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LETTERS TO THE EDITOR

RISK OF GASTROINTESTINAL NON-HODGKIN'S LYMPHOMA AT DIAGNOSIS OF CELIAC DISEASE

To the Editor,

Several studies have shown an increased incidence of malignancy in patients with celiac disease (CD). Such a trend is detectable both in Europe, where CD has a high prevalence, and in the USA, where CD was considered uncommon until recently.[1,2] We report some data taken from an Italian group of patients affected by CD that show that there was a higher risk, with respect to the general population, of developing intestinal non-Hodgkin's lymphoma. In the present study, 1968 patients diagnosed with CD over a 20-year period (between January 1982 and December 2002) in 20 Italian clinical centers specializing in gastrointestinal diseases were observed. The diagnosis of CD was made according to revised ESPGHAN criteria as follows:[1] (i) histological evidence of atrophy of duodenal or jejunum mucosa;[2] (ii) recovery at control biopsy after a gluten-free diet;[3] and (iii) serological positivity for AGA IgG or IgA and EMA IgG.[3]

For each patient, demographic data and symptoms, and concomitant pathology results at the time of the diagnosis of CD were collected. Each patient gave their informed consent to take part in the study and the protocol was approved by the ethics committee of each participating center.

Of the 1968 patients, 20 patients had already been diagnosed with gastrointestinal non-Hodgkin's lymphoma at diagnosis of CD (17 cases of intestinal non-Hodgkin's lymphoma and three cases of gastric non-Hodgkin's lymphoma). We found CD predominantly affected men (2:1). The standardized morbidity ratio (95% CI) for the malignancy results was 6.25 (3.8–9.6).[4] In addition, we noticed that the risk of developing gastrointestinal non-Hodgkin's lymphoma correlates with the age at diagnosis. The mean age of the patients with a non-Hodgkin's lymphoma at diagnosis of CD is 46.1 ± 13.8 years, whereas the mean age of the control group, represented by the patients with no neoplasm at diagnosis, is equal to 36.6 ± 13.7. Therefore, the delayed diagnosis of CD is likely to be a risk factor for developing a lymphoma, probably because of the prolonged exposure to gluten.

These data are consistent with those of other studies, indicating a relationship between CD and the development of gastrointestinal non-Hodgkin's lymphoma. It is noteworthy that most of these cases occur before the diagnosis of CD.[5,6] Celiac disease is often an asymptmonatic condition, with a high rate of associated pathologies and malignancies, often diagnosed before CD is diagnosed.

Non-Hodgkin's lymphomas are very frequent and can develop in sites other than the gastrointestinal tract, such as the skin, spleen, liver and central nervous system. The mechanism for the development of non-Hodgkin's lymphoma in patients with CD is not known. However, chronic inflammation and antigenic stimulation, increased intestinal permeability and the release of inflammatory cytokines have been suggested.[1] In our group of patients, we found only one case of extra-intestinal non-Hodgkin's lymphoma, a lymphoma of the spleen.

Sometimes the concomitant pathologies suggest the diagnosis of CD. Our results stress the importance of implementing programs aimed at making an early diagnosis of CD in order to prevent its complications, even if the best method of screening has yet to be determined.

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PREDICTORS OF SHORT-TERM OUTCOME OF SPONTANEOUS BACTERIAL PERITONITIS IN TURKISH CIRRHOTIC PATIENTS

To the Editor,

Despite the introduction of new antibiotics, earlier diagnosis and treatment, spontaneous bacterial

peritonitis (SBP) still continues to be a serious infectious complication in cirrhotic patients with ascites.[1–4] Short-term mortality, which denotes the proportion of patients who die from SBP during hospitalization, has remained unchanged during the past decade. This is caused by severe hepatic dysfunction in affected patients.[5] Therefore, we aim to introduce the prognostic factors determining short-term survival in Turkish cirrhotics with SBP. The patients who took part in this research study were from Trakya University Hospital, Edirne, Turkey and Haccettepe University Hospital, Ankara, Turkey.

The medical records of 87 consecutive cirrhotic patients (107 episodes) with SBP that were treated at the two university hospitals between 1999 and 2001 were reviewed retrospectively. The patients were diagnosed with cirrhosis according to their clinical signs and symptoms, laboratory findings and radiological evaluation. The patients underwent a liver biopsy whenever coagulation parameters permitted. Paracentesis for ascitic fluid (AF) culture and cell count were carried out in all cirrhotic patients with ascites on the first day of their hospitalization and in each appropriate clinical requirement. The diagnostic criteria for SBP was fever, abdominal pain, encephalopathy or other signs of peritonitis associated with AF neutrophil (PNL) count (>250 cells/mm^3) in the absence of surgically treatable causes of secondary peritonitis. Ascitic fluid cultures were collected and put into aerobic and anaerobic blood culture bottles at the bedside of the patients (10 cc AF drawn). The patients with culture-negative neutrocytic ascites (CNNA) were also included in the study. There appeared to be no statistically significant difference among the patients treated at the two different university hospitals, with regard to continuous and categorical variables, but alcoholic etiology was more prevalent in the European part of Turkey (5/41 [12%] at Hacettepe University versus 17/46 [37%] at Trakya University).

