnova: None. A.A. Barilo: None. A.A. Feizer: None.
Background: A possible protective role of the Helicobacter pylori (H. pylori) has been proposed in the development of allergic disease. The objective was to analyze the association between H. pylori infection and allergic diseases in Latvian population.

Material/Methods: Data from participants (40-60 years) of the GISTAR study in Latvia were collected 2016-2019. Participants had been tested for H. pylori (C13- UBT); data on gender, age (<50 vs ≥50 years), net income (<250€ vs ≥250€), education (upper secondary/low vs professional training/higher), smoking (never vs current/former), body mass index (BMI <25 vs ≥25 kg/m²), waist circumference (females: ≤88 cm vs >88 cm; males: ≤102 cm vs >102 cm), self-reported allergic disease were obtained by questionnaire. H. pylori prevalence was compared for participants with/without self-reported allergic disease. Factors that showed association (P < 0.09) with presence of asthma in univariate analysis were included in multiple logistic regression model adjusting for H. pylori status.

Results: In total, 2317 participants (mean age 52.27, SD ±6.86) were included in the analysis. Allergic disease was reported by 12.56%(291/2317) of which asthma-5.1%(117/2317), atopic dermatitis-0.04%(1/2317), allergic rhinitis-0.13%(3/2311), other allergies-5.96%(138/2317). Asthma was significantly inversely associated with female gender (6.2% vs 3.2%; P = 0.001), in those without asthma vs with asthma (54.1% vs 36.8%; P = 0.001); no difference was found with respect to the age (<18 vs ≥18 years) of asthma diagnosis (28.6% vs 38.5%; P = 0.39). H. pylori, gender, age, smoking, income, education, number of siblings in the family, waist circumference, BMI were included in multiple logistic regression model. In multivariate analysis asthma was significantly inversely associated with H. pylori (OR = 0.5; CI 95%: 0.3-0.7; P = 0.001), lower income (OR = 0.6; CI 95%: 0.4-0.9; P = 0.02); positively associated with female gender (OR = 1.6; CI 95%: 1.0-2.5; P = 0.05); sibling in a family (OR = 1.9; CI 95%: 0.9-3.9; P = 0.09); higher waist circumference (OR = 2.1; CI 95%: 1.4-3.3; P < 0.001).

Conclusions: Overweight and presence of siblings in a family are associated with the development of asthma. Lower prevalence of H. pylori infection among asthma patients suggests that bacteria could have immunomodulative role in respect to the development of allergy.


EP7.09 | Strain specific pathological changes in the liver of mice infected with Helicobacter pylori (H. pylori)

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Background: Helicobacter pylori (H. pylori) is the most common infectious pathogen of the gastroduodenal tract. H. pylori infection is correlated to the number of human diseases, including liver diseases. However, the data available is not unrevealing the mechanism through which H. pylori affects the development of hepatobiliary diseases and direct hepatotoxicity. We hypothesize that H. pylori infection could lead to cellular morphological alterations with the progressive liver function deterioration in the mice liver.

Results: Our data suggests that different strains of H. pylori i.e., SS1 and HPARE induced different grades of pathological changes in the liver samples of the infected mice. SS1 is pro-inflammatory, induces the structural changes of the hepatocytes but is weakly carcinogenic while HPARE is highly necro-inflammatory, regulates the hepatic morphogenesis, structural collapsing, necroptosis contributing to the fatty liver disease, bridging fibrosis, incomplete cirrhosis and liver cancer. The critical findings in our data were: bile duct abnormalities, active extramedullary hematopoiesis (EMP) suggesting the chronic infection induced by H. pylori leading to angiogenesis and neutrophil & erythrocytes altered profile along with nuclear hypersegmentation. Additionally, this study detected an abnormal distribution of Collagen type IV and excess deposition of the extracellular matrix into the space of Disse which results in distortion of the liver architecture. Moreover, immuno-histological analysis showed shorter-less distinct spirals and gull wing-like shape bacterium in the portal and sinusoidal areas. Conclusion: Our results indicate potential role of H. pylori in the induction of hepatobiliary diseases and the importance of bacterial strain in the severity of the pathology.

S. Khalid: None. L. Seeneevasen: None. E. Sifré: None. C. Varon: None. P. Spuul: None.
Helicobacter pylori (H. pylori) is a Gram-negative bacterium that colonizes the human gastric epithelium in about half of the world’s population. Several studies have associated H. pylori infection with the progression of liver cirrhosis and hepatocellular carcinoma. We have previously shown that different H. pylori strains, with distinct pathogenic outcomes, induce invadosomes with distinctive features in infected primary hepatocytes and in hepatoma Huh7 cell line. Due to the ability to degrade extracellular matrix, invadosomes are considered to be potential structures of proteolytic cell invasion. The central aim of our study is to investigate the mechanisms behind invadosome formation and to understand the subversion of liver cell functions upon H. pylori infection. Our study shows that H. pylori strains with cagPAI are able to significantly activate NFκB and upregulate the expression of IL8 and IL6 in infected Huh7 cells. Importantly, the inflammatory response correlated with the invadosome formation. NF-κB pathway inhibitor BAY11-7082 successfully managed to subdue the invadosome formation in cells infected with CagA-positive strains. CagA-positive strains also upregulated the expression of CD44. Wound healing scratch assay indicated slowed cell migration of infected Huh7 cell compared to untreated control cells and altered adherence junctions between confluent Huh7 cells infected with different H. pylori strains. Interestingly, adherence junction component β-catenin was relocated to radial junctions and around invadosomes. Our results suggest that pathogenic H. pylori strains induce increased inflammatory response in infected hepatocytes leading to actin cytoskeleton remodeling, hybrid epithelial/mesenchymal phenotype and could therefore contribute to the progression of liver diseases.

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Background: Prior to discovery of Helicobacter pylori-associated gastritis, an excess of concurrent (1961) and previously documented (1965) peptic ulcer was observed in idiopathic parkinsonism (IP).

Methods: This systematic review used an EMBASE database search, with search strategy according to PRISMA guidelines. Oxford Centre for Evidence-Based Medicine (OCEBM) based questions addressed, using the cumulative stratified evidence, were on inter-relationship of Helicobacter and IP, benefits of eradicating Helicobacter in IP and outcome of not treating.

Results: Twenty-one of 204 articles reviewed met inclusion criteria. The common a priori assumption that any benefit from Helicobacter eradication results from improved levodopa bioavailability is unjustified: increased bioavailability is unproven and benefit occurs even in anti-parkinsonian treatment-naïve patients. The inter-relationship of Helicobacter and IP is well-established (Level 1 OCEBM evidence), based on registry surveys. Previous Helicobacter infection appears as or more important than current to IP aetiopathogenesis. H. pylori virulence-markers (associated with autoimmunity and immune tolerance) influence risk, severity and progression of IP. The birth-cohort effect for virulence-marker antibodies, seen in controls, is obliterated in IP, suggesting causality. Successful H. pylori eradication in IP is disease-modifying, but not preventing (Level 1, based on systematic reviews of randomised-controlled-trials, observational study with dramatic effect and non-randomised-trials). Hypokinesia regresses with eradication, overall motor-severity lessens. Eradication may influence gastrointestinal microbiota adversely, unlocking development of rigidity. Failed eradication worsens hypokinesia, as does presence/persistence of H. pylori at molecular-level only. Adequate prognostic assessment of not treating Helicobacter is prevented by short follow-up.

