
Hp may play a role in NUD but to date treatment trials have suffered from methodologic flaws. We previously validated a symptom score for use as outcome measure in Clin Epidemio 1993;46:273) in NUD studies. In this study Hp-positive patients (N=51) suffering from NUD were randomized to triple therapy (bismuth subsalicylate (BSS) 300mg qid, amoxicillin 500mg tid, metronidazole 500mg tid) or identical looking placebo. Placebo was especially made up for BSS. Patients symptom score were taken and endoscopy performed (biopsies taken for culture and histology) on day 7 and both baseline, 4 weeks (visit 2) and 6 months (visit 3). If patients continued to be Hp-positive they were actively treated and again reviewed 6 weeks later (visit 4).

Results: Four patients (two active two on placebo) dropped out of study before visit 2. Hp was eradicated in 28 of 27 (94%) patients in active group and 1 of 22 (5%) of placebo group. Although symptoms did improve throughout the study there were no significant differences at visit 2, 3, or 4 in outcomes (measured in 7-point scales: global assessment, eight upper GI symptoms (e.g. epigastric pain, belching, postprandial bloating) and overall quality of life between treatment groups. It has been suggested that it may take longer for symptoms to improve following eradication, but no continuous improvement in symptom severity over time was found between visit 2 and 3 in Hp eradicated patients. The study did have 80% power to detect a two point difference on the seven point symptom scores that were.

Conclusion: Eradication of Hp does not improve NUD symptoms when compared to placebo.

DECISION ANALYSIS OF THE ECONOMIC IMPACT OF H PYLORI ERADICATION REGIMENS. N. Yats, B. Fennerty. University of Wisconsin, Milwaukee, WI & Oregon Health Sciences University, Portland, OR, USA.

We compared a decision analysis model to examine the economic outcomes of various eradication regimens for H pylori related DU over a 2 year period & determined the relative costs of each treatment strategy. Methods: The decisional models were developed & validated using standard decision analysis mathematical "roll-back" techniques using DATA software. Clinical success was defined in the model as eradication of H pylori & no recurrence. The probability of success or failure for each regimen was based on published clinical study eradication rates, treatment regimen compliance, antibiotic resistance rates, annual complication rates. Health care utilization costs were calculated using published figures for costs (e.g. HCFA) including costs of primary therapy & complications but not indirect costs. Each regimen level was compared & cost & its economic impact.
COMPARISON OF PCR TO OTHER ROUTINE INVASIVE METHODS IN THE DIAGNOSIS OF Helicobacter pylori INFECTION. AP Lalge1,3, E Godfroid1, A Fauchonnel1, A Burette1, J-P Butzler1, A Bollen1, Y Glupczynski4, 1 - Service de Genétique Appliquée - ULB - Nivelles, 2 - Nouvelle Clinique de la Basilique - Brussels, 3 - Hôpital Universitaire Saint Pierre - Brussels, 4 - Hôpital André Vésale - Montigny-le-Tilleul - BELGIUM

The detection of H. pylori in gastric biopsy specimens by PCR has been suggested by many authors as a highly specific and sensitive method for H. pylori diagnosis. However, in most series only a limited number of gastric biopsy specimens have been tested. The aim of the present study was to compare a PCR system targeting H. pylori ureC gene to the other routinely invasive techniques, culture, rapid urease test and Giemsa staining of histological sections, in H. pylori diagnosis. Gastric biopsy specimens from 104 consecutive untreated dyspeptic patients (mean age 55.3 ± 3.9, range 19 to 93) were submitted to the four invasive assays. H. pylori was detected in biopsy specimens of 40 (38.5%), 38 (36.5%), 36 (34.6%) and 35 (33.7%) of the patients by PCR, culture, rapid urease test and Giemsa staining of histological sections, respectively. All culture positive specimens also had their biopsy positive specimens by PCR. Furthermore, biopsy specimens from two culture negative patients yielded positive PCR results. The sensitivity and specificity values of PCR compared to those of culture were 100% and 97.0%, while those of rapid urease test and Giemsa staining of histological sections were 89.5 and 97.0 and 89.5 and 98.0, respectively. These findings indicate that PCR is a very useful tool for the diagnosis of H. pylori infection especially where culture is not available.

SEREOLOGICAL TESTS FOR DIAGNOSING HELICOBACTER PYLORI (Hp) ERADICATION. M Ferrana, F Viscarello, D Basso*, G Leonardi*, N Dal Bo', S Salandin, S Kustatscher, GA Grasso, G Battaglia, M Pehaux, P Di Mario, Deps. of Gastroenterology of Padua, #Venice and *IRCCS Castellana Grotte (BA); Dept. of *Clinical Biochemistry of Padua, Italy

Histology is currently considered the gold standard in the diagnosis of Hp-infection; other accurate methods are expensive (Breath-test) or for experimental studies (PCR).