The age range of the patients was 17–90 years (mean age, 53.5 ± 14.5 years), and there were 65 men and 22 women. The underlying cause of chronic liver disease was diagnosed as hepatitis B in 52 (61%) patients, hepatitis C in five (6%) patients, hepatitis delta in two (2%) patients, alcohol abuse in 22 (25%) patients and other causes in eight (9%) patients. Seventy-eight (92%) of the patients were suffering from Child's C and eight patients had Child's B (9%). The signs and symptoms of the patients are summarized in Table 1. Seventy-eight SBP episodes (73%) were treated with i.v. cefotaxim and the other 29 were treated with appropriate antibiotics or with combinations according to the clinical and microbiological requirements. The patients were classified into two groups for statistical analysis; patients who survived ($n = 64$, 78%) and patients who died ($n = 23$, mortality rate 22%) (Table 1). The percentages of the patients whose deaths were directly attributed to SBP before resolution of infection was 15% (16 patients). The remaining seven patients died as a result of variceal bleeding (three) and multiorgan failure (four). In 33/107 episodes (30%), at least one type of bacteria was isolated from AF (Table 1). The most common organism isolated from AF was *Escherichia coli* in 16 (49%) episodes followed by *Staphylococcus aureus*

Table 1 Clinical characteristics of survivors (computed as episodes) versus non-survivors at the time of diagnosis of spontaneous bacterial peritonitis (SBP)

Variables	No. survivors	No. non-survivors	*P*
No. patients (episodes)	64 (84)	23 (23)	ND
Male : Female	64:20	18:5	NS[†]
Age (years)	54 ± 14	52 ± 17	NS[‡]
Abdominal pain	32/84 (38%)	16/23 (80%)	0.002[†§]
Encephalopathy	44/84 (52%)	20/23 (87%)	0.002[†]
Ileus	1/84 (1%)	5/23 (22%)	<0.0005[†]
WBC count (/mm3)	8550 ± 7200	19 100 ± 34 600	0.01[‡]
BUN (mg/dL)	34 ± 31	79 ± 79	<0.0005[‡§]
Creatinine (mg/dL)	1.1 ± 0.6	2.1 ± 1.4	<0.0005[‡§]
Triglyceride (mg/dL)	83 ± 74	99 ± 40	0.018[‡]
Total bilirubin (mg/dL)	5.5 ± 8.9	11.6 ± 11.5	0.002[‡]
AST (U/L)	77 ± 55	158 ± 153	0.003[‡]
SBP episodes	84 (100%)	23 (100%)	ND
CNNA	57 (68%)	17 (74%)	NS[†]
Culture positivity	27 (32%)	6 (26%)	NS[†]
Gram-negative	17	3	NS[†]
Gram-positive	10	3	NS[†]
AF cell count (/mm^3)	2469 ± 5415	16 484 ± 41 520	0.039[‡]
PNL count (/mm^3)	1355 ± 1800	13 900 ± 40 000	0.029[‡]

$P < 0.05$, statistically significant. [†]χ^2 test. [‡]Mann–Whitney U-test. [§]Independent predictors of short-term mortality. AF, ascitic fluid; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CNNA, culture-negative neutrocytic ascites; ND, no data; NS, not significant; PNL, polymorphonuclear leukocytes; WBC, white blood cell.

Table 2 Accuracy of prediction of short-term hospital mortality

	Cut off	AUC	Sensitivity	Specificity
AST (U/L)	>108	0.704	56.5	82.1
Creatinine (mg/dL)	>1.1	0.783	82.6	75.5
Abdominal pain	>0.0	0.701	80.0	60.3

AST, aspartate aminotranferase; AUC, area under the receiver–operator curve.

in five (15%) episodes. Fever was largely associated with Gram-positive infection (12/20 [60%] in Gram-positive *vs* 5/29 [21%] in Gram-negative infection; $P < 0.002$). In the present study, we did not find clinical significance of any type of AF culture isolate on short-term prognosis. It has been suggested that nosocomial and staphylococcal infections are associated with a higher mortality rate than community-acquired infections and non-staphylococcal infections.[6] The short-term hospital mortality did not differ according to Gram-positive or -negative culture results. The prognosis was also similar at short term for the patients with both CNNA and culture-positive SBP (mortality rates were 17/74 [23%] and 6/33 [18%], respectively) ($P > 0.05$). So, we believe convincingly that CNNA is as serious as SBP and must be considered as serious as culture-positive SBP. When all of the clinical characteristics of the patients with CNNA and culture-positive SBP were comparatively analyzed, no statistically significant difference was observed between the groups. As a rule according to the results of the present study, to treat all cirrhotic patients with AF cell count >250/mm^3, it must be assumed that they have culture-positive SBP.

