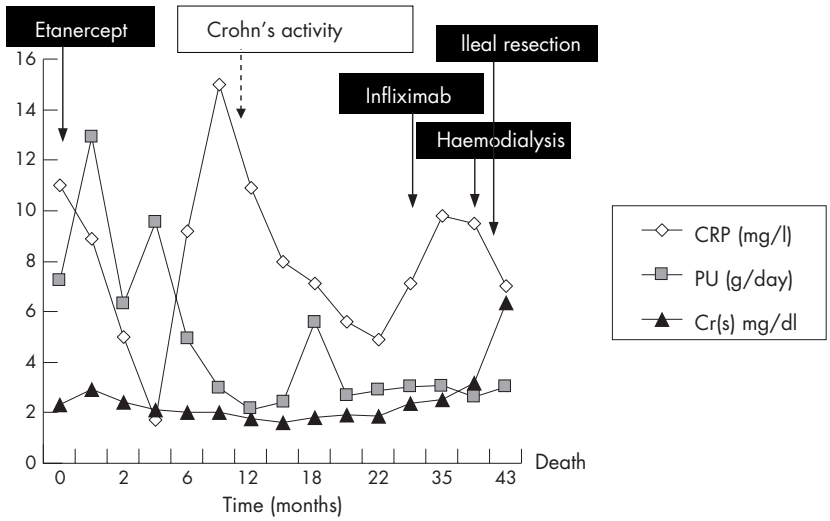

Figure 1 Serum C reactive protein (CRP), serum creatinine (Cr(s)), and proteinuria (PU) in our patient after anti-tumour necrosis factor treatment.

We chose etanercept initially because a case had been reported of a good response of AA amyloidosis to etanercept in 2001[2] and the results of the clinical trial showing lack of efficacy of etanercept in Crohn's disease had not been published.[3] This case illustrates the requirement in secondary amyloidosis for exhaustive control of any inflammatory activity, and highlights the fact that delay in providing effective treatment may prove fatal. Etanercept also lacks effectiveness for preventing reactivation of Crohn's disease. Accordingly, we should always choose infliximab for this type of patient, even if Crohn's disease has been inactive for a long period.

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Conflict of interest: declared (the declaration can be viewed on the *Gut* website at http://www.gutjnl.com/supplemental).

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Author's reply

In response to the comments of Fernandez-Nebro *et al* to our case (*Gut* 2006;**55**:744–5), we would like to make some additional observations.

We demonstrated a patient with systemic amyloidosis associated with Crohn's disease significantly responding to infliximab. Specifically, infliximab not only decreased serum amyloid protein level but also ameliorated renal function of the patient. With regard to the effect of infliximab on renal function, Anelli and colleagues[1] also demonstrated a patient with rheumatoid arthritis and renal amyloidosis whose serum creatinine level significantly improved (from 2.8 to 1.3 mg/dl) after treatment with infliximab. However, it is notable that serum creatinine level of our patient rapidly improved after treatment with infliximab.

Fernandez-Nebro *et al* suggested an association of other concomitant factors, such as volume depletion or drugs, which can affect baseline creatinine levels. However, the patient had not received any drugs that might affect baseline creatine levels for at least six months before treatment with infliximab. Also, we suggested that volume depletion might be involved in the progression of his renal dysfunction as the patient was admitted to our hospital because of acute progression of renal dysfunction during exacerbation of Crohn's disease. Thus he underwent parental nutrition soon after admission. Central venous pressure of the patient on the 10th hospital day was within normal limits (5.5 cm H_2O). An appropriate volume of infusion was provided for the patient but his renal function did not improve until he received an infusion of infliximab.

Based on these findings, we considered that progression of his renal dysfunction was not caused by drugs or volume depletion alone and that infusion of infliximab was clearly involved in improvement of his renal function. Progression of renal dysfunction in our patient appeared to be correlated with exacerbation of Crohn's disease and a high concentration of serum amyloid (762 μg/ml; normal range <8 μg/ml). It has been suggested that tumour necrosis factor blocking agents might not only reduce the synthesis of amyloid precursors but also slow amyloid deposition.[2] In addition, Bosca and colleagues[3] demonstrated that infliximab decreased the degree of amyloid deposits. Although the detailed mechanism is unknown, infliximab may work more effectively on patients with acute progression of renal dysfunction with high serum amyloid levels, such as in our patient.