Conclusions: We conclude that Helicobacter is a pathophysiological driver of IP.

R.M. Tucker; A.D. Augustin; B.H. Hayee; I. Bjarnason; D. Taylor; C. Weller; A. Charlett; S.M. Dobbs; R.J. Dobbs

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EP7.12 | *H. pylori* associated to irritable bowel syndrome (IBS)

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**Background/Aims:** The role of *H. pylori* in the pathogenesis of the Irritable Bowel Syndrome (IBS) was investigated in 38 subjects, these patients were diagnosed and treated for IBS, without any improvement.

**Methods:** Colonoscopy, Biopsy and CLO-test (Rapid Urease Test).

**Results:** Male 16 (42%) Female 22 (58%) *H. pylori* positive 19 (50%) *H. pylori* negative 19 (50%). One patient in this study previously presented Colonic Carcinoma.

**Conclusion:** All patients *H. pylori* positive received treatment and showed improvement including the patient with colonic carcinoma, which in the beginning presented 99% of stricture and after 1 month of treatment the stricture decreased to 50%. Accordingly to this research we consider that the presence of *H. pylori* in the colonic mucosa is another important co-factor in the pathogenesis of IBS and it should be considered in the development of colonic carcinoma.

A. Barrios: None. F.A. Barrios: None. A. Alvarez: None. E. Mendez: None.

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**ELECTRONIC POSTER ROUND 8: PAEDIATRIC CONDITIONS**

EP8.01 | The peculiarities of stomach mucosa in *Helicobacter pylori* schoolchildren

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*H. pylori* plays an important role in the development of different forms of gastritis pathology: gastritis, ulcer disease, stomach mucosa metaplasia and stomach cancer.

**Aim of the Research:** To study the character of endoscopic and morphological changes in *H. pylori* schoolchildren.

**Materials and Methods:** Our work shows the results of medical investigation for 338 schoolchildren of Siberia (Russia) with gastrointestinal complaints in the ages from 7 to 17 years. All of them had been performed gastroscopy. For biopsy we used the samples from stomach antrum and body. Gastritis was evaluated using histology indices in accordance with Sydney classification. The analysis of statistical meaning of the differences between qualitative signs was carried out by $\chi^2$ criterion.

**Results:** The prevalence of *H. pylori* in the examined schoolchildren amounted 57.7%. Among them ulcer disease was diagnosed in 2.1%, among non-infected 2.8% ($P = 0.6558$). Erosive gastritis was diagnosed in 9.2% of the infected subjects and in 10.5% without the infection ($P = 0.7$). Gastritis was diagnosed after the data of histological tests. In children with *H. pylori*-associated diseases in stomach body we diagnosed gastritis of the 2-3 stages in 38.5% cases, in non-infected cases in 18.9% ($P = 0.0001$); in antrum 67.2% and 22.4% ($P = 0.0001$) correspondingly. In *H. pylori* subjects mucosa metaplasia was found in 2.1%, in non-infected ones in 2.8% ($P = 0.6558$).

**Conclusion:** In *H. pylori* schoolchildren of Siberia the associations of the infection with ulcer diseases and metaplasia have not been marked. In bacterial invasion they showed the strengthening of gastritis activity in both antrum and body of a stomach.

T.V. Polivanova: None. V.A. Vshivkov: None.

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EP8.02 | Cytokines in *Helicobacter pylori*-associated gastritis in children with a family predisposition to gastric pathology

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**Aim of the Research:** To study the level of cytokines IL-2, IL-4, IL-6, IL-8, IL-18, IL-1β, FNO-α in the blood serum of *H. pylori*-associated gastritis in children with a family predisposition to gastric pathology.

**Material and Methods:** 159 children with a morphologically confirmed diagnosis of gastritis at the age of 7-17 years were examined. The data on the presence of gastric diseases in relatives was obtained. The children underwent gastroscopy with a sampling of biopsies from the gastric mucosa. Gastritis was diagnosed in accordance with the Sydney classification. Diagnostics of *H. pylori* in biopsy sections was carried out after Giemsa staining. Serum cytokine concentrations determined by ELISA. Significance of feature differences was analyzed using the Mann-Whitney test.

**Results:** Studies of the cytokine profile in blood serum in schoolchildren did not show significant differences depending on *H. pylori* infection. However, in children with *H. pylori*, in case of a family history of gastric pathology, there was an increase in IL-6 ($P < 0.05$), whose functional duties in the body are the activation of the immune response in the acute phase of inflammation. This is an increase in IFN-α ($P < 0.01$) – a cytokine that functionally ensures the launch of the body’s immune responses to damage; and a decrease in interleukin 8 ($P < 0.05$), which plays an important role in the innate immunity system.

**Conclusion:** The peculiarities of the cytokine regulation of *H. pylori*-associated inflammatory process in the gastric mucosa in children with a family predisposition to gastric pathology were obtained.

T.V. Polivanova: None. V.A. Vshivkov: None.
EP8.03 | Cytokeratins CK20, CK7 in assessing the severity of lesions of the gastric mucosa in schoolchildren with Helicobacter pylori-associated gastritis

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Aim: To study the expression of CK20, CK7 proteins in the gastric mucosa in schoolchildren with H. pylori-associated gastritis.

Material and Methods: Eighty-nine children with gastroenterological complaints aged 7-17 years were examined. A gastroscopy was performed with a biopsy from the antrum and the body of the stomach. Gastritis was diagnosed in accordance with the Sydney classification. H. pylori was determined by morphological method after staining biopsy sections according to Giemsa. The immunohistochemical method was used to record the expression of cytokeratins (CK20, CK7) in the gastric mucosa. The analysis of statistical meaning of qualitative characteristics was made by $\chi^2$ criterion under $P < 0.05$.

Results: Expression of CK20 in the antrum mucosa was found in children with H. pylori-associated gastritis in 19.6% and in 7.9% without H. pylori ($P = 0.1423$). The expression of CK7 in the antrum was detected in 43.1% and 34.2% of the examined, respectively ($P = 0.3938$). In the gastric mucosa, expression of the CK20 protein was recorded in 15.7% in schoolchildren with H. pylori and 2.6% in schoolchildren without bacteria ($P = 0.0724$); expression of CK7 protein was noted in 37.3% and 18.4% of cases, respectively ($P = 0.0627$).

