

Postscript

LETTERS

Diagnosis of Wilson's disease: an experience over three decades

The paper by Gow *et al* (*Gut* 2000;46:415-19) discussed the diagnosis of Wilson's disease in 30 patients presenting to two different clinical facilities over 28 years (1971-1998). Because a paper of this type is likely to be viewed as an authoritative guide, it is important that the information be valid. For that reason, I call attention to the following significant errors in the paper.

The authors report urine copper values of 5, 4, 7, 4, 5, 2, and 2 μg per 24 hours in seven patients in their table 1. These data cannot possibly be valid. The normal range for urine copper is 20-50 μg per 24 hours, and these patients are far below the lower limit of normal. In performing several thousand 24 hour urine copper tests on patients and normal subjects in our own laboratory, I have never seen one below 10 μg per 24 hours, except in copper deficiency. The patients in table 1 have Wilson's disease, the opposite of copper deficiency, making the data even more unbelievable. I also do not believe the data for two additional symptomatic patients in their table 1 who are reported to have urine copper values of 55 and 44 μg per 24 hours. Urine copper values in untreated symptomatic Wilson's disease patients are invariably over 100 μg . That is our experience in all of 88 newly diagnosed neurologically presenting patients (urine copper range 107-1593 $\mu\text{g}/24$ hours) and in all of 18 newly diagnosed patients with hepatic presentation (urine copper range 106-1880 $\mu\text{g}/24$ hours). Not all liver disease patients with urine copper values over 100 μg will have Wilson's disease but all untreated patients with Wilson's disease with clinically presenting liver disease will have a value over 100 μg . If a patient with liver disease does not have a value that high, then look to another diagnosis, or to a laboratory error. No doubt the latter is the case here because the values in seven of the patients are not biologically reasonable. One caveat: if the patient has been treated with a chelating agent, even briefly, and then the drug stopped, there is often a rebound period when urine copper will drop below 100 μg .

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The editors will decide as before whether to also publish it in a future paper issue.

I also question the diagnosis of Wilson's disease in two of the patients in this series. Patient Nos 20 and 22 had liver disease but were negative for Kayser-Fleischer rings, had a non-elevated urine copper, and normal caeruloplasmin levels. The diagnosis was supposedly made in patient No 20 by elevated liver copper. Non-Wilsonian chronic liver disease can also elevate hepatic copper.^{1,2} The diagnosis in this patient is uncertain, and disproved if urine copper is truly low. Patient No 22 also had elevated liver copper and a "positive radiolabelled copper plasma clearance test." Again, hepatic copper is not proof of the diagnosis. The abnormal radiocopper test is supportive but Wilson's heterozygotes, comprising about 1% of the population, are often falsely positive.³ Urine copper of 44 μg , if valid, would rule out the diagnosis.

In summary, if the data in the current paper were to be believed (nine of 22 patients with low or normal copper values), it would inappropriately denigrate the great usefulness of urine copper assay in the diagnosis of symptomatic Wilson's disease. Secondly, the liver copper value is not an absolute in the presence of chronic liver disease. Sometimes a therapeutic trial is the only way to come to a decision in these patients.

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Author's reply

Dr Brewer in his letter raises two points regarding our recent paper (*Gut* 2000;46:415-9). Most importantly, he questions the diagnosis of Wilson's disease in two patients. Both of these patients were very unusual in that they were both older than 40 years at diagnosis, had caeruloplasmin levels within the normal range, no Kayser-Fleischer rings, and low urinary copper excretion. The diagnosis in these two interesting patients is discussed in the paper. To summarise the discussion: in both patients the diagnosis was based on the findings of markedly elevated liver copper levels (634 and 1037 $\mu\text{g}/\text{g}$ dry weight liver, normal liver copper 15-60 $\mu\text{g}/\text{g}$). In addition, the first patient had a family history of cirrhosis and the second had a positive radiolabelled copper plasma clearance test. In this situation the primary differential diagnosis relates to elevated liver copper from chronic cholestatic liver disease. In both of these patients, chronic cholestatic liver disease was excluded by the absence of cholestatic biochemistry plus a lack of cholestatic findings on histological examination of the explanted liver. The important point to emphasise with these cases is the lack of

sensitivity of caeruloplasmin, urinary copper excretion, and Kayser-Fleischer rings in the diagnosis of Wilson's disease.