Our patient received an additional infusion of infliximab eight weeks later and serum creatine levels have been less than 3 mg/dl for approximately 41 weeks. Unfortunately, we were not able to assess the effect of infliximab on proteinuria because he could not collect total volume of urine before treatment with infliximab.

As Fernandez-Nebro *et al* demonstrated, systemic amyloidosis is a serious and fatal complication of Crohn's disease. It is critical to control the intestinal inflammation of Crohn's disease to prevent progression of renal dysfunction due to amyloidosis. In this context, we also believe that infliximab should be the first choice for patients with systemic amyloidosis associated with Crohn's disease.

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Anti-outer membrane of porin C and anti-I2 antibodies in indeterminate colitis

In a previous prospective study on the diagnostic value of serological markers in patients with indeterminate colitis (IC), we demonstrated that those who remained classified as IC during prospective follow up were more often negative for anti-*Saccharomyces cerevisiae* antibodies (ASCA) and atypical perinuclear antineutrophil cytoplasmic antibodies (pANCA) compared with patients with an eventual diagnosis of Crohn's disease (CD) or ulcerative colitis (UC).[1] New antibodies directed against luminal bacterial components have been reported in inflammatory bowel disease. Anti-OmpC IgA (directed against outer membrane porin C of *E coli*) and anti-I2 (directed against *Pseudomonas fluorescens*) have both been found in approximately 50% of CD patients[2–4] and

Table1 Marker combinations in the study population

	n	CD	UC	IC
Four positive markers				
pANCA+/ASCA+/OmpC+/I2+	1	1	0	0
Three positive markers				
pANCA+/ASCA+/OmpC+/I2−	1	0	0	1
pANCA−/ASCA+/OmpC+/I2+	3	1	0	2
pANCA+/ASCA−/OmpC+/I2+	3	1	0	2
Total	7	2	0	5
Two positive markers				
pANCA+/ASCA−/OmpC-/I2+	3	1	0	2
pANCA−/ASCA+/OmpC-/I2+	8	2	2	4
pANCA+/ASCA+/OmpC−/I2−	2	1	1	0
pANCA−/ASCA−/OmpC+/I2+	6	1	1	4
pANCA−/ASCA+/OmpC+/I2−	1	1	0	0
pANCA+/ASCA−/OmpC+/I2−	1	0	1	0
Total	21	6	5	10
One positive marker				
pANCA+/ASCA−/OmpC−/I2−	12	4	6	2
pANCA−/ASCA−/OmpC−/I2+	12	1	2	9
pANCA−/ASCA+/OmpC−/I2−	11	3	2	6
Total	35	8	10	17
No positive markers				
pANCA−/ASCA−/OmpC−/I2−	23	4	2	17
Total	87	21	17	49

IC, indeterminate colitis; CD, Crohn's disease; UC, ulcerative colitis; pANCA, perinuclear antineutrophil cytoplasmic antibodies; ASCA, anti *Saccharomyces cerevisiae* antibodies; anti-OmpC, antibodies against outer membrane porin C of *E coli*; anti-I2, antibodies against *Pseudomonas fluorescens*.

are both associated with ileal disease, increasing disease duration, and more severe disease that requires small bowel surgery.[5 6]

We investigated if anti-OmpC and anti-I2 were additive to ASCA and pANCA in the definitive diagnosis of patients who were included in our initial cohort of IC, and if patients who remained unclassified over time also lacked response to these microbial antigens. For additional testing of anti-OmpC and anti-I2, the serum of 93/97 patients included in the initial cohort was available. Further clinical evolution was possible in 90/97 patients and seven were lost to follow up. Additionally, serum from 93 healthy individuals (female/male 54/39; mean age 41.8 years (range 24–77)) and 64 inflammatory controls (female/male 29/35; mean age 57.8 years (range 20–92)), including infectious gastroenteritis (n = 28), diverticulitis (n = 23), ischaemic colitis (n = 4), acute self limiting colitis (n = 1), pseudomembraneous colitis (n = 2), and other non-specific colitis (n = 6), were tested for anti-I2 and anti-OmpC to investigate the specificity of these markers.

