LETTERS TO THE EDITOR

Immunocytochemical detection of Helicobacter pylori in formalin fixed tissue biopsy specimens

We have read with great interest the many excellent articles published in this journal concerning Helicobacter pylori infections. Several of these reports have used histological examination of tissue sections to identify the bacillus. Several of these special histochemical stains do not distinguish H pylori from other spiral Gram negative organisms which have now been reported to occur in the stomach.

Previous reports have shown the usefulness of immunocytochemical methods that use monoclonal antibodies in the identification of H pylori. These antibodies however, are not commercially available nor do they seem to be applicable to formalin fixed, paraffin wax embedded tissue biopsy specimens. Preliminary studies performed in our laboratory using a commercially available monoclonal antibody (Bioproducts for Science, Inc., Indianapolis, USA) which reacts with a formalin resistant flagellar epitope on Helicobacter organisms produced promising results in identifying H pylori in formalin fixed, paraffin wax embedded tissues.

We have now investigated the gastric biopsy specimens from 130 patients for the presence of H pylori with this monoclonal antibody. The use of a sensitive avidin-biotin immunoperoxidase labelling system allows the monoclonal antibody to be used at a dilution of 1/200000, making it very cost effective. Crisp, clear immunoreactivity is obtained which is easily recognised (figure). Immunolabelled specimens can be screened under low power magnification due to the high signal:noise ratio. No labelling of other Gram negative bacteria or normal tissue elements has been observed.

In a recently concluded study with Drs Scholes and Santogade at St. Luke’s/ Roosevelt Hospital Center (New York) we had the opportunity to apply our immunocytochemical technique to 115 biopsy specimens from 51 patients where tissue was also cultured for the presence of H pylori. All 24 patients with a positive tissue culture had positive immunoreactivity with Helicobacter monolonal antibody. Immunoperoxidase labelling identified five additional positive specimens that were culture negative. There were no false negative results.

Immunoperoxidase labelling has now become the routine method for H pylori identification in biopsy specimens at our institution. This method is attractive because all tissue fragments can be submitted to the surgical pathology laboratory in formalin fixative, thereby reducing potential sampling errors. The resulting immunoreactivity is easy to identify, making screening for H pylori less tedious. H pylori organisms can also be distinguished from contaminating bacteria. Based on our experience with this monoclonal antibody and immunoperoxidase labelling, we recommend immunocytochemical staining for the sensitive and specific identification of H pylori in processed tissues.

RF CUITRN
CA PEDEPEREN
GA KRYZMYOWSKI
BM BORAN
Anastomosis Pathology Division, Department of Pathology, Hartford Hospital, Hartford CT 06115-9729 USA

5 Cartus RW, Al-Annabi N, Morin SG, Pedersen CA, Ludwig ME. Immunocytochemical idenification of Campylobacter organisms in formalin-fixed, paraffin-embedded tissues utilizing a commercially available monoclonal antibody (MAb). Gastroenterology 1989; 96: 75A.

AgNOR counts in intraendometrial neoplasia

Many classifications of endometrial hyperplasia have been suggested, including that of intraendometrial neoplasia (IEN grades I-III). Although this term is not in common use and has been criticised, we have found it to be a simple and reproducible way of describing increasing grades of architectural, cellular, and nuclear atypism in dysplastic endometrial proliferation—that is, confined above the myometrium. Recently the enumeration of silver stained nuclear organiser regions (AgNORs) in tumour pathology has yielded a wide range of conflicting results. Consequently we performed the AgNOR technique on IEN lesions, as well as on normal and frankly malignant endometrial tissue.

This technique was applied to 56 endometrial curettage samples which were grouped by a separate observer into the following categories: normal proliferative (n = 10); cystic glandular hyperplasia (n = 10); IEN I-III (10 cases for each grade); and well differentiated adenocarcinoma (n = 6). Myometrial invasion was ruled out in IEN III by subsequent examination of the hysterectomy specimens. One hundred randomly selected cells were counted for each case using a x 100 oil immersion lens.