Conclusion: In children with H. pylori-associated gastritis, there is a pronounced tendency to increase the expression of CK20, CK7 in the stomach. This reflects an increase in proliferative processes and, obviously, is associated with an increase in the progression of the pathological process in the stomach in children with H. pylori-associated gastritis.

V.A. Vshivkov: None. T.V. Polivanova: None.

EP8.04 | Levels of IL-2, IL-4 with erosive and ulcerative lesions of the gastroduodenal zone associated with Helicobacter pylori in schoolchildren with pathology of the digestive tract in parents

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Cytokines are involved in the regulation of both immune responses and plastic processes in the organism.

Aim: To study the levels of IL-2, IL-4 with erosive and ulcerative lesions of the gastroduodenal zone associated with H. pylori in schoolchildren with pathology of the digestive tract in parents.

Material and Methods: A gastroscopy was performed with a biopsy taken from the gastric mucosa in 179 schoolchildren with gastroenterological complaints. The age of the examined was 7-17 years. Data were obtained on the presence of digestive tract diseases in parents. H. pylori was determined by morphological method after staining biopsy sections according to Giemsa. Concentrations of cytokines IL-2, IL-4 were determined in blood serum by ELISA. Significance of feature differences was analyzed using the Mann-Whitney test.

Results: Among schoolchildren with erosive-ulcerative lesions associated with H. pylori, IL-4 was higher with a family burden in the pathology of the gastrointestinal tract ($P = 0.006$) and higher than in schoolchildren without a destructive process ($P = 0.008$). In addition, among children with an erosive-ulcerative process and with a hereditary predisposition in the presence of H. pylori, IL-2 replication increased ($P = 0.039$); in children with H. pylori and with a hereditary predisposition in the presence of an erosive-ulcerative process, IL-2 replication also increased ($P = 0.038$).

Conclusion: In schoolchildren with an erosive-ulcerative process associated with H. pylori and a hereditary predisposition to diseases of the digestive tract, there is an induction of IL-2, IL-4 replication at the systemic level.

V.A. Vshivkov: None. T.V. Polivanova: None.

EP8.05 | Is CDX2 in children an immunohistochemical marker for the adverse course of gastritis?

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Transcription intestinal differentiation factor (CDX2) in adults is closely associated with atrophy and intestinal metaplasia of the gastric mucosa. In this regard, protein is a marker of these processes. In children, the question has not been studied.

Aim: To study the association of CDX2 with morphological changes in the gastric mucosa in children with gastritis.

Material and Methods: A gastroscopy was performed with a biopsy from the antrum and the body of the stomach in 89 schoolchildren with gastroenterological complaints aged 7-17 years. Diagnosis of gastritis was carried out according to the modified Sydney classification. H. pylori was determined in biopsy sections after Giemsa staining. Immunohistochemical determination of CDX2 using CDX2 antibodies (Clone: DAK-CDX2, 1:50), and EnVision Detection Systems Peroxidase/DAB imaging system (Daco, Denmark).

Results: CDX2 expression was observed in 4 (4.5%) examined children. Cases of the appearance of the protein concerned only the mucosa of the antrum. In the mucous membrane of the body of the stomach, CDX2 was not detected. In all children, gastritis was a morphological pathology. The relationship of protein expression with gastritis activity has not been established. The frequency of H. pylori...
in schoolchildren was 57.3%. Among children with CDX2 expression in the antrum, *H. pylori* was detected in 100.0% \( (P = 0.0659) \).

**Conclusion:** There is a pronounced trend in the effect of *H. pylori* on the expression of CDX2 in the gastric mucosa in childhood. There is a need for prospective studies of children with the expression of this protein in the gastric mucosa.

T.V. Polivanova: None. V.A. Vshivkov: None.

**EP8.06 | The relationship of blood leptin with *Helicobacter pylori* in children**

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The leptin mechanism is considered an important factor in the progression of gastritis and the formation of carcinogenesis of the stomach.

**Aim of the Research:** To assess the association of leptin circulating in the blood with *H. pylori* infection in children of the Europoid and Mongoloid populations.

**Materials and Methods:** Children: 24 Europoids and 60 Mongoloids aged 7-17 years with gastritis without obesity in the Central Asian region (South Siberia) were examined. A gastroscopy with a biopsy and blood sampling were performed. Diagnosis of gastritis was carried out according to the Sydney classification. The presence of *H. pylori* was determined after Giemsa staining. Plasma leptin was determined by an enzyme immunoassay (Human Adiponectin ELISA reagent kit, BioVendor). Inter-cohort comparison of the indices was carried out using Mann-Whitney criterion under \( P < 0.05 \).

**Results:** Circulating leptin in the blood was 3.5 (0.1-16.4) ng/mL in children with *H. pylori* and 7.4 (0.9-30.2) ng/mL without *H. pylori* \( (P = 0.199) \). The lowest leptin values were found in infected Europoids – 3.0 (0.1-15.4) ng/mL, in uninfected Europoids amounted to 12.4 (0.1-32.2) ng/mL \( (P = 0.205) \). Among the Mongoloids, the leptin level in the infected was 7.7 (1.2-18.0) ng/mL, in the uninfected, it was 7.0 (2.7-19.0) ng/mL \( (P = 0.820) \).

**Conclusion:** In children of the Europoid and Mongoloid populations, there is no association of circulating leptin in the blood with *H. pylori*. V.A. Vshivkov: None. T.V. Polivanova: None.

**EP8.07 | Chronic *Helicobacter pylori* infection in Siberian adolescents with gastroesophageal reflux disease (GERD)**

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Recently, much attention has been paid to the problem of the GERD and *Helicobacter pylori* (*Hp*) infection connection. Assumptions are made about the protective role of this bacterium concerning the development of GERD in adult patients. However, in the children’s population, such studies are not enough, and they have conflicting results. In this regard, we aimed to establish the frequency of *Hp* infection in Central Siberian children with GERD.

**Methods:** 553 adolescents aged 11-17, referred to a pediatric gastroenterology Centre (Krasnoyarsk, Siberia, Russia), were screened by the Russian version of Gastroesophageal Reflux Disease Questionnaire (GerDQ). According to the sum of the scores for the six GerDQ questions adolescents were split into three groups: (1) no GERD \( (n = 414) \), (2) GERD with low impact \( (n = 98) \), and (3) GERD with high impact \( (n = 41) \). *Hp* presence in antral biopsy and anti-*Hp*-cagA antibodies in plasma were tested in 416 and 473 adolescents, respectively. Chi-square test was used.

**Results:** *Hp* presence in antral biopsy progressively increased with GERD impact: no GERD group – 51.8 (46.2-57.3) %, GERD with low impact – 60.3 (49.1-70.4) %, and GERD with high impact – 70.6 (53.7-83.1) %. \( p_1-3 = 0.037 \). No connection was established between GERD and anti-*Hp*-cagA antibodies presence.