Criteria for diagnosis of IC in the present study as well as criteria for change in diagnosis to CD or UC during further clinical follow up have been described in detail previously.[1] Recently, however, a proposal towards a novel classification of IC has been made.[7] This should be taken into account for future studies in IC. Both anti-OmpC and anti-I2 were determined by an ELISA assay. For anti-OmpC antibody determination, samples were sent to Prometheus Laboratories Inc. (San Diego, California, USA).[8] For anti-I2 antibody determination, samples were sent to Dr Carol Landers (Los Angeles, California, USA).[3]

Mean disease duration for patients was 11.4 years (range 2.5–40.5). After 2.5 years of prospective follow up, disease in 41/90 (45.6%) patients has evolved towards either CD (n = 23) or UC (n = 18) after a mean disease duration of 5.3 years (range 1 month–26 years). Additional follow up of 1.5 years resulted in six more patients in whom the diagnosis was changed to CD and four more patients to UC. The remaining 49 (54.4%) patients remained classified as IC (mean disease duration 13.9 years (range 2.5–40.5)).

With the four markers tested, various combinations of responses were found (see table 1).

Calculations were made based on those patients in whom clinical as well as serological data were complete (n = 87). These 87 patients represented 21 CD, 17 UC, and 49 IC patients. Of all marker combinations, pANCA−/ASCA−/anti-OmpC−/anti-I2− was most frequently present in 23 of 87 (26.4%) patients. In 17/23 (73.9%) seronegative patients, the diagnosis remained IC. Only six of 23 (26.1%) patients in this seronegative group were given a final diagnosis of CD (n = 4) or UC (n = 2). The proportion of patients with at least one antibody response present that evolved towards a specific diagnosis was significantly higher: 32/64 (50%) patients (p = 0.047). The prevalence of anti-I2 in the IC cohort, healthy controls, and inflammatory controls was 41.9% (39/93), 17.2% (16/93), and 31.3% (20/64), respectively. Consequently, the respective sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of anti-I2 in the IC cohort were 41.9%, 76.4%, 48.1%, and 71.6%. The prevalence of anti-OmpC in this IC cohort, healthy controls, and inflammatory controls was 17.2% (16/93), 2.2% (2/93), and 25% (16/64), respectively. Respective sensitivity, specificity, PPV, and NPV of anti-OmpC in patients with IC were 17.2%, 88.5%, 47.1% and 64.4%. The prevalence of anti-I2 and anti-OmpC in this study cohort was lower than the reported prevalence of these markers in CD.[2 3 6] This is not unexpected as both markers are more often found in the presence of ileal CD. However, a large proportion of controls, especially inflammatory controls, also expressed anti-OmpC and anti-I2 antibodies.

The results of the present study indicate that by adding the antibodies anti-OmpC and anti-I2, the predictive capacity of serological tests only increases marginally and specificity drops significantly. More importantly, our results confirm the presence of a large group of IC patients who are negative for serological markers and may represent a separate phenotype.

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H pylori negative MALT lymphoma patients successfully treated with antibiotics: doubts about their *H pylori* negativity

We read with interest the report by Raderer and colleagues (*Gut* 2006;**55**:616–18). The authors reported on six patients with localised gastric *Helicobacter pylori* negative mucosa associated lymphoid tissue (MALT) lymphoma, successfully treated with eradication therapy. They suggest that patients with early stage *H pylori* negative gastric MALT lymphoma might benefit from antibiotics as the sole treatment modality and, as explanation for their findings, they propose the hypothesis of a role in gastric MALT lymphoma of bacteria other than *H pylori* and potential immunomodulatory effects of antibiotics.

We are surprised that the authors did not consider the possibility of false negative diagnostic tests for *H pylori*. As no single test is accepted as the gold standard for diagnosis of *H pylori* infection,[1 2] they correctly used more than one: histology, urea breath test, stool antigen test, and serology. However, none of these tests has a sensitivity of 100%,[1 2] and all but serology may be affected by an altered gastric microenvironment, such as hypochlorhydria.[1 3 4] The authors give no information about previous use of antisecretory drugs in their patients but it is highly probable that patients with leading symptoms such as epigastric pain and bleeding were treated with proton pump inhibitors or H_2 antagonists before diagnosis of MALT lymphoma and this treatment may have led to negative results for the urea breath test, stool antigen test, and histology for *H pylori* infection.