Statistical analysis was performed using the Mann-Whitney U test (figure). The lowest AgNOR counts were seen in normal (median 2.99) and hyperplastic (median 3.12) tissue; intermediate counts in IEN I (median 3.58); II (median 4.19), and III (median 4.93); and highest counts were obtained in adenocarcinoma (median 5.72). There was considerable overlap among the groups. Significant differences (p < 0.05) existed between the following groups: (1) benign endometrium (proliferative, cystic glandular hyperplasia) and IEN; (2) benign endometriosis and adenocarcinoma; (3) IEN I and the two other IEN grades; (4) IEN I and adenocarcinoma; (5) IEN II and adenocarcinoma.

IEN denotes dysplastic intraendometrial growth including adenocarcinoma in situ (IEN III), which is considered to be a precursor to adenocarcinoma. Despite the lack of morphological criteria to predict the outcome of endometrial dysplastic lesions, morphometrical studies indicate that cases with larger and more pleomorphic nuclei progress to cancer. Our results show that AgNOR counts may reflect on increase in nuclear activity with this increase in nuclear atypism and hence IEN grades.

Our conclusion is that despite significant differences, AgNOR counts are of no use for diagnosis of any single case in the studied groups because absolute differences between counts for the various categories were small.

Reproducible test for detecting Helicobacter pylori in frozen samples

Retrospective investigations of Helicobacter pylori in stored samples might help us to understand the aetiological role of Helicobacter pylori in different lesions with which it has been associated.1 Helicobacter pylori, however, is a fastidious micro-organism, and it is difficult to store it without pronounced loss of viability.2 Some investigators have studied the presence of Helicobacter pylori in stored samples using stained sections,3 but no one has investigated the reproducibility of a method to detect Helicobacter pylori in stored samples. The aim of this study was to evaluate prospectively the accuracy of a simple and rapid urease test and culture techniques for the detection of Helicobacter pylori in infected frozen gastric biopsy specimens.

A total of 52 antral gastric biopsy specimens were included in the study. The biopsy specimens were ground in 1 ml of 20% sterile glucose chilled transport medium with the pH adjusted to 6.8. A fraction of each specimen was processed for culture and rapid urease test assay. The remaining parts of the homogenised specimens were divided into three groups and stored at −70°C. The groups were reanalysed at different times (two, four, and six months after endoscopy) before using the rapid urease test and culture.

The culture was performed on Skirrow’s Campylobacter agar (Difco Laboratories, Detroit, Michigan), tryptase soy agar (Difco) supplemented with 7%, horse blood, 1%, IsoVitalex, and with or without antibiotics (10 mg/l vancomycin, 5 mg/l trimethoprim lactate, and 2500 IU/l polymixin B), chocolate agar, and Thayer-Martin agar (BBL) with 1%, IsoVitalex and 2%, haemoglobin. Plates were incubated at 37°C for up to seven days in a Campy Pak (BBL) generating microaerophilic or a Campylobacter gas generating Kit (Oxoid Ltd, London, England).

The rapid urease test used in a modification of that used by Arvind et al.,3 and recently validated in our laboratory,4 can be performed with very small amount of homogenised tissue incubated at 55°C.

The culture of the fresh homogenised specimens identified Helicobacter pylori in 42 of the 52 biopsy specimens tested, a positive rapid urease test being observed in all 42 that were positive by culture. The rapid urease test performed after two, four, and six months of freezing was also positive in all the samples that were positive before storage. Although the rapid urease test was 100%, reproducible at any time, a decrease in the activity of the enzyme was observed.

The culture of the stored specimens that were obtained in the fresh analysis identified micro-organisms in 14 of 16 (88%) specimens after two months, 14 of 15 (93%) after four months, and 10 of 11 (91%) after six months. The viability determined by the number of colony forming units was reduced from 20% to 80% after freezing. This loss, however, was related to the initial number of colonies rather than the time of freezing.