**Conclusion:** A direct connection between GERD presence/severity and chronic *Hp* infection in Siberian adolescents was revealed. We suppose that *Hp* eradication may be helpful, especially in adolescents with severe GERD according to GerDQ assessment.

S. Tereshchenko: None. M. Shubina: None. N. Gorbacheva: None.

**EP8.08 | Efficacy of standard triple therapy in the eradication of *Helicobacter pylori* in Siberian children: A single centre experience of treatment outcomes**

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Standard triple therapy remains the recommended treatment regimen for *Helicobacter pylori* (*Hp*) eradication in children. Eradication success rate and efficacy vary depending on bacteria resistance rate. We aimed to study the efficacy of standard triple therapy in the *Hp* eradication in children from Central Siberia, in the region with high *Hp* chronic infection prevalence (50-70%), according to our previously reported study \([1]\).

**Methods:** 31 children with recurrent abdominal pain complaints aged 9-17, referred to a pediatric gastroenterology centre (Krasnoyarsk, Siberia, Russia) were screened.
Siberia, Russia) were tested for Hp presence by antral biopsy and then prospectively retested by Hp antigen ELISA monoclonal test in stool after triple therapy in 1.5-3 months. Twenty children were diagnosed with erosion or peptic ulcer of the duodenal bulb by upper endoscopic examination, and another 4 had erosion or peptic ulcer of the stomach. Nine patients were diagnosed with GERD. Standard triple therapy (IPP + amoxicillin + clarithromycin) was used.

**Results:** Upon repeated testing, the HP antigen was detected in 3 children (9.7%). Thus, the level of successful eradication in our study was 90.3%. In 19 children recurrent abdominal pain stopped completely, in 8 children a decrease in the frequency of pain was observed, and in 4 children the pain did not stop.

**Conclusion:** Thus, the success of HP eradication after triple therapy in Central Siberia children showed a sufficient level of achieved eradication (90.3%), which corresponds to the recommended standards.


S. Tereshchenko: None. E. Anisimova: None. M. Shubina: None. N. Gorbacheva: None.

**EP8.09 | Effect of Helicobacter pylori on the formation of erosive-ulcerative lesions of the gastroduodenal zone, GERD and their associative relationship**

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The role of Helicobacter pylori in the formation of erosive and ulcerative diseases of the gastroduodenal zone in adults is proved. The association of infection with GERD is a moot point.

**Aim:** To study the effect of Helicobacter pylori on the formation of erosive-ulcerative lesions of the gastroduodenal zone, GERD and their association in children.

**Material and Methods:** Four hundred and forty-five schoolchildren with gastroenterological complaints aged 7-17 years were examined. Everyone underwent gastroscopy with a sampling of biopsies from the antrum and body of the stomach. Diagnosis of Helicobacter pylori was carried out by morphological method after Giemsa staining. GERD was diagnosed according to the Montreal Consensus.

**Results:** The frequency of Helicobacter pylori in the examined children was 56.9%. Erosive-ulcerative lesions of the gastroduodenal zone in schoolchildren with Helicobacter pylori were diagnosed in 21.7% of cases, without infection in 14.6% (P = 0.055), 22.1% of children with infection and 19.3% without infection had clinical manifestations of GERD (P = 0.462). Moreover, in the group of schoolchildren infected with Helicobacter pylori, the association of erosive-ulcerative lesions of the gastroduodenal zone with GERD was noted in 27.3%; in the group of schoolchildren without Helicobacter pylori in 32.1% of cases (P = 0.644).

**Conclusion:** Thus, Helicobacter pylori in schoolchildren is associated with a tendency to increase erosive-ulcerative lesions of the gastroduodenal zone. The effect of Helicobacter pylori on the formation of GERD and on the association of erosive and ulcerative diseases with GERD has not been established.

T.V. Polivanova: None. V.A. Vshivkov: None.

**EP8.10 | Application of liquid probiotic concentrate for gastroduodenitis of Helicobacter etiology in children**

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The aim was to study the effectiveness of “Biovestin-lacto” in the complex treatment of Helicobacter pylori-associated gastroduodenitis in children. The drug is made from skimmed milk fermented with pure cultures of bifidobacteria of two strains B. adolescentis MC-42, B. bifidum 791 with the addition of lactobacilli of the pharmaceutical strain L. Plantarum 8RAZ and its metabolism products. The advantage of the drug used is a high concentration of Bifidobacterium adolescentis (109 CFU/mL), Lactobacillus plantarum (108 CFU/mL).

For this purpose, 60 children aged 9-12 years were examined with a verified diagnosis of gastroduodenitis associated with Helicobacter pylori using clinical, morphological and paraclinical diagnostic methods. Based on random samples, two groups were formed: first received standard eradication therapy, and the second additionally received “Biovestin-Lacto”. Control of eradication was performed 6-8 weeks after the therapy. The use of “Biovestin-lacto” contributed to a significant improvement in the effect of eradication therapy (100% eradication in group 2), while in the control group 1 of children after treatment, infection with Helicobacter pylori was detected in 14.3%. The association with the confidence (P < 0.02) of the effect of probiotic in treatment on the severity of chronic gastroduodenitis (activity, intestinal metaplasia, insemi nation) was revealed. Scientific assumptions: the liquid form containing both lacto – and bifidobacteria contributes to the maximum effect of restoring the microbiocenosis of the stomach and intestines by directly acting on the infectious agent (by reducing the inflammatory process and mucosal regeneration) and reducing the adhesion of Helicobacter pylori to the gastric mucosa.

**EP8.11 | H. pylori infection in symptomatic Mexican children**

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**Background**: The approach of *Helicobacter pylori* infection must be individualized because the association with symptoms is still controversial. Its prevalence, clinical presentation and endoscopic findings in Mexican children are not well known.

**Objective**: To determine the main gastrointestinal symptoms and endoscopic findings in children with *H. pylori* infection, and to find a correlation between them.

**Methods**: A retrospective cross-sectional study over 2 years was carried out. We included 174 children from a third level hospital from Mexico, who underwent endoscopy, histology study, PCR, and culture. *H. pylori* infection was proven according to ESPGHAN/NASPGHAN consensus.

**Results**: A *H. pylori* prevalence of 27.5% was found in the studied population. There is not a statistically significant association between *H. pylori* infection and symptoms. Among the most frequent endoscopic findings is antral nodularity (62.5%) which is statistically associated with infection (P = 0.000), this finding has a sensitivity of 62.5% (CI 45.76-77.24%), specificity 70.6% (CI 62-29-78.98%), PPV 44.78% (CI 32.12-57.43%) and NPV 83.18% (CI 75.62-90.73%).

**Conclusions**: The symptoms should not guide the diagnostic approach. The presence of antral nodularity is a good predictor of *H. pylori* infection, being an indicator of broadening the approach with other invasive tests.


