Letters to the Editor

a day. At this time the platelet count was $256 \times 10^9/l$. Over the next nine days the cellu-
litis improved slightly, but on the ninth day a few purpuric lesions were seen on the
lower limbs. These extended within 24 hours and the platelet count on the tenth day was $3 \times 10^9/l$ with a normal haemaglobin and white cell count. A bone marrow aspirate taken at this time showed hypercellularity and increased megakaryocytes, consistent with accelerated platelet destruction. Cephalo-
mandole was then stopped. Four units of platelet concentrates were given, and cepha-
lexin was started in an oral dose of 1g five times a day 12 hours later. The platelet count increased steadily thereafter and returned to normal within four days. Cepha-
lexin was continued at the same doses during the subsequent three months and the
platelet count remained normal throughout.

Drug dependent platelet antibodies were shown using an immunofluorescence pro-
cedure with about 5 mg of cepha-
mandole added to the incubation mixture of patient's serum and platelets. Patient's serum and the antibiotic solution were tested separately as controls.

Other drugs given at the same time as cephamandole were frusemide, Slow K, sul-
indac, ibuprofen, panadine, anginine, palfium, isosorbide dinitrate and rifampicin.

All of these had been given for lengthy peri-
ods prior to the onset of thrombocytopenia, and with the exception of rifampicin, were continued subsequently without any adverse effects.

The table shows the results of the platelet antibody immunofluorescence test (PIFT)
performed in the presence of various cepha-
losporins and rifampicin, which was includ-
ed as this was the only other drug dis-
continued at the same time as cepha-
mandole. Platelet bound antibody was shown in the presence of cepfepazone and
moxalactam, as well as cephamandole, but not in the presence of cefoxitin, cephalothin,
cephalexin or rifampicin. The former three
antibiotics possess a common thiom-
ethyltetrazole group on the R2 side chain that is absent from the latter drugs.

The patient had had no known previous
exposure to cephalosporins or any other
drug possessing a thiomethyltetrazole
group. Cephazolin, however, which he had
been previously given in a single dose, pos-
sesses a similar tetrazole group on the R1
side chain. Unfortunately, insufficient serum
was available to perform the PIFT in the
presence of cephazolin, or a similar side
chain structure isolated from the antibiot-
cs, but it is possible that this antibiotic, given 18 months earlier induced an initial immune response.

This seems to be the first reported case of
cephalosporin sensitivity selectively
involving only those drugs with a thio-
ethyltetrazole group on the R2 side chain.
The apparent rarity of this occurrence
would not seem to justify changing pre-
scribing habits for cephalosporins.

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References

1 Granick HR, McGinnis M, Halterman R. Thrombocytopenia with sodium cephalo-
2 Sheiman L, Spielvogel AR, Horowitz H. Thrombocytopenia caused by cephalotin
3 Naraqi S, Raiser M. Nonreacrence of cephal-
alin—associated granulocytopenia and
4 Von Dem Borne AEG Kr, Verheugt FWA, Oosterhof F, et al. A simple immuno-

Table Platelet antibody
immunofluorescence test using patient's
serum and pooled normal platelets

<table>
<thead>
<tr>
<th>In the presence of:</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>—</td>
</tr>
<tr>
<td>Cephamandole*</td>
<td>++</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>++</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>++</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>—</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>—</td>
</tr>
<tr>
<td>Cephalixin†</td>
<td>—</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>—</td>
</tr>
</tbody>
</table>

*Associated with thrombocytopenia in patient.
†Used to treat patient without adverse effects.

Rapid diagnosis of Campylobacter pyloridis infection

A characteristic feature of Campylobacter
pyloridis, an organism implicated as the
aetiological agent of gastritis and possibly
gastric ulcers also, is the secretion of large
quantity of extracellular urease.1 Hazell and
Lee2 postulated a role for this enzyme in the
pathogenesis of these diseases. Other
investigators used this property to facilitate
rapid diagnosis of C pyloridis infection by
testing for the presence of preformed urease
in biopsy specimens taken at endoscopy.3 4

We report here our findings from two sepa-
rate studies, one done in London and one
in Belo Horizonte, Brazil, on the reliability
of the urease test as a rapid diagnostic test
for infection associated with C pyloridis. In
the two studies mucosal biopsy specimens were
taken from the gastric antrum, duodenum,
and oesophagus from patients attending the
endoscopy clinic for investigation of upper
gastrointestinal symptoms.