Also, serology, in particular enzyme linked immunosorbent assay (ELISA), may give negative results in patients with previous exposure to *H pylori* infection.[1] In fact, in patients with gastric cancer and atrophic body gastritis, immunoblotting can document past *H pylori* infection in patients classified as *H pylori* negative by ELISA serology.[5 6] The authors do not mention what type of serological approach they used, but it is plausible that sera immunoblotting might have revealed a previous contact to *H pylori* in their MALT lymphoma patients.

Furthermore, neutrophils are a very sensitive indicator of the presence or absence of *H pylori* and disappear within days of cure of infection.[7] Thus it would be interesting to compare this histological feature before and after eradication treatment as a reduction in the inflammatory activity after treatment may be interpreted as the presence of *H pylori* infection before therapy.

Finally, in *H pylori* negative MALT lymphoma patients, a high rate of t(11;18)(q12;q21) has been reported,[8] which has been described as identifying patients whose lymphoma will not regress following antibiotic treatment.[9] In the case series of Raderer *et al*, only one of the patients showed this genetic alteration. The very low presence of genetic changes together with the good response to antibiotics is another reason to question *H pylori* negativity.

In conclusion, we believe that the authors' interpretation of their results is incomplete as in our opinion the hypothesis—that *H pylori* infection was present but not revealed due to intrinsic limits of diagnostic methods—is more plausible than that given by the authors regarding bacteria other than *H pylori* or immunomodulatory effects of antibiotics.

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Conflict of interest: None declared.

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Author's reply

In their comment on our recent paper on antibiotic treatment in apparently *Helicobacter pylori* negative patients with gastric mucosa associated lymphoid tissue (MALT) lymphoma (*Gut* 2006;**55**:616–18), Lahner *et al* raise the question of whether our patients might indeed have been rightfully considered *H pylori* negative or whether the methods applied in our series were insufficient. In fact, this is an important argument which is extremely difficult to counter as the presence of an agent is easier to prove than its absence. Following their line of argument to the ultimate conclusion, one might speculate whether such a thing as *H pylori* negative gastric MALT lymphoma does indeed exist. Philosophical considerations notwithstanding, however, I still think that to the best of the current knowledge a *H pylori* negative status can be assumed with a sufficient amount of certainty for the large majority of our six patients, if not for all. As the authors have correctly pointed out, we used four individual tests to assess *H pylori* status in our series, as no single gold standard has been defined.

In view of the fact that some tests might indeed be influenced by antisecretory drugs, it is important to note that only two of our patients had undergone treatment with a proton pump inhibitor before diagnosis of MALT lymphoma (one three and the other five weeks before diagnosis). In addition, the gastric mucosa was conspicuously devoid of any inflammatory changes in three cases, initially even suggesting secondary gastric involvement with MALT lymphoma which could nevertheless be ruled out by meticulous staging in all patients. In the other patients, none had evidence of histological characteristics of ex-*H pylori* gastritis while one had changes associated with C-gastritis (and in fact was related to intake of diclofenac prior to diagnosis).

As Lahner *et al* have correctly pointed out and as we have also discussed in our report, only one of our six patients was positive for t(11;18), which appears to be a lower percentage than recently reported for *H pylori* negative gastric MALT lymphomas.[1] In view of the extremely small sample size in our report due to the rarity of such patients, our results are not necessarily implausible simply for statistical reasons. Interestingly, the definition of *H pylori* negativity in the study on t(11;18), in which we have also participated,[1] is readily accepted by Lahner *et al* while they doubt the validity of our interpretation of such patients as being *H pylori* negative. In fact, the criteria used were almost identical, or even less stringent, as stool antigen testing was not done in the 17 patients included in the multicentre analysis.[1] Thus the fact of a lower rate of t(11;18) in our series as opposed to the other study[1] is not a valid argument against a potential *H pylori* negativity in our report.

While the authors are nevertheless correct in suggesting that a small proportion of such patients might indeed be false negatives due to the shortcomings of available testing, we believe that to the best of our current knowledge the combination of histology, breath test, serological testing using ELISA, as well as stool antigen leaves a relatively narrow margin for doubt. However, we agree that one cannot rule out with complete certainty that a slight probability of missing prior contact with *H pylori* remains when using this approach. In terms of clinical management, however, the results of our series are still valid (that is, that patients with absence of proof of *H pylori* infection might also benefit from antibiotic treatment and are not necessarily immediate candidates for more aggressive oncological treatment).

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