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**EP9.01 | Treatment of non-erosive reflux disease and change of esophageal microbiome: A prospective multicenter study**

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**Introduction**: The pathogenesis of non-erosive reflux disease (NERD) has not been fully evaluated. We aimed to evaluate the treatment response of proton pump inhibitors (PPI) in patients with NERD and changes in microbial composition and biologic marker expression on esophageal mucosa after PPI therapy.

**Methods**: Patients were diagnosed with NERD and enrolled and received 20 mg of esomeprazole for 8 weeks. The treatment response was evaluated using the patient assessment of upper gastrointestinal symptom severity index questionnaire at baseline, week 4, and week 8. Esophageal mucosal markers and oropharyngeal and esophageal microbiomes were analyzed in patients who had required upper gastrointestinal endoscopy for screening.

**Results**: In 62 enrolled patients, complete and partial response rates at week 8 were 60.0% and 32.7%, respectively, for heartburn, and 61.8% and 29.1%, respectively, for regurgitation. After 8 weeks of PPI therapy, the expression level decreased in several inflammatory cytokines, including IL-6, IL-8, and NF-κB. In the microbiome analysis, Streptococcus, Haemophilus, Prevotella, Veillonella, Neisseria, and Granulicatella were prevalent regardless of timing (baseline vs week 8) and organs (oropharynx vs esophagus). However, the overall composition of the oropharyngeal microbiome was distinguished from that of the esophageal microbiome (P = 0.004). After the PPI therapy, this difference was not identified.

**Conclusions**: Half-dose of PPI for 8 weeks was effective for symptom control in NERD. It reduced the expression of several inflammatory cytokines in the esophagus. There was a significant difference in the microbial composition between oropharynx and esophagus in patients with NERD; however, it disappeared after PPI therapy.

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### EP9.03 | Helicobacter pylori and the possible probiotic Lactobacillus salivarius co-exist in Estonian gastric biopsy sample

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*Helicobacter pylori* (H. pylori) is a gastric pathogen inducing the development of chronic gastritis, peptic ulcers, and gastric cancer. Several studies have proved the probiotic effects of *Lactobacillus* species against *H. pylori* and their positive contribution in *H. pylori* eradication. Little is known about gastric microbiota inhabitants. Here, we report a case of *H. pylori* and *Lactobacillus salivarius* (L. salivarius) co-existence in a Caucasian female patient. Gastric biopsy sample was homogenised in Brucella broth and plated on Columbia Blood Agar supplemented with Vancomycin, Trimethoprim, Cefsulodin, and Amphotericin B. Culture presented as a mixture of small translucent colonies and slightly bigger greyish-white colonies. Only bigger colonies were possible to cultivate further. Colony PCR with CagA and VacA specific primers proved the presence of *H. pylori*, whereas MALDI-TOF-MS colony identification and later 16S rDNA sequencing resulted as *L. salivarius*. The genotype of *H. pylori* was verified with PCR from DNA extracted from co-culture and was CagA-positive with EPIYA-A, EPIYA-B¹, and EPIYA-C motifs, VacA-positive with s1/m2 alleles and UreB-positive. Whole genome sequencing (WGS) of the sample showed that the 2.05 Mb *L. salivarius* genome consists of one chromosome, one megaplasmid and four smaller plasmids. Only 0.1% of WGS reads matched with *H. pylori* genome. Further research needs to be done to verify whether the results show a co-culture of *H. pylori* and *L. salivarius* or extensive genetic transfer between the two bacteria.

K. Roots: None. L. Kasak: None. K. Suurmaa: None. I. Sarand: None. P. Spuul: None.

### EP10.01 | Effect of fecal microbiota transplantation on clearance of carbapenem-resistant enterobacteriaceae

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**Background and Aim:** Carbapenemas-resistant Enterobacteriaceae (CRE) carriage could be the fatal pathogen as hospital infection. Fecal microbiota transplantation (FMT) has been reported to be a possible option for decolonization. This study aimed to whether FMT is effective to eradicate intestinal colonization of CRE.

**Methods:** Retrospective review was performed 337 patients with CRE digestive tract colonization from August 2018 to July 2019. Among them, 9 patients were received FMT for clearance of CRE or Clostridium difficile infection, and 29 patients were selected as control groups, that were confirmed two consecutive CRE colonization and followed for more than 1 month. Successful decolonization was determined by at least three consecutive negative rectal swabs [culture] within 1 month.

**Results:** Median follow-up days after CRE colonization was 50.5 days (interquartile range [IQR] 36-95 days). In FMT group, median duration of carriage of CRE before FMT was 26 days. (IQR 10-67 days) A total of 14 patients (35.8%) presented free of CRE colonization within 1 month; 5 patients (55.6%) at FMT group and 9 patients (31.0%) at control group (P = 0.183). One week after FMT, 5 of 9 patients were free of CRE colonization. Only 1 patient received FMT showed CRE recolonization at 28 follow-up days. Compare to CRE conversion rate within 1 week, FMT group was significantly higher than control groups (44.4 vs 6.9%, P = 0.02).

**Conclusion:** FMT could be effective for CRE decolonization in intestinal tract. These data should be confirmed by larger cohorts and randomized trials.

Y. Shin: None. J. Lee: None. H. Kim: None.

### EP10.02 | Gut microbiota depending on the atopic dermatitis severity in children in Eastern Siberia

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**Background:** In recent years there is increasing interest in the relationship of the microbiota with atopic dermatitis (AD).

**Aim:** To study the intestinal microbiota depending on the atopic dermatitis severity in children in Eastern Siberia.

**Material and Methods:** The study group comprised 32 children with atopic dermatitis aged from 4 months to 18 years from Krasnoyarsk Territory (East Siberia of Russia). In our study 50.0% (16) children...
were female. AD severity were determined using the AD scoring system SCORAD. We formed 2 groups depending on the disease severity: 1 group (n = 21, mean age 3.0 ± 1.1 years) – children with AD mild and moderate severity (SCORAD ≤ 40), 2 group (n = 11, mean age 8.0 ± 0.9 years) – children with AD severe severity (SCORAD > 40)

The gut microbiota distribution was performed by bacteriological culture-based methods.

**Results:** In our study the amount of *E. coli* with reduced enzymatic properties more than 50% of the total number of *E. coli* was elevated in 2 group compared to 1 group: 18.1%(n = 2) and 0% infants, *P* = 0.04. Carriage of Staphylococcus aureus was more often in 1 group compared to 2 group: 100%(n = 21) and 81.8%(n = 9), *P* = 0.04. However, the counts of Staphylococcus aureus >10^5 was more often in 2 group compared to 1 group: 27.2%(n = 3) and 4.7%(n = 1), *P* = 0.04. Carriage of Candida albicans was more often in 2 group compared to 1 group: 63.6%(n = 7) and 28.5%(n = 6), *P* = 0.05.

**Conclusions:** Thus, we identified features of AD severe severity: elevated level of *E. coli* with reduced enzymatic properties, carriage of Candida albicans and Staphylococcus aureus >10^5.