In the study at St Charles's Hospital, Lon-
don 199 biopsy specimens were available from
111 patients. Specimens were pro-
cessed immediately or kept at 4°C for not
longer than two hours before processing in
the laboratory. The same specimen was used for
all the tests. It was crushed before being
first inoculated on to blood agar and C pyloridis
selective media for culture at 37°C in
microaerophilic conditions for up to six
days, then used to make a smear for Gram
staining, and finally placed in 0.5 ml of
Christensen's 2% urea broth and left at
room temperature for up to 24 hours to
detect preformed urease. A colour change
from brown to pink indicated a positive test.

In the Brazilian study 67 sets of biopsy
specimens were taken from 51 patients for
analysis. Each set consisted of three speci-
mens, one for culture, one for Gram stain,
and one for the urease test. The previously
crushed and ground specimens were pro-
cessed fresh in the endoscopy unit. The
media used and the method of reading the
results were the same as those in the London
study.

Detection of C pyloridis by culture or Gram
stain, or both, was taken as the stan-
dard with which the urease test had to be
compared. Table 1 shows that the urease test
has a specificity of 88% and a sensitivity of
74%, when applied to biopsy specimens from
the gastric antrum. These contrast with the
figures of 100% and 88%, respectively,
reported by McNulty and Wise.3 5

When oesophageal and duodenal biopsy
specimens are also included in the analysis,
the specificity of the urease test is found to
be 86% while the sensitivity is only 59%,
with a false positive rate of 28% (table 2). These are almost identical with the figures of

Table 1 Detection of C pyloridis in gastric
antrum

<table>
<thead>
<tr>
<th>Culture or Gram stain for C pyloridis</th>
<th>Urease</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>37</td>
<td>4</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>29</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>33</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

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Table 2 Detection of C pyloridis in gastric antrum, duodenum and oesophagus

<table>
<thead>
<tr>
<th>Urease</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>44</td>
<td>17</td>
<td>61</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>108</td>
<td>138</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>125</td>
<td>199</td>
</tr>
</tbody>
</table>

86% and 60% for specificity and sensitivity, respectively, obtained from our Brazilian study (Table 3). Processing specimens immediately in the endoscopy unit and using the same specimen for the urease test seem to make no appreciable difference to the sensitivity and specificity of the test. The increase in false positive results may be explained by the fact that in the oesophagus and the duodenum the suppressive effect of gastric acid on the growth of contaminant microbial flora may not be as great as that in the gastric antrum.

On the basis of our results, we cannot recommend the biopsy urease test as a reliable and rapid test to assist in the diagnosis of C pyloridis infection, at least in sites other than in the gastric antrum. We also agree with Morris et al1 that the test is really no faster than an adequate Gram stain, as most of our positive urease tests took longer than three hours to become positive.

Table 3 Detecting C pyloridis in gastric antrum, duodenum and oesophagus (Brazilian study)

<table>
<thead>
<tr>
<th>Urease</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>32</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>Negative</td>
<td>21</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>14</td>
<td>67</td>
</tr>
</tbody>
</table>

Simple half-Gram stain for showing presence of Campylobacter pyloridis in sections

Like Gray et al1 we have abandoned the Warthin-Starkey technique for identifying gastric Campylobacter pyloridis in tissue sections, because it is unpredictable, time consuming, and expensive. As an alternative to their modified Giemsa technique we can also recommend a simple half-Gram method that we have been using for the past six months, and which shows well the characteristic morphology of the organisms (figure).

Paraffin embedded sections are dewaxed, taken to water, and stained for 30 seconds in a 1/20 aqueous dilution of Hucker's stain (one part 10% alcoholic crystal violet plus four parts 1% ammonium oxalate). After a rinse in water they are treated with Lugol's iodine for 60 seconds, washed in tap water, then blotted and allowed to dry thoroughly before clearing in xylene and mounting in DPX.

Most of our patients investigated for the presence of C pyloridis have paired biopsy specimens taken, one for histology and one for culture. The biopsy specimen for culture is also ground, and the suspension is plated on 5% blood agar and also on to fastidious anaerobe agar (Lab M), containing nalidixic acid 10 mg/l, vancomycin 2-5 mg/l, and 5% horse blood. The plates are incubated for seven days at 37°C in an atmosphere of nitrogen containing 5% oxygen and 6% carbon dioxide. Isolates are identified by colonial and morphological appearance, and a rapid urease reaction.

The results of the histological half-Gram method correlated with those of culture in 91% of cases, which is similar to the experience of Marshall et al2 using the Warthin-Starkey stain. Of 102 paired biopsy specimens received, 45 were negative and 48 positive by both methods. Seven positive by the half-Gram method were culture negative, and in two cases culture was positive but no bacteria could be seen in half-Gram stained sections of the paired biopsy specimens.

References


Isolation of Campylobacter: what are we missing?

Numerous selective media have been described for the isolation of campylobacters, almost all containing several antibiotics as inhibitory agents. A method

Figure

Gastric pit. (Half-Gram).