AIM of our study was to identify the most suitable serum index of Hp-eradication among gastrin, Pepsinogen Group A (PGA), C (PGC) and Immunoglobulins anti-Hp (IgG).

METHODS: 472 Hp-positive subjects (288 duodenal ulcer; 53 Gastric ulcer; 131 gastritis) entered the study. All underwent different medical treatment schedules with either Amoxicillin or Azithromycin or Clarithromycin plus Metronidazole plus either Omeprazole or Bismuth for one or two weeks. Endoscopy was performed basally and 2 months after the end of therapy; 6 gastric biopsies were taken from gastric antrum and body to assess Hp-infection by histology (Giemsa modified stain) and rapid urease test (CLO-test). Blood samples were obtained at the same periods to measure gastrin, PGA, PGC (RIA Methods, Sorin Biomedica, Saluggia, Italy) and IgG (RADIM, Pomezia, Italy) levels.

RESULTS: after treatment 322/472 pts. were eradicated (Group 1) while in the remaining 150 pts. Hp-infection was still present (Group 2). The table reports mean values ± standard deviation and statistical analysis (Friedman's test for paired data) of each parameters before and after the treatment.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Before</th>
<th>After*</th>
<th>p &lt; 0.05</th>
<th>Group 2</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrin</td>
<td>64.9±31</td>
<td>53.5±18</td>
<td>0.012</td>
<td>65.0±21</td>
<td>54.5±13</td>
<td></td>
</tr>
<tr>
<td>PGA</td>
<td>133.4±77</td>
<td>83.7±45</td>
<td>0.054</td>
<td>127.5±74</td>
<td>113.5±65</td>
<td></td>
</tr>
<tr>
<td>PGC</td>
<td>19.2±11.4</td>
<td>14.2±1</td>
<td>0.002</td>
<td>19.3±10.6</td>
<td>14.2±1.4</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>57.8±42</td>
<td>42.1±37</td>
<td>0.001</td>
<td>58.8±40</td>
<td>56.2±41</td>
<td></td>
</tr>
</tbody>
</table>

According to the percentage of reduction, PGA was the most accurate index of Hp-eradication (ROC curves). A decrease higher than 38% was the most sensitive (75.5%) and specific (74.5%) value in predicting eradication of Hp-infection with diagnostic accuracy of 75.2%.

CONCLUSION: PGC levels may be a useful tool in the follow-up of Hp-infected patients; 2) PGC represents an early marker of Hp-eradication. Significant variations occur within two months after the end of the treatment when IgG levels remain still higher.

ONE YEAR MONITORING OF HELICOBACTER PYLORI (Hp) ERADICATION BY SERUM PEPSSINOGEN A AND C AND Hp IgG AS MARKERS OF Hp ERADICATION AND IN THE FOLLOW-UP OF THE PATIENTS. Aim of this study was to evaluate the relationship between Hp-eradication and PGA, PGC and IgG levels and their usefulness in the follow up of Hp-eradicated patients. 476 pts (262 DU, 53 GU, 27 DU+GU, 134 gastritis, mean age 55 yrs range, 17-80, 322M:154 F) entered the study. All the pts were positive for Hp-infection by two tests: histologic examination of six antral and body biopsies (Giemsa modified stain), the urea test (CLO-test). In order to eradicate the bacterium, the pts were treated with different schedules of Omeprazole, Amoxicillin or Azithromycin or Clarithromycin and Metronidazole for one or two weeks. Two months and twelve months later, an endoscopic control was performed. In the follow-up of the patients. The samples were collected by the 17th, 34th, 6th, 12th month of the eradication period. After the eradication period, the patients were treated with different schedules of Omeprazole, Amoxicillin or Azithromycin or Clarithromycin and Metronidazole. The follow-up of the patients. The samples were collected by the 17th, 34th, 6th, 12th month of the eradication period. After the eradication period, the patients were treated with different schedules of Omeprazole, Amoxicillin or Azithromycin or Clarithromycin and Metronidazole. The follow-up of the patients. The samples were collected by the 17th, 34th, 6th, 12th month of the eradication period. After the eradication period, the patients were treated with different schedules of Omeprazole, Amoxicillin or Azithromycin or Clarithromycin and Metronidazole.
VALIDATION OF TWO NEW RAPID BLOOD TESTS FOR *H. PYLORI* AE Dugan, A Knifton, RPH Logan, CJ Hawkey, RFA Logan, Div Gastroenterology & Dept Public Health Medicine, University Hospital, Nottingham.

