

PostScript

LETTERS

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Upper gastrointestinal bleeding and surrogate end points

In their paper, Hawkey *et al* (*Gut* 2001;49:372-9) report on the use of blood in the stomach as a surrogate of hard end points such as rebleeding, need for surgery, and death in a group of patients with upper gastrointestinal bleeding randomised to placebo, tranexamic acid, lansoprazole, or both lansoprazole and tranexamic acid. Using logistic regression analyses, they found that blood in the stomach was predictive of clinical outcome and that the active medications decreased the gastric pool size at endoscopy. The authors suggest that although the medications did not significantly improve the rates of clinical outcomes, they would probably do so in a trial of sufficient sample size given the effect achieved on the surrogate endoscopic end point. These conclusions should be viewed with caution, if not with skepticism, for several reasons. Firstly, it is interesting that the authors chose the presence of blood in the stomach as a substitute end point based on a previous study in which no statistical difference was found for the prevalence of this endoscopic sign alone between patients randomised to placebo and omeprazole.¹ Rather, in that study, the presence of a constellation of findings defined as "any sign" (blood, active bleeding, or stigmata) showed a significant reduction in favour of the omeprazole group. The current trial should then be viewed as an exploratory attempt to assess prospectively the independent prognostic contribution of blood in the stomach as a correlate of well defined clinical events. Thus the validity of extrapolating that the active medications will favourably impact on clinical outcomes because they reduce the gastric blood pool in the same cohort of patients in whom a correlation between the surrogate end point and clinical outcomes is being assessed is questionable.

Secondly, no clear definition is provided for what is meant by high or low risk groups, although this factor (risk classification) appears to be a consistent predictor of endoscopic and clinical outcomes. If this categorisation varies among different caregivers, how

can we make sure what the regression analyses are truly predicting as it relates to risk status?

Thirdly, there seems to be an imbalance between the number of independent variables and the number of predicted outcomes in the logistic regression analyses. For instance, estimation of rebleeding in the model had only 39 predicted events and at least eight factors selected as potential determinants of clinical outcome (blood in the stomach among them). Such a disproportion between predictors and predicted variables is known to cause overestimation or underestimation of the regression coefficients, thus distorting the estimated effect.^{2,3}

Finally, application of a marker as a surrogate end point requires demonstration of its accuracy (correlation with the clinical end point) and precision (reproducibility of the marker), with rather restrictive criteria applying to the definition of "surrogate end point".^{4,5} Unfortunately, substitute end points often do not predict the true clinical effects of interventions, and sobering examples remind us that they also turn out to be inadequate markers for clinical practice.⁶ Hawkey *et al* address in the introduction of their paper the issue of sample size requirements to find significant differences on hard clinical end points. This seems to be a recurring academic exercise in the discussion of intervention studies for gastrointestinal bleeding because trials almost invariably end up with sample sizes that fall short of providing the answers to the relevant questions. The average sample size in a recent meta-analysis of 21 studies of pharmacological treatment for upper gastrointestinal bleeding was only 170,⁷ and that number is larger than the sample size included in the majority of endoscopic treatment trials.

Large scale studies have long been claimed in the field of therapeutic intervention for ulcer bleeding.⁸ This important clinical research area may not be advanced any further if conduction of trials with a small sample size continues; persistence with this methodological approach is sure to delay further progress. Perhaps we should follow the bold and altruistic examples of other fields in medicine, such as that of cardiology, which in one international effort alone assembled over 40 000 patients in order to clarify the benefits of different thrombolytics in the treatment of acute myocardial infarction.⁹ In the long run, the conduction of such trials will be the only defence against misleading reliance on uncertain substitute end points, and will provide at the same time the opportunity to reach firm conclusions derived from direct measures of outcome.¹⁰

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Author's reply

The choice of the amount of blood in the stomach was made prospectively in the design of this trial so the results are more than exploratory. However, we agree that the possibility that blood in the stomach is predictive of drug effects needs to be verified prospectively. The purpose of our study was to try to identify a relatively quick screening method that did not involve several thousand patients. Our study was started before the BSG guidelines on high and low risk patients and was made on individual clinical judgement. However, as noted in the paper, doctors in our hospital receive a card indicating high risk factors (tachycardia, hypotension, gastric ulceration, oesophageal varices, and comorbidity) guiding them about risk factors. Finally, we agree that several thousand patients would be needed to show a clinical end point. We conducted our study with a view to a two stage approach to drug treatment—show an effect on the surrogate end point before designing the large trial to help to focus a choice of agents for research.