Among the 16 clinical, laboratory and microbiological variables, it was found that the white blood cell count, the serum aspartate aminotransferase (AST), bilirubin, blood urea nitrogen, creatinine, triglyceride levels and AF total cell count, AF PNL count, encephalopathy, ileus and abdominal pain were statistically significant predictors of short-term mortality by univariate analysis (Mann–Whitney *U*-test and χ^2 test with Yate's correction where necessary; Table 1). The variables reaching statistical significance univariately were further evaluated by stepwise logistic regression analysis. Only the three variables – serum creatinine ($P < 0.001$), AST ($P < 0.001$) and abdominal pain ($P < 0.001$) – were determined to be independent predictors of mortality. Logistic regression correctly predicted the outcome in 83.2% (89/107) episodes with a sensitivity of 60% and specificity of 90% for death. The serum creatinine value, which was more than 1.1 mg/dL, and AST value, which was more than 108 U/L, in the presence of abdominal pain were associated with a poor outcome (Table 2). The Zweig and Campbell method was used for comparisons of the areas under the receiver–operator curves of creatinine, AST and abdominal pain (Fig. 1). No statistically significant difference was found among the areas under the receiver–operator curves of creatinine, AST and abdominal pain ($P > 0.05$). In a similar study by Llovet *et al.* in 1993, it was suggested that absence of abdominal pain was associated with a significant mortality, but the current study reveals the opposite.[7] The association of a poor outcome with abdominal pain possibly reflects the severity of peritoneal inflammation.

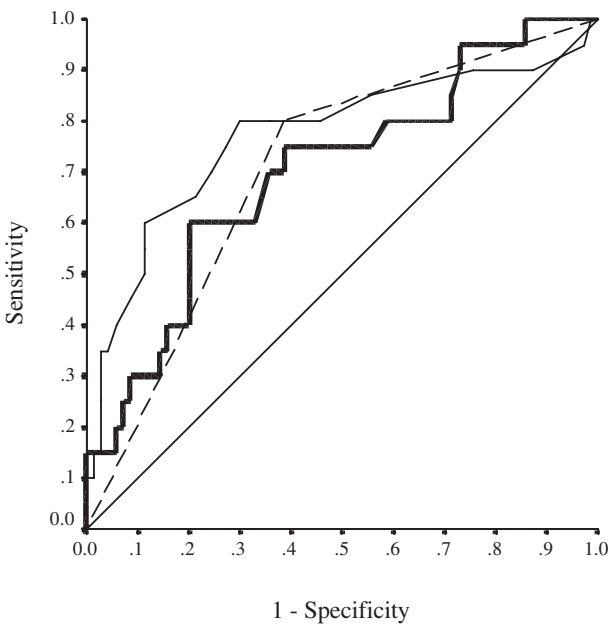

Figure 1 Receiver–operator curves predictimg short-term hospital mortality in cirrhotic patients with spontaneous bacterial peritonitis according to (▬▬) aspartate aminotransferase, (——) serum creatinine and (— —) abdominal pain.

In conclusion, CNNA has a similar short-term outcome as culture positive SBP. The high short-term mortality from SBP still challenges physicians as an important problem. When a cirrhotic patient presents with SBP associated with renal failure, increased serum transaminases and abdominal pain, physicians should be aware that these patients will have a high probability of short-term hospital mortality. The patients should be closely followed, preferably in an intensive care unit. Regarding the insufficient number of liver donors for a life-saving transplantation, efforts towards developing new strategies to prevent high mortality as a result of this infection are still needed.

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TWO NOVEL MUTATIONS (2976INSA, 4311INSA) OF *ATP7B* IN A PATIENT WITH WILSON'S DISEASE COEXISTING WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

To the Editor,

We present a patient with Wilson's disease who had early onset of the disease, accompanying hepatological and neurological manifestations and glucose-6-phosphate dehydrogenase deficiency with mutations 2976insA and 4311insA in exon 13 and 21 of *ATP7B*.

Wilson's disease is an autosomal recessive disorder of copper metabolism, resulting in hepatic cirrhosis and neuronal degeneration.[1] There is extensive heterogeneity of symptoms within Wilson's disease, with some patients presenting with primarily hepatic problems, some with primarily neurological impairment and some with both types of symptoms. The age of onset varies from childhood to early adulthood (3–16 years) for hepatic presentation, and usually 12 years or more of age for neurological problems.[2] The Wilson's disease gene has been mapped to chromosome 13q14.3. Molecular cloning of WD gene, *ATP7B* (WD, MIM# 2779000, L25591), has provided new insight into the mechanism of copper transport in humans, as well as new molecular tools for continued investigation of normal and abnormal copper metabolism. The Wilson's disease gene is encoded by 21 exons, spanning more than 80 kb on chromosome 13q14.3. The *ATP7B* gene has been shown to encode a novel member of the family of cation-transporting P-type ATPase. Analysis of the patient's mutations revealed an enormous molecular heterogeneity consisting of a very small number of frequent mutations that are population specific, as well as a much greater number of rare individual alleles.[3] The defect in copper ATPase results in excess accumulation of copper intracellularly, particularly in the liver and brain, presumably because of the generation of metal dependent oxyradicals and to metal ion antagonism. The production of hydroxyl radicals associated with cellular accumulation of copper can damage cell membranes, DNA, mitochondria and proteins.[4] Glutathione (GSH) has a protective role against toxicity of metals by chelating them.[5,6] Glucose-6-phosphate dehydrogenase plays an important role in maintaining the glutathione in a reduced state.