Introduction: A rapid, reliable blood test for *H. pylori* would reduce unnecessary investigations for dyspepsia since duodenal ulcer disease and gastric cancer are both rare in the absence of *H. pylori*. We therefore assessed two new rapid tests, Flexsure and the Helisal against routine tests for *H. pylori*. Centrifugation of blood is recommended for Flexsure. Since this is not routinely available in primary care, we evaluated its performance on serum formed from blood specimens allowed to stand for a short period.

Methods: Blood was taken from 100 patients presenting for endoscopy. To reflect conditions in primary care the Flexsure was tested on the serum formed from blood allowed to stand for a half, one and three hours. Helisal was compared on 53 of these specimens. Both tests were performed blind to the *H. pylori* status; Helisal was considered present if histology (antral and corpus; H&E, toluidine blue) and CLO, culture, or C14 urea breath test were positive. Results that were negative on Flexsure or Helisal were retested using routine ELISA serology (Helic-G).

Results: On standard testing 57 patients were positive for *H. pylori*, 41 negative and 2 were indeterminant.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helisal</td>
<td>1/1 1 1 hour</td>
<td>3 hours</td>
</tr>
<tr>
<td>Flexsure</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>63%</td>
<td>66%</td>
</tr>
<tr>
<td>Specificity</td>
<td>92%</td>
<td>10%</td>
</tr>
</tbody>
</table>
| Invalid & false negative Flexsure results were more likely to occur on early testing, suggesting insufficient serum had formed. Only 1 patient with *H. pylori* was negative on all serological tests.

Conclusion: Flexsure testing of serum formed from unspun blood is an alternative if centrifuging facilities are not available. It has greater sensitivity at 3 hrs and similar specificity to Helisal.


In the present study we prospectively evaluated the accuracy of the 13C-UBT for the detection of *H. pylori* infection by using two different test meals. Material and Methods. A total of 181 consecutive patients (age range 16-73 years, 92 male, 89 female) submitted for the evaluation of the *H. pylori* status in our gastrointestinal function laboratory were included in the study. All patients had previously undergone endoscopy and *H. pylori* infection testing by the urease rapid test, histology and culture from antral biopsies. 53 patients had macroscopic signs of gastritis. 5 had gastric ulcer, 20 duodenal ulcer and the remaining 103 patients a normal finding at endoscopy. The 13C-UBT was performed by giving 75 mg of 13C-urea 10 min after administration of either 200 ml of a commercially available semiliquid meal Meritene* (n=108 patients) or 200 ml of citric acid 0.1 N (n=73 patients). The 13CO2/12CO2 ratio in a basal breath sample was compared with that of a sample collected at 30 min after the administration of 13C-urea. The ratio increase (delta, D) was corrected according to the weight of the patient (nD). A positive test is defined by a nD >0.25. The sensitivity (S), specificity (Sp), positive and negative predictive values (PPV and NPV) and overall accuracy (QA) were calculated for both test procedures. Results. 111 patients were found to be *H. pylori* positive by at least two tests among urea rapid test, histology and culture. The 13C-UBT was performed in 69 of them with Meritene and in the other 42 with citric acid. Results are summarized in table.

<table>
<thead>
<tr>
<th>Test meal</th>
<th>S</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meritene</td>
<td>86%</td>
<td>95%</td>
<td>97%</td>
<td>97%</td>
<td>99%</td>
</tr>
<tr>
<td>Citric acid</td>
<td>95%</td>
<td>100%</td>
<td>94%</td>
<td>90%</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions. The accuracy of the 13C-UBT for the detection of *H. pylori* infection is substantially increased by using a citric acid solution as test meal instead of a semiliquid test meal.

STANDARDISATION OF THE 13-C-UREA BREATH TEST IN CHILDREN. S. Cadzarea, L. Corvaglia, E. Keppens *. Gastroenterology Department, Queen Fabiola Children's Hospital and * Geology Department, Free Universities of Brussels.

We use routinely 13-C-urea breath test (UBT) for the detection of HP infection in children and also as follow up of the efficacy of the treatment and in epidemiology studies in the siblings and parents of the symptomatic children. Since October 1988, 791 UBT were performed. Aim: to evaluate the sensitivity and specificity of UBT. Methods: A dose of 2 mg/kg bodyweight (max. 100 mg) is ingested after a normal meal followed by a fat-rich chocolate ice-cream; breath samples are taken at 0, 5, 10, 20 and 30 minutes, stored in 20 ml vacutainers and assayed by mass spectrometry. We reviewed all the UBT performed in children at the time of diagnosis for whom a corresponding HP biopsy culture was available.

Results:

- Biopsy culture
- Positive Negative
- UBT Positive 100 4 104
- Negative 0 80 80

Sensitivity 100%, specificity 95%, positive predictive value 96%, negative predictive value 100%.