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Gluten exposure and risk of autoimmune disorders

We applaud the paper from Sategna Guidetti *et al* (*Gut* 2001;49:502-5) showing that, contrary to what was described in a paediatric population,¹ there is no clear relationship between gluten exposure and risk of autoimmune disease (AID) in adult patients affected by coeliac disease (CD).

Although in our clinical experience we have seen CD patients developing AID after many years on a strict gluten free diet, we thought that the data published by Ventura and colleagues¹ should have been easily confirmed

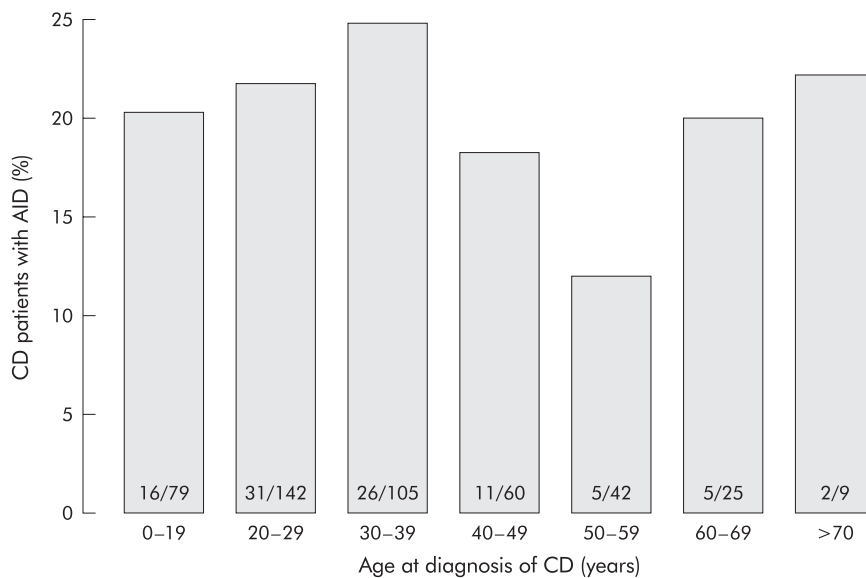


Figure 1 Prevalence of coeliac disease (CD) patients affected by autoimmune disease (AID) according to age at diagnosis of CD.

in an adult population. However, in common with Sategna Guidetti *et al*, this did not appear to be the case in the coeliac patients under our care. Moreover, although Sategna Guidetti *et al* could at least confirm the relationship between age at diagnosis of CD and risk of developing AID found by Ventura *et al*, we could not confirm this finding.

We retrospectively studied the notes of 462 adult patients affected by CD attending our outpatient clinic. All had been diagnosed on the basis of villous atrophy in jejunum biopsy specimens which improved after withdrawal of dietary gluten. There were 327 females and mean age at diagnosis of CD was 33.3 ± 15.6 years (range 1-79). Ninety six patients were affected by at least one AID. Age at diagnosis of CD did not differ between CD patients with and without AID (32.6 v 33.5 years; $p=0.6$). The prevalence of AID was not related to age at diagnosis of CD (χ^2 , $p=0.7$). However, an upward trend, similar to that shown by Ventura *et al*, was noted in the first three decades (fig 1).

Since we had previously shown that diagnostic delay is a very important feature in the history of CD,² we hypothesised that the longer the diagnostic delay the greater was the risk of developing AID. However, the prevalence of AID was not related to duration of diagnostic delay. Moreover, remarkably, diagnostic delay in patients with CD and AID was significantly shorter than that of patients with CD but not AID (9.1 v 13.2 years; $p=0.02$). We feel that this is a sensible result. The more reasons a patient has to be diagnosed, the more likely it is that he/she will be diagnosed sooner. However, this strongly argues against a relationship between the risk of AID and exposure to gluten.

Finally, as we agree with Sategna-Guidetti *et al* on the fact that dermatitis herpetiformis should not have been considered, we analysed our data both including and excluding this skin condition. The final outcome was unaffected.

In conclusion, our data in adult patients with CD confirm those of Sategna-Guidetti *et al*. Our only finding that was similar to that of Ventura *et al* was an upward trend in the first three decades. As patients affected by latent CD clearly prove that CD can start in adult life and not exclusively during childhood,³ this

upward trend could mean that the findings of Ventura *et al* are valid only for the paediatric age. However, as Londei points out, the only way to answer this question would involve an unacceptable prospective study.⁴

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Thiopurine metabolites and the role of thiopurine methyltransferase activity in inflammatory bowel disease

We read with interest the recent article by Lowry *et al* (*Gut* 2001;**49**:665-70) on the role of thiopurine methyltransferase (TPMT) activity and thiopurine metabolites in patients with inflammatory bowel disease (IBD). Lowry *et al* concluded that 6-thioguanine nucleotide (6-TGN) concentrations do not correlate with disease activity or leucocyte counts. Although in general terms this is a well designed trial and includes a large number of patients, analysis of the results presents a number of problems.