Dr Brewer's second point relates to reported low levels of urinary copper excretion in seven of our patients. In these patients the values were reported as $\mu\text{mol}/24$ hours when they should have been converted to $\mu\text{g}/24$ hours. The true levels for patient Nos 6, 7, 8, 11, 12, 17, and 20 are 312, 117, 437, 1250, 762, 125, and 100 $\mu\text{g}/24$ hours, respectively.

Dr Brewer also questioned the urinary copper excretion values in patient Nos 16 and 22. In both of these cases the data were rechecked and the reported values were found to be correct.

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Small intestinal bacterial overgrowth, intestinal permeability, and non-alcoholic steatohepatitis

In a recent issue, Wigg and colleagues (*Gut* 2001;48:206-11) reported that small intestinal bacterial overgrowth (SIBO), as diagnosed by a combined ¹⁴C-D-xylose/lactulose breath test, is significantly more common in patients with non-alcoholic steatohepatitis (NASH) than in control subjects without liver disease. The authors investigated the possible pathogenic significance of this observation by examining whether intestinal permeability and circulating levels of endotoxin and tumour necrosis factor α are increased in NASH patients with SIBO compared with those without. No significant differences in any of these parameters could be demonstrated in the two groups.

An important factor influencing the validity or otherwise of these findings is the diagnostic accuracy of the ¹⁴C-D-xylose and lactulose breath tests for SIBO. Our experience, using a sterile endoscopic technique to sample small intestinal secretions under direct vision, is that these breath tests lack sensitivity and specificity for culture proven SIBO.^{1,2} Endogenous CO₂ production and colonic metabolism of D-xylose are important factors inherently limiting the accuracy of the ¹⁴C-D-xylose breath test for SIBO.¹ Furthermore, reliance on the finding of "double peaks" in serial breath hydrogen or methane levels after ingestion of lactulose to improve the accuracy of the ¹⁴C-D-xylose breath test, or as a diagnostic marker in its own right, is problematic. In a study in which a scintigraphic tracer was administered concurrently

with lactulose, we found that each of the double peaks in breath hydrogen values may occur after the arrival of the test meal at the caecum, paralleling delivery patterns of fermentable substrate to caecal bacteria. A caecal source of each peak was suggested on 50% of occasions, rather than the first peak necessarily reflecting small intestinal metabolism of lactulose by overgrowth flora as purported. Conversely, a single rise in breath hydrogen levels commencing before the test meal reached the caecum was evident in 22% of subjects with culture proven SIBO. Thus both false positive and false negative diagnoses of SIBO may result.² Indeed, as pointed out in the accompanying commentary (*Gut* 2001;48:148–9), the prevalence of SIBO, as diagnosed by breath testing, in control subjects in Wigg *et al*'s study seems remarkably high.