In 10 patients the test was repeated, before treatment, on 4 successive times, with the same dose given either as a solution or as a tablet, fasting or after a meal. All 4 tests gave comparable reliable results but the post-fatty meal UBT did not always yield the highest curve.

Conclusion: UBT is probably the most reliable test for the detection of HP-infection in children and compares favourably with all other more invasive tests.

DIAGNOSIS OF H. PYLORI INFECTION IN PEPTIC GASTRIC ULCER. HOW TO DO IT PROPERLY? K. Seppälä, P. Sipponen, H. Nuutinen, T.U. Kosunen. Helsinki University Central Hospital and Departments of Pathology and Immunology, University of Helsinki, Finland.

A true peptic gastric ulcer is always associated with *H. pylori* positive chronic gastritis. According Sobala & Axon (1992) the percentage of *H. pylori* infection rates varied greatly among gastric ulcer patients (mean 77%, range 56-96%) in 15 studies consisting of 914 gastric ulcer patients.

In the present study we investigated the value of different diagnostic procedures for *H. pylori* infection before and after *H. pylori* eradication in 106 patients with chronic peptic ulcer.

Material and methods. The series included peptic ulcer patients in association with *H. pylori* gastritis (32 gastric ulcers, 19 prepyloric, 3 pyloric and 52 duodenal ulcers). *H. pylori* infection was considered to be present if at least two of the following tests were positive culture, histology, serology, and rapid urease test (Jatrox). To ensure high diagnostic reliability, several biopsies from antrum and corpus were taken for histology, culture and urease test.

Results. Before the eradication therapy *H. pylori* culture was falsely negative in 2% (2 of 106), histology in 10.4% (11/106), serology 7.5% (8/106). The urease test was negative in 11.3% (5/44). In gastric ulcer 40.6% (13 of 32) of patients had at least one false negative diagnostic pretreatment test. After successful eradication all patients were negative for *H. pylori* in both culture and histology and strongly decreased antibody titer.

Conclusion. In this series gastric ulcer patients with chronic gastritis had *H. pylori* infection as often than duodenal ulcer patients. Accurate diagnosis of *H. pylori* infection in peptic ulcer disease is essential, because the therapy is very dissimilar in *H. pylori* negative and positive ulcer patients.
EVALUATION OF HELICOBLOT 2.0 - A NEW COMMERCIAL WESTERN BLOT TEST FOR THE SEROLOGICAL DIAGNOSIS OF HELICOBACTER PYLORI INFECTION. Y. Gharpurey,1 C. Van den Born,2 S. Goulton,3 J.P. Butzler,2,3 A. Bunnett,2 Hôpital André Vésale, Montigny-le-Tilleul,1 Hôpital Brugmann,2 Hôpital St-Pierre,3 and Nouvelle Clinique de la Basilique,1 Bruxelles, Belgium.

AIM & METHOD: To evaluate a new commercial Western Blot (WB) kit (Helico Blot 2.0, Genelabs Diagnostics, Singapore) for the serodiagnosis of H. pylori infection. 1) on consecutive serum samples obtained from 76 untreated adults (range 18-85 yrs, mean, 52 yrs) undergoing routine endoscopy for symptoms relating to the upper GI tract, 2) on sera from 17 successfully treated patients followed 1-72 months after eradication, and 3) on selected serum samples obtained from 90 H. pylori-positive patients with peptic ulcer (DU or GU; n=40) or with NUD (n=50). In all three groups, the performance of the WB was evaluated in comparison with culture, histology, rapid urease test and ELISA (Cobas Core, Roche) staining. Patients were considered as H.pylori-ve either on the basis of a positive culture result or if two other tests were positive.

RESULTS: In the group of consecutive dyspeptic patients, 36 individuals were H.pylori-positive by culture and histology and 40 were H.pylori-negative. The sensitivity, specificity, negative and positive predictive values of the WB in this group was: 92, 88, 92 and 86% vs. 81, 83, 84 and 90% for the ELISA. Overall, the most common reactive bands in the WB tests were the 26.5 kD and the 116 kD proteins which reacted with 92% and 76% of the H. pylori-positive sera. After treatment, the WB had a positive predictive value for a successful eradication of 70% (i.e. 7/10 patients were negative) more than 3 months after treatment vs. only 40% for the ELISA (i.e. 4/10 patients were negative). In the subgroup of 70 H. pylori-positive group with defined clinical diagnosis, the overall sensitivity of the WB was 92.8% with no difference observed between NUD patients and ulcer patients. However, reactivity to the 116 kD (cagA) protein was found significantly more frequently in the ulcer group than in the NUD group (82% vs. 70%; p=0.01).