A $5^1/_2$-year-old boy was admitted to the Pediatric Gastroenterology ward of the Postgraduate Institute of Medical Education and Research, Chandigarh, India, with a 1-year history of recurrent episodes of jaundice. He was passing brown cola colored urine. An examination revealed normal vital signs. He was icteric and had palmar erythema and echymotic spots on his extremities. He had ascites but his liver and spleen were not palpable. His handwriting had deteriorated. Investigations showed hemoglobin count (9.0 g/dL), total leukocyte count (31 700/mm^3), normal differential count, platelets count (69 000/mm^3) and normal renal function tests. Liver function tests showed total protein (5.2 g/dL), albumin (2.0 g/dL), globulin (3.2 g/dL), serum bilirubin (14.7 mg/dL) with conjugated fraction (8.4 mg/dL), SGOT/SGPT 44/23 IU/I, alkaline phosphatase 15 KAU and deranged coagulogram. Ascitic fluid analysis showed evidence of spontaneous bacterial peritonitis. Glucose-6-phosphate dehydrogenase activity in erythrocytes from this patient was found to be 0.93 U/g Hb, which was markedly lower compared with the age-matched controls (5.9 U/g Hb) and showed evidence of intravasicular hemolysis. The patient had bilateral Kayser–Fleischer rings on slit lamp examination. An ultrasound examination of the abdomen showed an irregular outline and a coarse echotexture of the liver and the presence of ascites. An upper gastrointestinal endoscopy showed esophageal varices. The work up for Wilson's disease showed pre-D-penicillamine urinary copper 313 μg/24 h (normal <80 μg/24 h), post-D-penicillamine urinary copper 459 μg/24 h, serum ceruloplasmin 3.6 mg/dL (normal >20 mg/dL) and serum copper 50 μg/dL (normal >70 μg/dL). Serum copper not bound to ceruloplasmin was found to be 38 μg/dL (normal <15 μg/dL), indicating free copper in the blood.[2] Autoimmune markers, alpha-1 antitrypsin, hepatitis B and C were absent. An X-ray radiograph of the chest showed hilar lymphadenopathy and infiltrates in the parenchyma. The patient's Montoux test was highly positive. His blood culture was positive for *Staphylococcus* and *Pseudomonas*. In view of sepsis and pulmonary tuberculosis, the patient was started on ciprofloxacin and antitubercular drugs rifampicin, ethambutol and streptomycin. D-Penicillamine was also given. The patient's condition deteriorated further,

leading to sepsis, encephalopathy, electrolyte imbalance and finally death. Liver autopsy showed loss of architecture, Mallory's hyaline, neutrophilic and chronic lymphocytic infiltration, and hepatic fibrosis. Excess pigment as stained by orcein stain and rubeanic acid stain was seen in zone 1 of the lobules. Orcein stain showed diffuse granular positivity for copper associated protein. In the present case, the plasma GSH level was 0.64 µmol/g Hb, which were significantly lower than the normal value of 1.2 µmol/g Hb. Superoxide dismutase (SOD) is an important enzyme for the dismutation of super oxide anion free radicals ($O_2^- + 2H^+$) into harmless products ($H_2O_2 + O_2$). In this patient, the activity of SOD in red blood cells hemolysate was 0.352 units/g Hb, which was lower than the age matched controls 0.611 units/g Hb. Membrane lipid peroxidation was assessed by measuring malondialdehyde (MDA) in the RBC hemolysate and was found to be 0.069 µmol MDA/g Hb. It was significantly higher than the normal level of 0.03 µmol MDA/g Hb.

The patient was diagnosed with Wilson's disease, interestingly with glucose-6-phosphate dehydrogenase deficiency. Although both are recessive genetic disorders, G6PD is X-linked and Wilson's disease is autosomal. This patient showed WD phenotypes, as well as hemolysis for G6PD deficiency. We carried out the mutational analysis by single-strand conformational polymorphism (SSCP) and direct DNA sequencing in