Rather than seeking to establish the prevalence of SIBO in patients with NASH, as in the study of Wigg *et al*, we have investigated the prevalence of liver damage, as reflected by elevated liver enzyme levels in serum, in patients with culture proven SIBO.³ Biochemical evidence of liver injury was found in 0/11 patients with SIBO with salivary type bacteria only, 0/21 patients with SIBO with facultative anaerobic (*Enterobacteriaceae*) but not obligate anaerobic (*Bacteroides* spp) colonic type bacteria, and 1/8 patients with SIBO including *Bacteroides* spp. Alkaline phosphatase and gamma glutamyl transferase levels were elevated in this patient, although liver ultrasonography and cholangiography revealed no abnormality. Small intestinal permeability was increased and, together with liver enzyme abnormalities, normalised following eradication of SIBO with a metronidazole based antibiotic regimen. We concluded that liver injury, reversible with antibiotic treatment, occurs uncommonly in patients with SIBO, and only when the overgrowth flora includes obligate anaerobes such as *Bacteroides* spp, in keeping with earlier findings implicating such flora in the pathogenesis of liver injury associated with experimental SIBO in rodents.⁴ Liver injury associated with SIBO with *Bacteroides* spp was not a necessary consequence of increased small intestinal permeability, which was also evident in 50% of patients with SIBO with *Bacteroides* spp who had no evidence of liver damage.

Based on these observations, we suggest that future studies examining the prevalence of SIBO in patients with NASH and its possible pathogenic significance should use culture of small intestinal aspirate rather than breath testing as the diagnostic modality and focus on the presence or absence of overgrowth with obligate anaerobic flora such as *Bacteroides* spp. Such an approach would be preferable to simply assessing for any improvement in NASH following a therapeutic trial of metronidazole, as SIBO with *Bacteroides* spp and other obligate anaerobes is not always eradicated by a single course of antibiotic treatment. As small intestinal colonisation with *Bacteroides* spp depends on underlying intestinal dysmotility,⁵ factors other than SIBO are likely responsible for NASH in patients in whom intestinal motility is normal.

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Authors' reply

We thank Riordan *et al* for their own observations concerning the diagnosis of small intestinal bacterial overgrowth (SIBO) and liver injury associated with SIBO.

We agree with the suggestion that *Bacteroides* may be more important than other bacterial species in causing liver injury. This may be an explanation for our failure to detect endotoxin elevations in those patients diagnosed with both NASH and SIBO (endotoxin is derived only from *Escherichia coli* bacteria and not *Bacteroides* spp) (*Gut* 2001;48:206–11).

We must however correct their statement that no significant differences were found in our study between NASH patients and control subjects for tumour necrosis factor α . A statistically significant difference was found between these groups ($p < 0.001$) (*Gut* 2001;48:206–11).

Their comments highlight the longstanding difficulty in gastroenterology of diagnosing SIBO. Although the traditional 14C-D-xylose breath test has been associated with a high sensitivity in some studies,^{1,2} the specificity of this test is unacceptable in our experience. The high false positive rate associated with this breath test probably relates to catabolism of unabsorbed 14C-D-xylose by the colon, resulting in 14CO₂ expiration. In an attempt to retain sensitivity and improve specificity, we have developed a combined 14C-D-xylose-lactulose breath test. Lactulose, which is not absorbed and requires large bacterial concentrations for its catabolism to H₂ and CH₄, acts as an internal transit marker of colonic metabolism. Smaller bacterial concentrations only are required for the catabolism of 14C-D-xylose to 14CO₂. Thus in SIBO, catabolism of 14C-D-xylose by small intestinal bacteria will result in an early 14CO₂ peak prior to the colonic H₂ and CH₄ peaks. Specificity is improved because 14CO₂ peaks due to colonic metabolism of unabsorbed 14C-D-xylose can be identified when they rise simultaneously with H₂ and CH₄ colonic peaks.

When both the 14C-D-xylose breath test and the combined breath test were done in a group of 11 patients, only four had positive combined tests compared with nine positive 14C-D-xylose breath tests (*Gut* 2001;48:206–11). This suggests that the combined test has achieved a greater specificity. We feel that the combined 14C-D-xylose-lactulose breath test is a sensitive and specific non-invasive alternative to culture of small intestinal aspirates.