CONCLUSION: Helico Blot 2.0 is a suitable non-invasive assay for the serodiagnosis of H. pylori infection. This test has at least equivalent sensitivity and specificity to ELISA and provides additional information such as the full serological profile for reactivity to individual H.pylori proteins which the ELISA cannot provide.

CONFIRMATION OF SUCCESSFUL THERAPY OF H. PYLORI INFECTION: NUMBER AND SITE OF BIOPSY VS. A RAPID UREASE TEST. H.M.T. El-Zaatari, R.M. Genta, D.Y. Graham. Department of Medicine, V.A. Medical Center and Baylor College of Medicine, Houston, TX, USA.

Background: Although a number of tests have been described to detect the presence of H. pylori in biopsy specimens, studies of positive and negative value have largely been performed on untreated patients; the reliability of post therapy is unknown.

Methods: We examined the value of the number and site of biopsies performed and the method used for specimen evaluation post-therapy. Post antimicrobial therapy 141 patients with previously confirmed H. pylori infection had 3 biopsies taken, 2 from the antrum and 1 from the corpus. Individual slides were coded, randomized, and interpreted blindly by two pathologists. Further, in 143 patients a biopsy specimen was taken from the antrum and immediately inserted into the gel of the rapid urease test and the results were compared to those obtained from histopathology.

Results: In 71 patients H. pylori therapy was unsuccessful; in 61 (96%) all 3 sites were positive. The highest yield with a single large cup biopsy specimen was 94%, the lowest 91%. Two antral biopsies were negative in 4% (95% CI = 1% to 12%). The combination of a biopsy from the antrum inanria and one from the greater curvature of the corpus correctly identified all treatment failures. The rapid urease test was false negative in 5% (95% CI = 1% to 13%), there were no false positives.

Conclusion: Use of either the rapid urease test or 2 antral biopsies for evaluation of success of antimicrobial therapy for H. pylori infection will result in a false declaration of cure in at least 5% of cases. Three large cup gastric mucosal biopsies for histology are recommended for evaluation of the success of anti-H. pylori therapy.

DETECTION OF HELICOBACTER PYLORI IN GASTRIC BIOPSIES BY IN SITU HYBRIDIZATION. T.J. Karpuz, F.A.K. El-Zaatari, R.M. Genta, B. Yoffe, D.Y. Graham. Departments of Medicine and Pathology, VAMC and Baylor College of Medicine, Houston, TX.

Although histology is sensitive method for diagnosis of H. pylori infection it is not specific. False positive diagnoses are possible due to contaminating flora, while treatment potentially causes false negative results by low numbers of organisms or altered morphology (e.g., cocoid forms). We developed a non-radioactive in situ hybridization (ISH) method for detection of H. pylori and compared it with conventional methods for diagnosis of the infection.

Methods: Two 22-base primers were used to amplify approximately 500 base pair segment of 16S rRNA gene from H. pylori (KD26). PCR product was labeled with digoxigenin and used as a probe. Specificity of the probe was tested with dot blot hybridization with DNA from over 20 different clinical isolates of related and unrelated bacteria. For ISH, sections of paraffin embedded gastric biopsies were treated with proteinase K, hybridized with heat denatured probe, and bound probe was detected with alkaline phosphatase labeled anti-digoxigenin antibodies. Specificity of hybridization reaction was shown by negative reaction with irrelevant probe, without probe and after RNase treatment. A random sample of 15 biopsy specimens was studied in a blind fashion using the ISH method and the results were compared with those of culture and conventional histology. Results: Comparison of ISH and conventional histology showed agreement in all 15 specimens (4 negative and 11 positive). Between culture and ISH there was agreement in 13 cases (8 positive, 5 negative). Two cases were negative by culture but positive by ISH and histology.

Conclusion: Non-radioactive ISH provides a sensitive and specific method for detection of H. pylori infection. It should be particularly useful for confirmation of infection in cases unequivocal with other methods, and potentially in post-treatment evaluation.
IgG-ANTIBODIES AGAINST H. pylori AND PROGNOSIS OF EFFECTIVE ERADICATION

M. Caroli and A. Saggio - Department of Gastroenterology - Umberto I Hospital - Venice-Mestre - Italy

The behaviour of IgG-antibodies against H. pylori after eradication is still under study and no data are certain in the definition of a cut-off for therapeutic efficacy.

Purpose of this study was to identify the true possibility to use IgG anti-H. pylori antibodies as a marker of the healing of the infection.