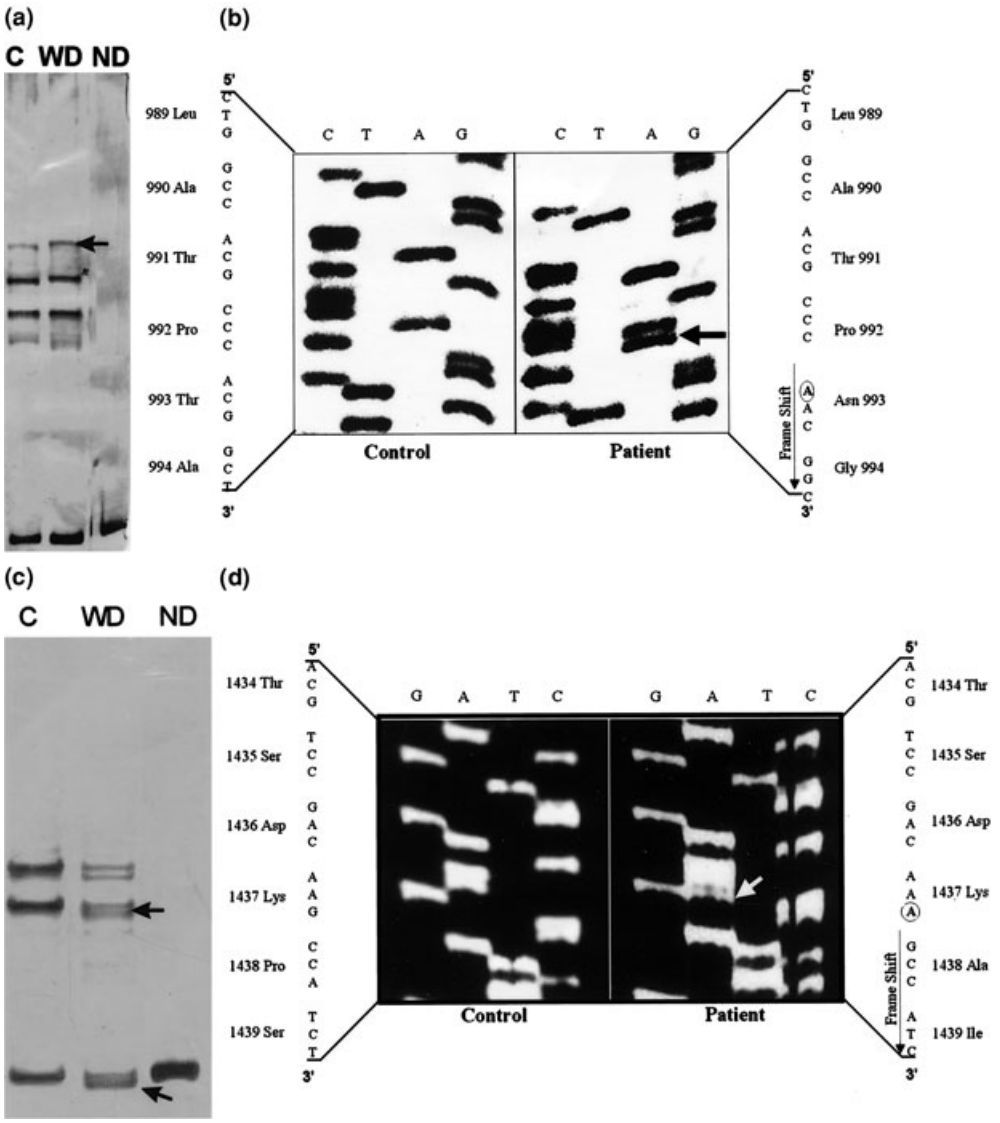

Figure 1 Detection of WD mutations 2976insA and 4311insA. (a) Single-strand conformational polymorphism (SSCP) analysis of exon 13. C, Normal subject; WD, Wilson patient; ND, non-denatured polymerase chain reaction (PCR) product. Arrow indicates the shifts of the bands. (b) Sequence of exon 13 from a WD patient and a control patient. The arrow in the WD sequence indicates insertion of adenine. (c) SSCP analysis of exon 21. C, control subject; WD, Wilson patient; ND, non-denatured PCR product. The arrow shows the hetroduplex formation at the bottom of the gel. (d) Sequence of exon 21 from a WD patient and a control patient. The arrow in the WD sequence indicates insertion of adenine.

this patient. For mutational analysis, genomic DNA was extracted from peripheral blood leukocytes following the standard procedure of the QIAamp DNA Blood Midi Kit (Qiagen GmbH, Hilden, Germany). Polymerase chain reaction (PCR) was used to amplify all 21 exons of *ATP7B* and their corresponding intron-exon junction, using specific primers in the flanking region. All amplified exons of the WD gene were subjected to mutational analysis by SSCP. The Wilson's disease PCR samples showed shifts, relative to normal samples of corresponding exons of *ATP7B* on SSCP which were subjected to DNA sequencing. The same primers used for SSCP analysis were used to generate asymmetric PCR products and sequencing. The sequence was determined using (^{35}S)α-dATP and the sequenase kit (US Biochemicals, San Diego, CA, USA).

Direct DNA sequencing of the PCR products that exhibited shifts showed insertion of two novel mutations, 2976insA and 4311insA, in exons 13 and 21, respectively (Fig. 1). These mutations are expected to alter the reading frame of the gene product and result in non-functional *ATP7B* protein. It is well accepted that the type of mutation in a specific region of the gene manifests a specific phenotype in the patient. Mutations 2976insA and 4311insA cause a frame-shift by changing threonine to asparagine at the 993 amino acid position in exon 13 and proline to alanine at the 1437 position in exon 21 of the *ATP7B*. Mutation 2976insA in exon 13 affects the sixth transmembrane domain and/or cation channel of *ATP7B*, which would affect the copper transport from the intracellular compartment to the extra-cellular compartment. The significance of the carboxy C terminus of *ATP7B* with respect to its biosynthetic role in copper delivery to the ferroxidase ceruloplasmin has recently been reported. To date, in the C terminus of *ATP7B*, only two missense mutations and one deletion have been reported in a WD patient.[7] Interestingly, the characterized 4311insA in exon 21 creates a downstream stop codon at amino acid position 1447. This change would result in a shortened functionless protein and is predicted to affect copper delivery. Mutation 2976insA has a deleted restriction site for the *Sec I* restriction enzyme in a WD patient; however, 4311insA did not create/delete any restriction site. The presence of mutations 2976insA and 4311insA were checked in 25 controls by restriction digestion and DNA sequencing. Both mutations were found to be absent in all. Therefore, the patient was a compound heterozygote and had mutations in both alleles, and both mutations cause frame-shift and would produce a shortened functionless WD protein. The defect in the sixth transmembrane domain and carboxyl region of *ATP7B* might result in excessive copper accumulation in the liver, brain and other organs.