A.A. Barilo: None. S.V. Smirnova: None. I.V. Borisova: None. A.A. Feizer: None.

**EP10.03 | Watercress and the gut**

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We are investigating the urease inhibiting potential of Watercress (*Nasturtium Officinale*) extracts. Bacterial urease converts urea to ammonia, and its extracellular presence on *H pylori* is a survival adaptation to the stomach's acidic environment. We have demonstrated multiple classes of urease inhibitors in watercress which may offer an adjunctive/alternative treatment of this colonisation. Phenethyl isothiocyanate is a known urease inhibitor and gives watercress its peppery taste. We are developing processing methods to maximise production of Phenethyl isothiocyanate, and other urease inhibitors. We aim to investigate the effects of a watercress-derived urease inhibiting extract on the gut microbiome in sarcopenic patients. Commonplace in agriculture is the use of synthetic urease inhibitors to increase the yield of animals. Unfortunately, synthetic urease inhibitors have a challenging side effect profile and their UK clinical use is limited. However, a plant-based extract which offers multiple urease inhibitors working synergistically may overcome this problem. The aim is to promote the cycling of urea into amino acids within the gut, thereby decreasing ammonia production. Improving gut efficiency in this way may be beneficial in sarcopenia, and in sports performance/recovery. Heparic encephalopathy (HE) through raised serum ammonia in liver cirrhosis is another therapeutic target. Current therapies include lactulose which increases gut transit and reduces fermentation to lower ammonia production while promoting proliferation of non-urease-producing bacteria such as *Lactobacillus*. Similarly, the antibiotic Rifaximin (another recognised HE treatment), lowers serum ammonia by eradicating urease-producing bacteria. A watercress extract to inhibit urease in the gut offers several potential therapeutic targets.

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**ELECTRONIC POSTER ROUND 11: MICROBIOTA AND INTESTINAL DISEASE**

**EP11.01 | Trimebutine maleate as a multidimensional-antibacterial agent against functional dyspepsia: A prospective multicenter randomized, double-blind controlled trial**

**J. Kountouras**; E. Gavalas; A. Papaeftymiou; I. Tsechelidis; S. Polyzos; S. Bor; M. Diculescu; A. Bochenek; K. Jadallah; J. Rozciecha; T. Karakan; P. Dabrowski; M. Tadeusz; Z. Spirchez; O. Sezgin; M. Gülten; N. Farsakh; M. Doulieris

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**Introduction:** Functional dyspepsia (FD) consists a multi-factorial disorder associated with inflammatory and neuro-motility gastrointestinal (GI) dysregulation also related with GI dysbiosis. This multicenter randomized double-blind study evaluated the efficacy and safety of trimebutine maleate (TM) in FD, following its suggested antibacterial role on microbiota.

**Methods:** 211 patients with FD diagnosed by Rome III criteria were randomized to receive TM 300 mg BID (108 patients) or placebo (103 patients) for 4 weeks. The symptoms’ relief was evaluated by
two clinical scales and gastric emptying (GE), as an objective index, was measured by the 99mTc-Meal Scintigraphy test (8 TM and 8 placebo participants in one center). All adverse events were recorded during this period.

**Results:** 185 (52.4% in the TM and 47.6% in the placebo arm) were analyzed. Although, both treatment arms showed a significant improvement in symptom relief (TM P-value: 0.015/placebo P-value: 0.041), TM effect during the second study period (last 2 weeks) was significantly better (P = 0.02). Likewise, the proportions of complete symptom relief and marked improvement were greater in the TM arm, though not significantly. Both groups showed similar effects in quality of life (P = 0.598). Regarding GE test, TM accelerated gastric emptying at 50 minutes (median emptying 75.5% TM vs 66.6% placebo, P = 0.036) and shortened the lag period though not significantly. Finally, safety evaluation showed similar percentages of mild adverse events.

**Conclusion:** TM appears to be effective and safe in treating FD. The potential mechanisms include the suggested motor effect in upper and lower GI tract and its bacteriostatic/bactericidal activity.

J. Kountouras: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; This work has been financially supported by Galenica A.E. Pharmaceutical Company, Greece. E. Gavalas: None.


**EP11.03 | Comparison of glucose and lactulose breath test results for detecting small intestinal bacterial overgrowth**

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**Background:** Lactulose and glucose breath tests are accepted in clinical practice for SIBO (Small intestinal bacterial overgrowth) detection. North American and Italian guidelines are used for result interpretation.

**Aim:** To compare the results of lactulose and glucose breath tests in SIBO detection, and to correlate test positivity to clinical symptoms.

**Materials and Methods:** This was a retrospective analysis of patients with abdominal symptoms having undergone diagnostics in Digestive Disease Centre GASTRO (2015-2019). Every patient underwent lactulose and glucose test. Breath samples were analysed with the Quintron BreathTracker SC analyser by determining breath hydrogen and methane concentrations and with the application of CO2 correction factor. The criteria set by the North American guidelines for SIBO were applied. Symptoms were recorded during the breath testing period.

**Results:** The study cohort consisted of 60 patients (50% males; median age 42 years). Median interval between the tests was 7 days. 41.7% and 13.3% were diagnosed SIBO with lactulose and glucose test, respectively. 6.7% tested positive with both breath tests. Elevated baseline hydrogen concentrations (>16 ppm) were present in 22 patients (11 patients for each test), however this elevation was maintained in 10 patients undergoing lactulose, and 3 – undergoing glucose test. Methane-producers accounted for 16.7%, 20% and 28.3% reported symptoms during lactulose test and glucose test.
The prevalence of *Helicobacter pylori* and protozoan invasions and the impact on the performance of enzymatic digestion

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The aim is to study the prevalence of *H. pylori* and protozoal infections in chronic gastroduodenal diseases (CGDD). Materials and methods. We examined 244 patients (130 adults and 114 children) with clinical manifestations with abdominal pain and dyspeptic syndromes. All patients were examined by immunochromatographic test for the qualitative detection of *H. pylori* in the feces, coprological and protozoological methods. Results. In the group of patients with CGDD the *H. pylori* antigen in the feces was detected in 116 (47.5%) patients. During protozooscopy examination of feces, the detection rate of *Lambia intestinalis* was 22.9%, in *Blastocystis* spp. – 13.1%, *Entamoeba coli* 10.2%, *Entamoeba* spp. 9.4%. Protozoan invasion was detected in the groups of *H. pylori*+ and *H. pylori*– patients: infection of *Lambia intestinalis* was diagnosed in 27.6% and 18.8% of cases respectively (P < 0.05), in *Blastocystis* – in 16.4% and 10.2% cases (P < 0.05). Mixed infection with *H. pylori* and pathogenic protozoa, along with increased manifestations of gastrointestinal, ileocecal, enteral and acholic syndrome, signs of pancreaticogenic and distal-coolic syndromes were detected. Monoinfection *Lambia intestinalis*, signs of enteral, ileocecal, and acholia with signs of ileocecal syndromes were detected, and when monoinfection *Blastocystis* spp. signs of enteral and ileocecal syndromes were revealed. Conclusions. It is shown that intestinal protozoan and bacterial-protozoan mixed infection (*H. pylori* + *L. intestinalis*; *H. pylori* + *Blastocystis* spp.) can have a negative effect on enzyme digestion and are important etiopathogenetic factors of diseases of the upper gastrointestinal tract.