We included 46 patients: 10 normal and 36 duodenal ulcer H. pylori positive volunteer patients. All were studied by endoscopy, urea test and histology before and two and twelve months after the end of a triple therapy and agreed to have their serum IgG-antibodies against H. pylori tested every 2 weeks to establish the cut-off level useful to define eradication. IgGs were expressed in U/ml and mean and confidence interval considered. 32 DU patients were eradicated (negative histology and urea test after 2 and 12 months), and eradication was confirmed by a normalization of serum IgGs (below 14 U/ml) after 9 months in all patients. The slope of the curve being greater for the higher values at the beginning, but not significant at any point, suggesting the 9 months point as the true cut-off.

IgG anti-H. pylori U/ml

<table>
<thead>
<tr>
<th>Controls</th>
<th>Eradicated</th>
<th>Not Eradicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>12.00</td>
<td>12.00</td>
</tr>
<tr>
<td>2</td>
<td>22.00</td>
<td>22.00</td>
</tr>
<tr>
<td>4</td>
<td>24.00</td>
<td>24.00</td>
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<tr>
<td>6</td>
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<tr>
<td>8</td>
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<td>38.00</td>
</tr>
<tr>
<td>20</td>
<td>40.00</td>
<td>40.00</td>
</tr>
</tbody>
</table>

Conclusions: High dose omeprazole administration results in an effective inhibition of urease activity in vivo. This observation may be of clinical importance as urease based diagnostic procedures may report false negative results for Hp.

A MONOCOCCAL ANTIBODY TO THE HELICOBACTER PYLORI 128 kDa VIRULENCE ASSOCIATED PROTEIN

J. C. Stephen, P. E. J. Larkins, B. J. Rathbone, University of Leicester, Leicester Royal Infirmary.

The 128 kDa protein of Helicobacter pylori, a product of cagA gene, is not found in all isolates. This protein appears to be a marker of virulence in that antibodies to this protein are found more commonly and in higher titre in Helicobacter pylori positive patients with ulcer disease than in Helicobacter pylori patients without ulcer disease. The presence of a local gastric antibody response to the 128 kDa protein correlates with the severity of histological gastric inflammation.

Using an Helicobacter pylori isolate with a relatively large amount of the 128 kDa protein, a preparation of purified polypeptide was obtained by electrophoresis, and used as antigen for monoclonal antibody production. After three fusions a satisfactory monoclonal characterized as IgG1 was obtained.

The monoclonal has identified using immunoblotting the 128 kDa protein in 30 clinical isolates known to be 128 kDa positive. The amount of protein varies considerably between isolates. One NCTC isolate thought to be negative for the 128 kDa protein by gel electrophoresis has been shown to be positive by blotting with the monoclonal. As well as being a very specific marker to help type Helicobacter pylori isolates, the 128 kDa monoclonal also provides a means to carry out a specific second generation Helicobacter pylori serological assay.

H. PYLORI UREASE ACTIVITY IS INHIBITED BY HIGH DOSE OMEPRAZOLE IN VIVO.

B. Stoch et al., E. Dominguez-Muñoz, T. Sauerbruch, P. Malfertheiner. Department of Medicine, University of Bonn, FRG and Department of Medicine, University of Magdeburg, FRG.

Omeprazole has an antibacterial effect on Helicobacter pylori (H. pylori) and selectively inhibits H. pylori urease activity in vitro. Aim of the present study was to investigate the effect of different doses of omeprazole on the H. pylori urease activity in vivo.

Methods: 12 patients with H. pylori-associated chronic gastritis were studied. H. pylori diagnosis was based on histology, CLO-test and culture from antral biopsies, serology and 13C- urea breath test (13C-UBT). Patients received omeprazole 40 mg (n=6) or 80 mg (n=6) p.o. for 5 days and 13C-UBT was performed on day 1, 3 and 5, 30 minutes after omeprazole administration. The 13C-UBT was performed with 200 ml 0.1 N citric acid and 75 mg 13C-urea in 50 ml water. Breath samples were collected before and 30 minutes after 13C-urea administration. Results were expressed as normalized delta (δ) and compared by student t-test. A positive 13C-UBT was defined by a δ, > 0.25. Results: Significant inhibition of urease activity was observed only under high dose omeprazole administration (figure) and three patients had a negative 13C-UBT on day 5.

Conclusions: High dose omeprazole administration results in an effective inhibition of urease activity in vivo. This observation may be of clinical importance as urease based diagnostic procedures may report false negative results for Hp.