We concluded that these two mutations, 2976insA and 4311insA, and depleted levels of GSH in a patient might lead to an increase in free copper levels, which have been shown to enhance DNA damage in biological systems via formation of hydroxyl radicals. Copper is a potent catalyst of the Haber–Weiss reaction, in which hydrogen peroxide is converted to the hydroxyl radical and rapidly reacts with polyunsaturated fatty acid residues of cell membrane, thiol-containing proteins and nucleic acids. In the present study, cell membrane damage was also evident from marked enhancement of MDA. In addition, GSH has a very strong affinity for Cu and forms stable complexes, which have been shown to act efficiently in the donation of Cu (I) to copper chaperon (CSS) and subsequently to copper-zinc SOD. Therefore, reduced activity of SOD could be possible as a result of low levels of GSH. An observed reduction in G6PD could be a prominent factor for low levels of reduced GSH because G6PD deficiency is known to influence GSH status in humans and cause hemolysis. All of these findings suggest that novel mutations 2976insA and 4311insA might exacerbate copper-induced toxicity by alteration of G6PD, and antioxidant status, which might lead to the early onset of the disease with hepatological and neurological manifestations, as well as susceptibility to pulmonary tuberculosis.

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CURE RATE OF HIGH DOSE OMEPRAZOLE AND AMOXICILLIN THERAPY FOR TREATMENT-RESISTANT *HELICOBACTER PYLORI* INFECTION

To the Editor,

For patients in whom primary attempts to eradicate *Helicobacter pylori* (*H. pylori*) have failed, especially in the setting of clarithromycin and metronidazole resistance, the optimum treatment regimen is unclear. Bismuth containing quadruple therapy is potentially effective but, in Australia, bismuth subcitrate as part of a combination pack is no longer available. Dual therapy with omeprazole and amoxicillin has been reported as a possible second or third line option,[1] but experience with such high doses of omeprazole is very limited. We designed a pilot study to prospectively evaluate the safety and efficacy of high dose omeprazole with amoxicillin in a group of patients who had treatment resistant *H. pylori*.

Patients were chosen if there had been at least one failed attempt to eradicate *H. pylori* infection. This group was comprised of new referrals to a gastroenterology clinic for management of their infection and pre-existing clinic patients who had ongoing infection and may have participated in previous trials of eradication therapy. An endoscopy to exclude an active peptic ulcer had been carried out within the previous 12 months and was only repeated if new upper gastrointestinal tract symptoms were present or culture of *H. pylori* was required. Current *H. pylori* infection was confirmed by a positive culture result obtained at endoscopy or by the string test (Entero-test HP, HDC Corporation, CA, USA). The bacterial isolates were tested to determine the antibiotic susceptibility levels of each strain by the disc diffusion method. This was carried out prior to participation in the present trial. If this bacterial diagnosis was more than 3 months prior to trial entry, active current infection was confirmed by positive 14-C urea breath test (PYtest, Tri-Medical, Perth, Australia). Trial exclusion criteria included the following: penicillin allergy; use of other antibiotics in the trial period; pregnancy; and lactation. Pre-existing proton pump inhibitor (PPI) was permitted (many subjects required the agent for dyspepsia symptom relief), with a changeover to omeprazole for the duration of the trial drug administration. The local institutional research ethics committee approved the conduct of the study and written informed consent from each patient was obtained prior to entry into the trial.

Omeprazole 40 mg and amoxicillin 1 g were given three times daily for 14 days. The drugs were to be taken together, with no particular regard for meal times. Pre- and post-treatment interview, physical examination and a pill count was carried out to assess compliance, tolerability and adverse events.

Six weeks after the completion of the treatment course, subjects were interviewed to assess late adverse events and a urea breath test (UBT) was carried out according to the manufacturer's instructions. A positive result was considered indicative of persistent infection. In the event of an indeterminate result, a repeat UBT was carried out 7 days later, ensuring that acid suppressive therapy had been ceased. Subjects who remained positive were offered counselling and further management at our clinic. Subjects with a negative UBT at 6 weeks were offered further testing 12 weeks post-treatment to confirm persistent eradication.