Firstly, the results for metabolite concentrations and metabolic pathways deserve more attention. A number of competitive enzymes, including TPMT, are involved in the complex multistep thiopurine metabolism and result in the synthesis of varying concentrations of

active or inactive end metabolites which have both toxic and non-toxic properties.¹ Secondly, thiopurine users can be broadly divided into clinically overlapping responders, non-responders, and therapy intolerant groups which in turn correspond to concentrations of these end metabolites.^{2,3} Exclusion of any of these end metabolite dependent patient subpopulations can significantly influence study outcome.

Failure to find a significant association between thiopurine related toxicity or disease activity and 6-TGN concentrations by Lowry *et al* could be explained by the median whole blood 6-TGN concentration (136 pmol/ 8×10^8 red blood cells (RBC)) in the study population. This is well below the pharmacologically suggested therapeutic concentration of 230 pmol/ 8×10^8 RBC.^{2,3} In a separate study, Dubinsky and colleagues⁴ were able to show that significantly high isolated 6-TGN concentrations were not related to toxicity. They in fact demonstrated a clinical improvement with increasing 6-TGN concentrations while toxicity was directly proportional to increasing 6-methyl mercaptopurine ribonucleotide (6-MMPR) concentrations. Lowry *et al* ignored this important preferential metabolic pathway observed in certain patient subpopulations.

We feel that carefully designed future trials are needed for further elaboration of these interacting factors by inclusion of all patient subpopulations and consideration of important metabolites. Alternatively, 6-thioguanine, a thiopurine compound not subject to the 6-MMPR catabolic pathway, may offer a more realistic approach to study the correlation between 6-TGN concentrations and disease activity in IBD patients.⁴

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Author's reply

There are numerous factual inaccuracies in Qasim *et al*'s letter which merit response. Firstly, they state that "A number of competitive enzymes, including thiopurine methyltransferase (TPMT), are involved in the complex multistep thiopurine metabolism and result in the synthesis of varying concentrations of active or inactive end metabolites which have both toxic and non-toxic properties". They reference a nine year old review article for this statement.¹ While there is no argument that the metabolism of azathioprine and 6-mercaptopurine is complex, and that it involves multiple enzymes, there is very

little valid information about which metabolites are toxic or non-toxic. The clear bias of Qasim *et al* is that various metabolites are either active or inactive and that some have toxic properties while others are non-toxic, but this has not been definitively proved in statistically valid clinical studies.

Secondly, Qasim *et al* state that "thiopurine users can be broadly divided into clinically overlapping responders, non-responders, and therapy intolerant groups, which in turn correspond to concentrations of these end metabolites. Exclusion of any of these end metabolite dependent patient subpopulations can significantly influence study outcome". The two papers quoted have exactly the same limitations as the current study (exclusion of patients who received azathioprine or 6-mercaptopurine for less than four months, and exclusion of patients with leucopenia or increased serum hepatic or pancreatic enzymes before initiation of therapy).²⁻⁴ As nearly half of the cases of thiopurine related leucopenia, most cases of elevated liver transaminases, and virtually all cases of pancreatitis and allergic reactions occur within the first four months of therapy, the study design for both of these studies would have excluded many patients who were intolerant to azathioprine or 6-mercaptopurine. Thus it is unlikely that the exclusion criteria in our study, which were virtually the same as in the studies of Cuffari and colleagues² and Dubinsky and colleagues,³ can account for the differences in the results and conclusions.

Thirdly, Qasim *et al* state that the lack of an association between thiopurine toxicity or disease activity and 6-thioguanine nucleotide (6-TGN) concentrations in our study could be explained by low whole blood 6-TGN concentrations. The authors should refer to paragraph 4 of the discussion on page 699 of our paper that indicates that our group has performed a study that shows a strong direct correlation between the assay used by Cuffari and Dubinsky and the assay used in our study (unpublished data). A conversion factor of 1.6 is required to convert the results of our study to those of the Cuffari and Dubinsky studies. Once that conversion factor is applied, the results of our study would yield a mean red blood cell (RBC) 6-TGN concentration of 261 pmol/8×10⁶ RBC, which is well above the pharmacologically suggested therapeutic concentration of 230 pmol/8×10⁶ RBC referred to by Qasim *et al*. Thus, as we clearly stated in the discussion of our paper, differences in assay methodology are unlikely to account for the discrepant results.