FlexSure™HP WHOLE BLOOD TEST- A RAPID METHOD TO DETECT ANTIBODIES AGAINST HELICOBACTER PYLORI


BACKGROUND: A novel, immunochromatographic serologic test-FlexSure™HP which detects serum antibodies against Helicobacter pylori in a rapid test format was described previously (Schrier, et al., Clin. Chem. 40:1022, 1994). A rapid, convenient, point-of-use FlexSure™HP whole blood test (currently under development) that can use either a drop of whole blood, serum or plasma is described. METHODS: The test incorporates a blood separation mechanism in place of the current sample pad. One drop of venous whole blood containing heparin or EDTA, or capillary blood from a finger stick is applied to the sample pad. The cellular components of the blood are retained by the separation mechanism while the serum or plasma passes into the test strip and chromatographs up the strip to the capture zone. The separation mechanism is capable of yielding 25-30 uL of plasma from 50 uL of whole blood. The capacity of the separation mechanism exceeds 100 uL of whole blood. Following the addition of the whole blood, the procedure is the same as the FlexSure™ HP serum test. Test results are interpreted 4-6 minutes after closing the test device. RESULTS: A group of 211 volunteers provided matched whole blood and serum samples and were tested with the HM-CAP EIA, FlexSure™ HP serum, and FlexSure™ HP whole blood tests. Of the 211 samples, 74 were positive, 131 were negative and 6 were indeterminate by HM-CAP. The indeterminate samples were removed from the analysis. The FlexSure™ HP serum and whole blood test agreement with the HM-CAP EIA was 93.7% and 93.2%, respectively. Agreement between the FlexSure™ HP serum and whole blood tests was 96.9%. CONCLUSION: FlexSure™ HP whole blood is a simple, rapid, point-of-use, visually read test that requires only a drop of whole blood from a finger stick or venipuncture. Further development of this non-instrumented, rapid diagnostic technology is expected to provide a cost-effective method for immediate diagnosis of H. pylori infection in patients with peptic ulcer disease and related upper G.I. disorders. The test would also be expected to facilitate testing at sites that do not have laboratory equipment required for conventional serologic testing (i.e., centrifuge, microtiter plate reader, etc.).
THE 13C-UREA BREATH TEST FOR EARLY ASSESSMENT OF HELICOBACTER PYLORI ERADICATION.


Introduction. The 13C-urea breath test (13C-UBT) is an accurate method to non-invasively detect the actual presence of Helicobacter pylori (H. pylori) infection. For assessment of eradication, 13C-UBT is currently performed, as suggested for other accurate methods such as histology, urease test and culture, four weeks after treatment withdrawal in order to avoid false negative results due to partial suppression of the infection and subsequent recolonization. However there are at present no studies considering the possibility that an accurate method such as 13C-UBT could detect true eradication at an earlier stage. Aim. To determine whether 13C-UBT is capable to assess H.p. eradication earlier than the conventionally adopted four week interval after the end of treatment. Methods. 58 patients (31 males, 27 females; range age 25-69, mean age 48 yrs) with non-ulcer dyspepsia and H.p. infection, participating in an ongoing randomized double blind, double dummy eradication study, underwent upper GI endoscopy and were evaluated by urease test, histology and culture before treatment and four weeks after withdrawing medications. 13C-UBT was performed (European standard protocol, positive result = excess Δ 13CO2 excretion > 3 per ml) before treatment and every week for four weeks after withdrawing medications. Results. In 23 out of 58 patients H.p. eradication was established at four weeks after treatment by negative urease test, histology, culture and 13C-UBT (excess Δ 13CO2 excretion = 14.5±0.17(mean±SE) per ml). In all 3 patients in whom eradication was assessed at four weeks after treatment the 13C-UBT was already negative at one week (excess Δ 13CO2 excretion = 17.2±0.18(mean±SE) per ml). And in all but 3 patients in whom successful eradication was not achieved the 13C-UBT was positive when the first week (excess Δ 13CO2 excretion = 31.4±0.32(mean±SE) per ml), 3 patients had a negative test at one week (excess Δ 13CO2 excretion = 1.8±0.7(mean±SE) per ml) which however turned to be positive at the second week evaluation. Conclusions: 13C-UBT can assess Helicobacter pylori eradication as soon as one week after treatment withdrawal. When performed at 2 weeks it showed the same sensitivity and specificity of the test performed at four weeks after withdrawing medications.

A SIMPLIFIED, RELIABLE UREA BREATH TEST.


INTRODUCTION. Non-invasive tests to detect H. pylori are necessary for epidemiological studies, investigation of dyspepsia in younger patients and in primary care and for follow-up after eradication treatment. The 13C urea breath test (13C-UBT) is highly sensitive and specific. However, the current recommended protocol for the UBT, including an overnight fast, the use of large reservoir bags and multiple positional changes and sample collections render the test unsuitable for widespread use.

AIM: The aim of this study was to assess the sensitivity and specificity of a modified, simple UBT.