None of the 10 Helicobacter pylori negative samples in the fresh examination gave a positive culture or rapid urease test when analysed at two, four, or six months. These results indicate that despite freezing and greatly reducing the viability of the micro-organism, the urease enzyme remains sufficiently active after six months of freezing to give positive results at 55°C. We conclude that the more rapid urease test is more accurate than culture in identifying Helicobacter pylori in frozen gastric mucosal specimens and, therefore, it is the most convenient method for retrospective studies.

S ADALLA, F MARCO, R PEREZ, JM PIQUE

Departments of Gastroenterology and Microbiology, Hospital Clinico, Villarruel 170, Spain

Acute phase response and deep lower limb venous thrombosis

The acute phase response with fever, a neutrophil leucocytosis and increased concentrations of plasma proteins may be induced by different infectious, ischaemic, immunological, malignant, or traumatic stimuli. One of the most practical methods for monitoring it is to quantify the serum C-reactive protein (CRP) concentration.1 The purpose of the present work was to compare the acute phase response in different types of deep lower limb thrombosis.

Forty five patients with deep lower limb thrombosis verified by ascending phlebography were studied. These patients did not have any other cause for the acute phase response.

TREATMENT PROTOCOL AT OUR HOSPITAL INCLUDES THE COMBINATION OF HEPARIN AND CUMARINE AND ONLY CUMARINE AFTER DISCHARGE. PERIPHERAL BLOOD LEUCOCYTE COUNTS ON AdMISSION WERE DETERMINED BY AN AUTOMATISED CALCULATOR, AND AXILLARY TEMPERATURES AND CRP CONCENTRATIONS WERE MONITORED THROUGHOUT. SERUM CRP WAS DETERMINED BY AN IMMUNONITROBIMETRIC METHOD (Orion Diagnostica, Espoo, Finland). The detection level for CRP at our hospital varied from <10 mg/l to <15 mg/l during the period concerned, but in those cases a serum CRP value of 5 mg/l was used for statistical purposes. Data were compared with the non-parametric Student’s t-test, Mann-Whitney U-test, and Pearson’s correlation analysis.

The 19 cases with iliofemoral thrombosis (group 1) were older than the 26 with the tibial or popliteal thrombosis, and the serum CRP response was higher in the former, but no such difference was seen in leucocyte responses or axillary temperatures (table). Age itself did not seem to explain the differences in CRP response between these two groups when examined by Pearson correlation analysis (r = 0.24, p = 0.24 in group 1; r = 0.24, p = 0.33 in group 2). Axillary temperature, CRP, and leucocyte responses were similar in the 22 men and 23 women. Serum CRP peaked over two days in all except four cases.

Analysis of the individual responses showed more than one third (35%) of the 26 patients in group 2 to have no CRP response (figure), while only three had a temperature of

Maximum serum C-reactive protein concentration in all cases of acute deep lower limb venous thrombosis. Group 1, iliofemoral; group 2, tibial or popliteal thrombosis. The horizontal lines denote means of serum CRP. The difference between the medians was significant (p = 0.02 [Mann-Whitney U-test]).

Characteristics of 45 patients with deep lower limb venous thrombosis

<table>
<thead>
<tr>
<th>Tibilial/Popliteal</th>
<th>Iliofemoral</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 26)</td>
<td>(n = 19)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Leucocytes*</td>
<td>8.7 ± 1.9</td>
</tr>
<tr>
<td>Leucocytes*</td>
<td>8.6 ± 1.6</td>
</tr>
<tr>
<td>T-test</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>t-test</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* maximum of peripheral blood leucocyte counts, ± 10^9/l; t maximum of axillary temperature, °C.

Letters to the Editor
AgNOR counts in intraendometrial neoplasia.

A Hansen and B Ostergård

*J Clin Pathol* 1990 43: 518-519
doi: 10.1136/jcp.43.6.518-b

Updated information and services can be found at:
http://jcp.bmj.com/content/43/6/518.2.citation

These include:
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/