G.S. Isaeva; None. E.V. Agafonova; None. R.A. Isaeva; None.

Fecal microbiota transplantation as treatment for recurrent *Clostridiodes difficile* infection in patients with inflammatory Bowel disease: Experiences of the Netherlands donor feces bank

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Background: In patients with inflammatory Bowel disease (IBD) the prevalence of co-infection with *Clostridiodes difficile* infection (CDI) is higher than in the general population due to the use of immunosuppressive medication and dysbiosis of the bacteria in the colon. Here we report the treatment course and efficacy of FMT provided by the Netherlands Donor Feces Bank (NDFB) for IBD patients with rCDI.

Methods: The NDFB was founded to facilitate FMT by providing ready to use donor feces suspensions for treatment of patients with rCDI in hospitals throughout The Netherlands. Prior to FMT, all patients were pretreated with vancomycin 250 mg for 4 days and bowel lavage. In patients with ulcerative colitis as comorbidity, prednisone was added when there was an IBD flare simultaneous.

Results: In total, 129 patients (of which 14 suffered from IBD) were treated with 143 FMTs for CDI with a cure rate of 89.9% after a single FMT (116/129). Fourteen IBD patients were treated with FMT (9 ulcerative colitis, 2 Crohn’s disease and 2 indeterminate colitis). 3/14 patients suffered from rCDI with an active episode of IBD. Of the 14 IBD patients treated with FMT, only one patient developed a relapse of a CDI infection within 2 months (total cure rate 92%). This cure rate does not differ from CDI patients without IBD.

Conclusion: In IBD patients with rCDI, FMT is equally effective compared to other patients with rCDI. In case of concurrent activity of IBD, pretreatment with prednisolone in combination with vancomycin appears to be effective.


A considerable rate of COVID-19 GI symptoms is associated with antimicrobial therapy: Findings from a large western prospective cohort

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Background: Gastrointestinal symptoms are commonly described in patients with COVID-19, but most reports are retrospective and come from Eastern countries.
Objective: Our aim was to evaluate prospectively gastrointestinal symptoms in Western patients with COVID-19.

Methods: We assessed gastrointestinal symptoms in all patients with COVID-19 at our hospital, through the Gastrointestinal Symptoms Rating Scale (GSRS), both at admission and after the start of therapy. Univariate and multivariate analysis for critical clinical picture and death were obtained.

Results: Of 420 enrolled patients, 247 patients (59%) reported at least one gastrointestinal symptom, of which the most common were diarrhea (37%), nausea (19%), urgency (17%), loose stools (16%) and Upper gastrointestinal pain (14%). One-hundred-and-seventeen patients (47%) were symptomatic at admission, while 130 patients (53%) developed symptoms only after the start of COVID-19 therapy. Upper gastrointestinal symptoms, including heartburn (15/117 vs 6/130, P = 0.007), acid reflux (15/117 vs 6/130, P = 0.02), hunger pains (10/117 vs 1/130, P = 0.003), nausea (43/117 vs 35/130, P = 0.04), and rumbling (11/117 vs 3/130, P = 0.02), were more frequent at admission, while diarrhoea appeared more commonly after antimicrobial therapy (57/117 vs 98/130, P = 0.02), were

Conclusion: In our cohort the majority of patients with COVID-19 presented with gastrointestinal symptoms, which had two separate patterns according to the timing of their appearance (at admission vs after antimicrobial therapy) and predicted a worse clinical picture. Therefore, gastrointestinal symptoms may be useful to disentangle characteristics and predict critical clinical picture.

Background: Clostridioides difficile infection (CDI) is the major cause of nosocomial diarrhea related to use of antibiotics in Brazil. The treatment of recurrent CDI is a challenge in countries where fecal microbiota transplantation (FMT) is not widely available. In addition, data on effectiveness and safety of TMF in emerging countries are scarce. The main objective of this study is to evaluate the efficacy of FMT in treatment of recurrent CDI in a Brazilian university center.

Methods: In a prospective pilot study, FMT was performed using frozen samples from our stool bank. Donors were screened according to international guidelines and national regulatory aspects. FMT success was defined as cessation of diarrhea within 8 weeks.

Results: Among the 63 patients with stage IE low grade gastric MALT lymphoma, 37 were excluded, ongoing follow-up (7), follow-up loss (7), transfer-out (4), surgery for gastric outlet obstruction (1), Diffused large B-cell lymphoma with feature of MALT (DLBCL-MALT) (4) and H. pylori negative gastric MALT lymphoma (14). In 18 out of patients lymphoma remission occurred at follow-up without any further therapy following H. pylori eradication. Only one patient who had achieved remission after H. pylori eradication had recurrence. The remaining all 7 patients treated with radiotherapy alone due to lymphoma persistence after successful eradication achieved complete remission. Median age was 55 years (range, 31-67). Median total radiation dose was 30.6 Gy (range, 30.0-30.6 Gy) delivered in 1.7-Gy fractions within 4 weeks to the stomach.

Conclusion: Radiotherapy is effective in achieving complete remission in low grade MALT lymphoma unresponsive to H. pylori eradication.

S. Suk: None. S. Park: None. D. Cheung: None. J. Kim: None.
Impact of high fat diets with different content of unsaturated fatty acids on hydrogen gas generation by the gut microbiota in rats

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Objective: Hydrogen gas is one of the metabolites by anaerobic microbial fermentation in the gut. Recent studies confirmed its antioxidant, anti-apoptotic, anti-inflammatory, cytoprotective and signaling properties. Consumption of different fatty acids can exert its biological effect by modulating the gut microbiome. The aim of our study was to identify the influence of diets on the endogenous hydrogen gas total production (sum of breath and flatulence).

Methods: Experiment was performed on male Wistar rats randomly divided into five groups. Each group was fed by the respective experimental diet for 18 weeks. Normal diet control («ND») and high-fat diets with different fats (30% fat): palm oil «PO», butter «B», trans fat «TF», plant-derived fat «PDF». Hydrogen levels were measured before and after diet supplementation for 18 week by breath test at 0, 3, 6 and 8 hours.

Results: Hydrogen excretion after diet administration «PO» and «PDF» showed significant higher hydrogen gas level that of «ND», respectively, 23.45 ± 3.01 ppm (P = 0.046) and 32.43 ± 6.24 (P = 0.033) ppm vs 15.82 ± 1.72 ppm.