The present study was carried out between November 2000 and February 2002. Fifteen subjects (six men,

Table 1 Patient details including previous treatments, antibiotic susceptibility results and outcome in this trial

Patient	Previous treatments	Amoxicillin susceptibility	Metronidazole susceptibility	Clarithromycin susceptibility	PPI pre-treatment	6 week UBT result
1	OAC	S	R	S	No	–
2	OAC × 2	S	R	R	No	–
3	OAC, RBcAC	S	R	R	No	–
4	BMT, OAC, NTZ	S	R	S	No	–
5	PAC, AORif, NTZ	S	R	R	Yes	+
6	BAMx2, OAC × 2	S	R	R	No	–
7	BMT, OAC × 2	S	R	R	Yes	+
8	BMT, OAC	S	R	S	No	–
9	OAC × 2, NTZ	S	R	R	Yes	+
10	BMT, OAC, OBMT	S	R	S	Yes	–
11	BMT, OAC, NTZ	S	R	R	No	–
12	OAC	S	R	R	No	+
13	BMT, OAC	S	R	R	No	+
14	BM, BMT, NTZ	S	R	S	Yes	–
15	BMT, OA (low dose), OMC	S	R	R	No	–

A, amoxicillin; B, bismuth subcitrate; C, clarithromycin; M, metronidazole; NTZ, nitazoxanide; O, omeprazole; P, pantoprezole; PPI, proton pump inhibitor; R, resistant; RBc, ranitidine bismuth subcitrate; Rif, rifabutin; S, sensitive; T, tetracycline; UBT, urea breath test.

nine women) aged 18–75 years (mean age 51 years) were enrolled. The median number of previous treatments was three (range 1–4). Indication for eradication of *H. pylori* was gastritis with dyspepsia (seven cases), dyspepsia (four cases), long-term PPI use in gastroesophageal reflux disease (two cases), family history of gastric carcinoma (one case) and previous duodenal ulcer (one case). Previous regimens used and antibiotic susceptibility profiles are shown in Table 1. All patients exhibited metronidazole resistance. No amoxicillin resistance was detected. Clarithromycin resistance was 66% overall, but 100% in those we could identify with previous clarithromycin-based treatment.

At 6 weeks post-treatment, 10/15 (67%, 95% confidence interval, 38–88%) subjects had a negative UBT. Eight of these 10 had a further negative UBT at 12 weeks with two patients lost to follow up. In five subjects with a clarithromycin-sensitive isolate, the cure rate was 5/5 (100%). In the setting of clarithromycin resistance, the cure rate was 5/10 (50%).

The treatment was generally well tolerated and all subjects completed the treatment. One subject missed only one dose of omeprazole and amoxicillin. Two patients reported mild headache and diarrhoea during treatment and another two reported that their reflux symptoms worsened following the trial (both subjects had negative UBT results at 6 weeks), which required an increase in their maintenance acid-suppressive therapy.

The present study of *H. pylori* eradication in patients with a median of three previous treatment failures shows that high dose omeprazole and amoxicillin is well tolerated and cures about two-thirds of infections. Our findings are similar to those of Ellenrieder *et al*. who achieved a 77% eradication rate in a group of patients with one previous treatment failure.[1]

In patients with duodenal ulcers receiving dual therapy, a relationship between omeprazole dose and efficacy of eradication was attributed to increasing intragastric pH. Amoxicillin is an acid labile antibiotic and its activity is enhanced in a more neutral pH environment. At an increased gastric pH induced by omeprazole, it has also been suggested that bacterial overgrowth in the stomach can occur and might displace *H. pylori* or be inhibitory to its growth.[2] Our trial used omeprazole 40 mg three times daily with the aim of maintaining gastric acid suppression throughout the day, and amoxicillin was given at the same times to improve compliance. Recently, attention has been drawn to the impact of CYP2C19 polymorphism on the metabolism of omeprazole and its implications for *H. pylori* treatment. The 'poor metabolizer' genotype, present in 12–70% of Asian populations, is associated with more profound gastric acid suppression and improved *H. pylori* eradication rates compared with the 'extensive metabolizer' genotype, which occurs in up to 95% of Caucasian populations. In the latter, Kita *et al*. have suggested that 80 mg omeprazole has a similar acid suppressing action as 20 mg in poor metabolizers and a daily dose of 160 mg per day might be needed for optimal anti-*H. pylori* activity.[3] In treatment-naïve subjects who were extensive metabolizers, Tanigawara *et al*. only achieved a 50% eradication rate with omeprazole/amoxicillin dual therapy.[4] The metabolism of rabeprazole is largely independent of CYP2C19 and Furuta *et al*, using rabreprazole and amoxicillin, have reported a 100% cure rate in 17 subjects who had previously failed one course of standard triple therapy, irrespective of the CYP2C19 genotype.[5]

The results of this and other studies suggest that dual therapy with amoxicillin and potent acid suppression is an option for treating *H. pylori* infection, particularly as it uses easily available components. While the importance of antibiotic resistance in difficult-to-treat *H. pylori* is well recognized, the role of CYP2C19 polymorphism and the optimal dosing of PPI is of increasing relevance to this subset of patients.

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HETEROTOPIC MESENTERIC OSSIFICATION: A RARE REACTIVE PROCESS

To the Editor,

Heterotopic ossification is an unusual process in which abnormal formation of bone is observed outside the skeleton. The histogenesis of intra-abdominal new bone formation from connective tissue caused by trauma supports a metaplastic process. Here, an extraordinary finding related to an osseous lesion of the mesentery and omentum in a 74-year-old patient, who was operated on for an intestinal obstruction, is described. The patient died after 1 month of hospitalization.