METHODS: Subjects undergoing endoscopy for investigation of dyspepsia or after a course of eradication therapy were recruited. Non-eradicating patients were administered a standard motility inhibiting liquid meal (10mL Celopepin®10mL Ensure). Immediately after, breath samples were collected in duplicate in 10mL vacutainer syringes. Subjects then received 100 mg of carbon urea dissolved in 100 mL of water. After sitting for 30 minutes, repeat breath samples were collected in vacutainers. An excess of 15002 excretion of 5 per mL was taken as a positive result. The UBT results were compared with antral and corpus histology (±), antral and corpus culture (±) and antral CLO test. The 'gold standard' was defined by the results of any two of the biopsy based methods.

RESULTS: 184 patients were recruited (98 male), mean age 42 years (range 17-79). 85 subjects had a positive UBT. 85 true positives and 4 false negatives. 80 subjects had a negative UBT; 78 true negatives and 2 false negatives. Therefore the sensitivity was 97.7% and the specificity was 95.1%.

CONCLUSION. This modified UBT is user-friendly, ideal for use in a primary care setting and is as reliable as other methods for the non-invasive detection of H. pylori.

DNA SEQUENCE OF CAG1, A NEW MULTIGENE LOCUS IMPLICATED IN H. PYLORI VIRULENCE.


The ~21 kb cagl ("second cytotoxin associated gene") locus (Akopyants et al., this meeting), like the previously described vacA (vacuolating cytotoxin) and cagA loci, seems to be characteristic of the most virulent H. pylori strains, i.e. those strains routinely recovered from patients with peptic ulcers or gastric cancer and less often from asymptomatic carriers. To help identify functions encoded by this locus, a cosmid carrying the entire cagl region plus flanking H. pylori DNA (39 kb) was sequenced via a shotgun-based automated DNA sequencing strategy. To ensure high accuracy, all regions were sequenced at least three times, and at least once on each strand. Open reading frames and sequence motifs of interest were identified using the standard internet-based BLAST and BLOCKS programs. The cagl region is gene-rich, as is typical of bacterial genomes, and 64% A+T, which is quite typical for H. pylori. Several inferred cagl region proteins exhibit high levels of homology to proteins found previously in other species. Apparent homologs include: cell surface proteins of Bordetella, E. coli, Neisseria and V. cholerae that participate in plasminogen activation and/or DNA transfer; the malarial MESA protein that binds the cytoskeleton and is found on infected erythrocyte surfaces; an NaH antipporter from E. coli; a regulator of dipetide permease operon expression in B. subtilis; a restriction endonuclease from E. coli; and the 12500 kD transposase encoded in S. typhimurium. The present cagl DNA sequence and its open reading frames will help guide further mutational, gene expression and histopathologic studies to better characterize cagl-encoded proteins, and tests of our expectation that some of them will affect the extent of tissue damage, inflammatory or immune responses, or host cell proliferation during H. pylori infection, and thereby help determine the severity of disease.

ACTIVATION OF HELICOBACTER PYLORI BOUND PLASMINOGEN BY TISSUE-TYPE PLASMINOGEN ACTIVATOR (tPA) - a virulence trait in peptic ulcer disease.

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Plasminogen is a plasma- and extracellular matrix protein, important in haemostasis as a main mediator of fibrinolysis. Activators such as tissue-type plasminogen activator (tPA), splits one peptide bond of the native plasminogen creating the active plasmin. Receptor bound plasminogen facilitates activation to plasmin and protects the molecule from inactivation by several bacterial plasminogen bind-eractivators, followed by activation to plasmin, which is assumed to be an important virulence mechanism for tissue penetration.

Binding of plasminogen to Helicobacter pylori was studied using 125I-labelled plasminogen. Activation of H. pylori cell bound plasminogen was performed by incubating plasminogen with the bacteria in presence of tPA. Conversion of plasminogen to plasmin was detected in a spectrophotometer at 405nm after adding the chromogenic substrate S-2251 (Chromogenix, Mölndal, Sweden).

All of the H. pylori strains tested bound 125I-plasminogen. H. pylori strain 17874 (NCTC 11637), was selected for further characterisation of the plasminogen binding. Inhibition of the interaction between H. pylori and plasminogen was observed after preincubation of the bacterial cells with non-labelled plasminogen, plasmin, lysine and the lysine analogue e-amino caproic acid. Our findings clearly showed that H. pylori binds specifically the loop structures of the plasminogen molecule. Plasminogen fragments, containing the loop structures, did also inhibited the plasminogen binding to H. pylori. Plasminogen bound to H. pylori activated to plasmin in the presence of tPA. No activation was observed when plasminogen or tPA were incubated with bacteria alone.

Formation of plasminogenase by H. pylori may be important to provide a powerful proteolytic mechanism for tissue penetration of peptic ulcers, since plasmin degrades not only fibrin but also matrix proteins such as collagen and fibronectin.