Conclusions: Results demonstrate that intake plant derived fats with higher percentage of unsaturated fats causes increased hydrogen production by the gut microbiota. The results can be explained by the increasing the abundance of hydrogen-producing microbes or by the inhibition of activity of methane generating ones. It was suggested that adherence to a diet rich in unsaturated fatty acids is useful for prevention diseases associated with oxidative damage.

A.Y. Ivanova: None. I. Shirokov: None. S. Trunov: None. N.Y. Samodurova: None. O. Medvedev: None.

EP14.01 | Circulating blood microbiome signatures in patients with liver cirrhosis: Association between clinical parameters, circulating taxa and inflammatory cytokines

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Aim of the Study: To assess structure of circulating microbiome in patients with liver cirrhosis.

Introduction: Intestinal microbiome to plays a significant role in the course of liver disease. Several studies have shown specific circulating microbiome profiles in patients with liver disease. We have aimed to assess circulating microbiome in patients with various degrees of portal hypertension (PH) and look for possible correlation with inflammatory cytokines and intestinal permeability markers.

Methods: Study was conducted in Department of Gastroenterology of Lithuanian University of Health Sciences, Kaunas Clinics. 58 patients with liver cirrhosis and 46 healthy control (HC) subjects were enrolled. 16S rRNA gene sequencing was used to determine bacterial composition of blood plasma samples. Levels of IL-6, IL-8, LPS and FABP2 were measured in cirrhotics and HC subjects.

Results: Blood microbiome in both PH patients and HC subjects was predominated by Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. α-diversity was significantly higher in cirrhotic patients. Bacterial community structure showed a significant clustering between HC and PH patients. Genus Prevotella was associated with higher degree of portal hypertension and worse liver function, while abundance of Escherichia/Shigella - with liver function. Levels of IL-6 and IL-8 correlated with Child-Turcotte-Pugh and MELD scores, while FABP2 – with PH level and MELD score, LPS discriminated cirrhotics and HC. Several taxa correlated with these markers.

Conclusions: Cirrhotic patients have distinct circulating microbiome profile. Levels of Prevotella and Escherichia/Shigella correlated with clinical parameters of liver cirrhosis. Several taxa correlated with inflammatory cytokines and intestinal permeability markers.

EP14.02 | Laboratory assessment of a device prototype for methane and hydrogen breath testing

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Background: Hydrogen Breath tests are limited due to the presence of specific microbes, which consume hydrogen (H2) to form methane (CH4). In this study we conducted laboratory assessment of a prototype device based on MOX sensors with modified surface and adjusted for methane and hydrogen detection.

Materials: A prototype was based on two MOX sensors with two different heating temperatures. It was necessary to decrease the cooling effect of inserted breath/gas samples since the temperature is the key to good sensor performance. To solve this problem the special circulation scheme was designed. The assessment of sensor performance was conducted with 150 mL gas sample. Signals were obtained using target gases in concentrations similar to exhaled concentrations (0-100 ppm with a step of 10 ppm for H2 and 20 ppm for CH4). Cross-reactivity was assessed by H2S (1 ppm) and acetone (20 ppm). To form required gas concentrations and conditions similar to human breath the calibration gases were mixed with humid air using a syringe.

Results and Discussions: The sensors showed high sensitivity to target gases. Detection limit for hydrogen was 1 ppm, for methane was 12.3 ppm. The hydrogen sensor showed no reaction toward CH4, acetone and H2S. The signal obtained by methane sensor toward H2S and acetone was significantly lower than the signal to methane. Humidity of gas sample showed no effect on the performance of the sensors.

Conclusion: The device prototype has promising results for simultaneous measurement of methane and hydrogen. The device in the future may be used for non-invasive diagnosis of gut disorders.

E. Kolomina: None. M. Dmitrienko: None. V. Pazenko: None. I. Jahatspanian: None.

EP14.03 | Multiparameter flow cytometric enumeration of the commercial probiotic products

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The key in probiotics manufacture is to indicate the amount of bacteria in the final product to ensure that the product adheres to regulatory standards and information on product label. Bacterial counting studies are necessary before each clinical trial for probiotics. The plate count method (PCM) is widely used in the probiotic industry to determine the number of microorganisms in a final product. However, this approach is limited by the long incubation time, labor consumption and lack of discrimination in the number of live, dead and viable but non-cultivated microorganisms (VBNC).

Flow cytometry method (FCM) is a new method for assessing the number of live bacteria in single and multi-strain probiotic products. All results were compared to the ISO 19344:2015 standard method which specifies a standardized method for the quantification of bacteria and analyses were conducted in parallel with the traditional PCM. The study has showed that FCM can be applied to probiotic enumeration and viability assessment. We focused on microbial cells viability and vitality based on different physiological parameters: membrane integrity and measurement of the activity of cellular oxido-reductases. No significant differences were found for AFU/g obtained using the two protocols for the samples analyzed, indicating a high degree of equivalence for data obtained for the different staining protocols that measure different parameters of bacterial viability. Results indicated that flow cytometry provides better performances than the traditional PCM. Furthermore using FCM we have opportunity for easier, faster and more accurate routine quality control to assess bacterial viability.


EP14.04 | In-depth comparison of different platforms for high throughput sequencing microbiome analyses of gastric mucosa

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Introduction: To understand the role of the microbiome in human disease, it is important to assess large sample numbers – potentially including different sample cohorts and geographical regions. For collection of the data, various different sampling approaches and protocols including bioinformatic platforms are applied which have the potential to introduce bias into the results obtained. We wanted to assess the variability introduced by applying different bioinformatic analysis platforms to the same data set.

Methods: Five research groups, from Antwerp (Belgium), Oxford (United Kingdom), Kaunas (Lithuania), Sydney (Australia) and Magdeburg (Germany) applied their custom-built pipelines (Qiime, mothur and dada2) and databases for taxonomy annotations (Greengenes, Silva and rdp) to the same input files (fastQ format). The data set comprised 16S rRNA amplicon sequencing from 79 gastric tissue samples from patients with normal gastric mucosa,
chronic gastritis and gastric cancer patients with/without *H. pylori* infection.

**Results:** Preliminary results shown high differences in the number of reads obtained after applying the different analysis pipelines. In addition, some differences in taxonomic annotation that might affect the statistical differences between groups were observed. For instance with regard to stomach, the genera *Campylobacter* or *Helicobacter* were not within the phylum Proteobacteria (as classified in Bergey's Manual of Systematic Bacteriology), but they are annotated as “Campilobacterota”. Comparison between different pipelines using different databases for taxonomic annotations was potentially associated with reporting bias.

**Conclusions:** Understanding the differences introduced by different bioinformatics pipelines is essential. A standardized analysis approach is needed for reproducible microbiome analysis from high-throughput sequencing data.

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Urea breath test: EP1.01, EP1.05
Urease: EP10.03

V
VacA: EP4.02
Vav2: EP3.03
Vonoprazan: EP2.04

W
Watercress: EP10.03