Heterotopic ossification occurs in a variety of body sites and tissues and is associated with trauma. Soft tissue injury can be caused by a number of mechanisms such as trauma (60.75%), small mechanic injuries, ischemia and inflammation.[1] The rapid growth of these lesions in the mesentery, which frequently arouses clinical suspicion, in conjunction with their hypercellularity, cytological atypia and mitotic activity resembling myositis ossificans, explains the use of the term 'mesenteritis ossificans' or 'heterotopic mesenteric ossification'. A more appropriate term for mesenteritis ossificans would be pseudomalignant osseous tumor of extraskeletal soft tissues. This was first described by Fine and Stout in 1956 on the basis of eight cases, but none occurred in the mesentery.[2] In 1992, Yannopoulos *et al*. described a case of multifocal heterotopic mesenteric ossification.[3]

A 74-year-old man suffering from hypertensive heart disease with a 1-year history of surgical reconstruction of an umbilical hernia, cholecystectomy and a recent prostatectomy presented with a small bowel obstruction (noticeable 4 days after the prostatectomy) and mild renal failure. Lysis of adhesions as well as resection of a small segment of the small bowel was carried out during a second operation. Ten days later, persistent obstructive symptoms led to a third laparotomy, which lasted 18 h. During this operation, we noticed that the adhesions between the small bowel and mesentery had a fibroosseous appearance, which made them too difficult for us to remove without injuring the small bowel. As a consequence, as well as the lysis of adhesions, the majority (80%) of the small bowel and the right colon up to the midtransverse colon were resected and sent for pathological examination. During the operation, a jejunocolic anastomosis was repaired. Postoperatively, the patient was placed in an intensive care unit with monitoring, total parenteral nutrition and i.v. antibiotics. The patient died from severe sepsis and liver and renal failure on the 6th postoperative day (after the third operation) in the intensive care unit.

Histologically, there were fibrous septa of variable thickening that entrapped adipose tissue, nerves and blood vessels within the mesenteric tissue. These fibrous septa were moderately cellular and mainly composed of fibroblasts (Fig. 1). Cellular fasciitis-like areas, which have been reported in other cases,[3,4] were absent in the present case. Trabeculae of osteoid rimmed by osteoblasts were noticed in proximity to the fibrous septa. The osteoblasts showed occasionally prominent nuclei but lacked malignant cytological features. In the present case, the osteogenic proliferation did not exhibit a 'zonal' architecture. Chronic inflammation throughout the process was minimal. Because the presence of foreign bodies arguably played a role in the pathogenesis of the present lesion, we should underline the histological absence of any foreign body granuloma in the examined sections. The surgical procedures used were unlikely to introduce any foreign bodies. Because the inflammatory reaction was generally unremarkable, the reactive nature of the lesion was questionable; a metaplastic nature seems more likely.

The difficulty and importance of microscopically distinguishing the present reactive lesion from a sarcomatous process is noteworthy. In the present case, the differential diagnosis was quite easy because no morphological features suggesting malignancy were observed. However, in other reported cases of heterotopic mesenteric ossification, where zone phenomena are prominent, it is important not to overdiagnose this reactive process as an extraskeletal osteosarcoma. The term 'zone phenomena' refers to the immature central portion of the lesion (characterized by hypercellularity, atypia and mitoses) progressively maturing toward the periphery, first forming a primitive osteoid, then a well-organized osteoid with prominent osteoblastic miming, and finally a mature lamellar bone.[4] This progressive maturation pattern excludes the diagnosis of malignancy in such cases.[4]

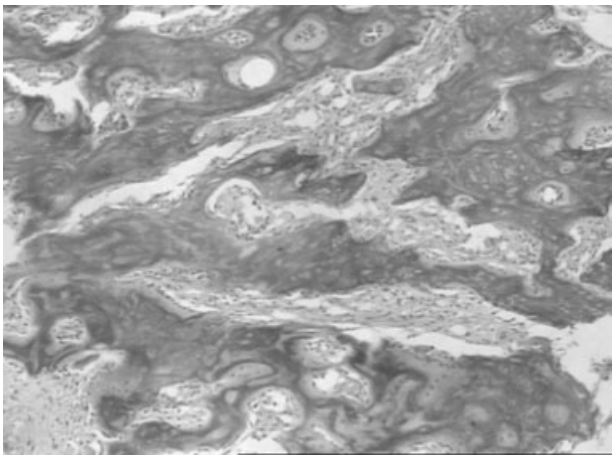

Figure 1 Osteoid trabeculae and fibrous septa (HE; original magnification, ×40).

The clinical highlight of the present case was the remarkable thickening of the bowel wall and the difficulty in lysis of the osteoid adhesions, which can also be observed in ossification of abdominal scars.[5] The rapid appearance (10 days after the 2nd operation) of the fibrous and osteoid structures is similar to other reported cases,[3,4] and could have been caused by traumatic results after the lysis of bowel adhesions in the second operation. It is of interest that in this reported case, ossification expanded to the root of the mesentery. Bone formation was noticed both between the mesentery and the small bowel wall, and between the small bowel loops. As in other reported cases,[3,4] the periphery of the omentum was involved but the abdominal wall was not.

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