We note the concern of Riordan *et al* on the use of double H₂ peaks for the diagnosis of SIBO, based on their observations with scintigraphic studies. In very severe SIBO, a double peak of H₂ and CH₄ may be produced due to lactulose catabolism by bacteria in both the small intestine and colon. As suggested by Riordan *et al*, double peaks may reflect catabolism of lactulose by colonic bacteria rather than by bacteria in the small intestine and then the colon. Diagnosis of SIBO in our study was based on early 14CO₂ expiration before the appearance of a H₂ or CH₄ peak in all cases. Double H₂ or CH₄ peaks were observed in only one of the 16 breath tests recorded as positive in our study. In this patient, significant 14CO₂ was expired prior to the first peak.

The studies quoted by Riordan *et al* have used cultures of small intestinal aspirates as the gold standard for the diagnosis of SIBO. Is aspiration of intestinal secretions under sterile conditions a satisfactory gold standard for the diagnosis of SIBO? This diagnostic method is not universally accepted. It is likely that the small volume of proximal intestinal contents aspirated does not accurately represent the bacterial flora of the entire small intestine. This may explain the problems with sensitivity and reproducibility described by some investigators.^{1,3,4} Lack of standardisation of specimen collection and the invasive nature of the test are further problems, particularly in the setting of studies involving a healthy control population. The use of culture of small intestinal aspirates as a gold standard to assess the performance of breath tests may therefore not be valid.

In view of the difficulties associated with diagnosing SIBO, the association of SIBO with NASH found in our study requires confirmation by other investigators. Studies using culture of small intestinal aspirate to diagnose SIBO, which can also provide qualitative bacterial information, will be complementary to our study using a combined 14C-D-xylose-lactulose breath test.

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CORRECTION

Abstract 6/12 in *Gut* 2001;49(suppl II):A33 contained an error. Q Song should be affiliated with institution 1 (University of Ulm). In abstract 8/09 (*Gut* 2001;49(suppl II):A47), the author list should read AT Dubois¹, C Seminomora¹, H Woreta¹, S Doi¹, I Carlstedt². ¹USUHS: Bethesda, MD, USA; ²University of Lund: Lund, Sweden.

NOTICES

Broad Medical Research Program—Inflammatory Bowel Disease Grants

Funds for inflammatory bowel disease (IBD) research are available immediately from the Broad Medical Research Program of The Eli and Edythe L Broad Foundation for innovative projects regarding etiology, therapy, or prevention. Grants totalling approximately US\$100,000 per year are available for basic or clinical projects. Larger requests may be considered. Initial letter of interest (no submission deadline), simple application, rapid (60 day) peer review, and funding. Criteria for funding includes new ideas or directions, scientific excellence, and originality. Early exploratory projects, scientists not currently working in IBD, and/or interdisciplinary efforts are encouraged. Further information:

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Malignancy Liver Tumours: Basic Concepts and Clinical Management

This Falk Workshop will be held on 24–25 January 2002 in Leipzig, Germany. Further information: Falk Foundation e.V. Congress Division, Leinenweberstr. 5, PO Box 6529, D-79041 Freiburg, Germany. Tel: +49 761 15 14 0; fax: +49 761 15 14 359; email: symposia@falkfoundation.de

European Association for the Study of the Liver: 37th Annual Meeting

The EASL Annual Meeting will be held on 18–21 April 2002 in Madrid, Spain. Further information: EASL Liaison Bureau, c/o Kenes International, 17, rue du Cendrier, PO Box 1726, CH-1211 Geneva, Switzerland. Tel: +41 22 908 04 88; fax: +41 22 732 28 50; email: info@easl.ch; website: www.easl.ch

Falk Symposium No 128: Exogenous Factors in Colonic Carcinogenesis

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Gastroenterology and Endotherapy European Workshop: XXth Anniversary

This course will be held on 17–19 June 2002 in Brussels, Belgium. Further information: Nancy Beauprez, Gastroenterology Department, Erasme Hospital, Route de Lennik 808, B-1070 Brussels, Belgium. Tel: +32 (0)20 555 49 00; fax: +32 (0)20 555 4901; email: beauprez@ulb.ac.be