Finally, Qasim *et al* state that "In a separate study, Dubinsky *et al* were able to show that significantly high isolated 6-TGN concentrations were not related to toxicity. They in fact demonstrated a clinical improvement with increasing 6-TGN concentrations while toxicity was directly proportional to increasing 6-methylmercaptopurine ribonucleotide concentrations. Lowry *et al* ignored this important preferential metabolic pathway observed in certain patients subpopulations." We disagree strongly with this statement. The study by Dubinsky *et al* was an uncontrolled open label pilot study in 10 patients with Crohn's disease.³ It is unreasonable to compare the results of our study (a study involving 170 patients) with the preliminary results from an open label pilot study in 10 patients.

We agree with Qasim *et al* on one point—that is, that a prospective trial is needed in which patients who are candidates for thiopurine therapy are randomly assigned to full dose azathioprine (2–3 mg/kg/day) or 6-mercaptopurine (1.5 mg/kg/day) with downward adjustments in dose, as clinically indicated for toxicity, versus dose adjustment to a target 6-TGN concentration range. The issue of patient subpopulations based on metabolite profiles is very preliminary (one published study in 10 patients) and this hypothesis needs further characterisation before a controlled trial can even be designed. In the meantime, routine measurement of RBC 6-TGN in patients being treated with azathioprine or 6-mercaptopurine is not necessary.⁶

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Serum leptin and body mass index in children with *H pylori* infection

Leptin, a protein product of the obese gene expressed primarily by adipocytes, is known to regulate food intake, energy expenditure, and body weight homeostasis. Leptin has recently been detected in rat gastric mucosa,¹ and elevated leptin levels have been found in the gastric mucosa of patients with *Helicobacter pylori* associated gastritis.² In a study on the effect of *H pylori* infection on gastric leptin expression, Azuma *et al* (*Gut* 2001;**49**:324–9) demonstrated that gastric leptin may play a role in weight gain after eradication of *H pylori* infection. I read their article with great interest and would like to add a comment concerning serum leptin and body mass index (BMI). The authors showed a significant increase in gastric leptin expression in patients with *H pylori* infection, and a significant reduction in gastric leptin expression with a concomitant increase in BMI after successful eradication therapy. On the other hand, serum leptin levels did not change significantly after eradication of *H pylori* infection. We have also examined the effect of *H pylori* eradication on serum leptin levels and BMI in 15 children with *H pylori* infection.

The subjects were 15 children who presented at our hospital with anaemia and gastrointestinal symptoms such as abdominal pain and nausea, and were diagnosed with *H pylori* infection from a positive ¹³C urea breath test and histological and culture examinations of the gastric mucosa. There were seven boys and eight girls, ranging in age from 6.2 to 14.2 years (mean 10.6). *H pylori* eradication therapy was achieved with amoxicillin, clarithromycin, and lansoprazole twice daily for seven days. Two months after therapy, successful eradication of *H pylori* was confirmed by a negative ¹³C urea breath test and histological and culture examinations. Serum leptin levels and BMI were evaluated before and six months after eradication therapy. There were no significant differences in serum leptin levels or BMI before (6.10 (2.69) ng/ml and 17.9 (2.66) kg/m², respectively) and after (6.92 (3.41) ng/ml and 18.6 (2.10) kg/m², respectively) eradication of *H pylori*. However, serum leptin levels increased in 7/9 patients who showed an increase in BMI, and decreased in 5/6 patients who showed a decrease in BMI. There was a significant (p<0.01, r=0.796) positive correlation between serum leptin levels and BMI in patients after eradication of *H pylori* infection.

Given the concomitant change in serum leptin levels with change in BMI and the positive correlation between serum leptin and BMI in patients after successful eradication of *H pylori* infection, our results corroborate those of Azuma *et al* by clearly ruling out the effects of serum leptin on anorexia. However, in contrast with their results in adults, we did not observe an increase in BMI after eradication. While we did not examine gastric leptin expression, some differences in gastric leptin expression or the response to leptin between adults and children may help explain why BMI did not increase in children after successful eradication. Azuma *et al* suggest that gastric inflammation induced by *H pylori* infection raises the level of gastric leptin expression. On the other hand, the immunopathogenesis of *H pylori* associated gastritis in children is considered to be different from that in adults.³ This may account for the difference in gastric leptin expression between adults and children.

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