PostScript

LETTERS

Is the downregulation of EphB2 receptor expression during colorectal tumorigenesis due to hypoxia?

Mutations in components of the Wnt pathway contribute to the aberrant activation of the β -catenin/T-cell factor-4 (TCF-4) complex and initiate most colorectal cancers.¹ Upon its activation, the β -catenin/TCF-4 complex induces the expression of target genes such as *c-MYC*, *cyclin-D1* and *EPHB* receptors.² EphB receptors are receptor tyrosine kinases (RTKs) that play an important role in the coordination of cell proliferation, migration and compartmentalisation along intestinal crypts.^{3,4} The main effectors of these functions in the intestine are believed to be EphB2 and EphB3 receptors.⁵ Although EphB receptors are targets of β -catenin/TCF-4, Batlle *et al.* reported the unexpected downregulation of EphB2 receptor expression at the colorectal “adenoma–carcinoma” transition, despite the evident nuclear localisation of β -catenin.⁶ Intriguingly, EphB receptors were found to play a suppressive role in colorectal tumorigenesis as their loss promotes tumour progression in mice.⁶ The authors proposed the existence of a secondary, yet unknown, silencing mechanism that underlies the downregulation of EphB receptors, thus allowing colorectal tumour progression.⁶ Such a mechanism, we suggest, could be instigated by alterations in the tumour microenvironment. Hypoxia, the state of low oxygen tension in tissues, is a hallmark of the microenvironment in solid tumours.⁷ Hypoxia is believed to represent a key regulatory factor for tumour growth, survival and aggressiveness.⁸

The cellular response to hypoxia is orchestrated by a master transcription factor known as hypoxia inducible factor-1 (HIF-1). We have recently shown that during hypoxia HIF-1 competes with TCF-4 for the direct binding to β -catenin in colon cancer cells, thereby resulting in the inhibition of β -catenin/TCF-4-mediated transcription with no obvious effect on β -catenin levels or nuclear localisation.⁹ Having shown that hypoxia inhibits β -catenin/TCF-4 activity in colon cancer cells in vitro and since EphB2 is a target of β -catenin/TCF-4; here we hypothesised that hypoxia may contribute to the silencing of EphB2 receptor expression during colorectal tumour progression in vivo.

To test this hypothesis, we initially examined the expression of EphB2 in a panel of normal and cancer tissue from the colon. Consistent with the previous report,⁶ we found that while EphB2 is expressed at the base of the normal colonic crypt where the Wnt pathway is known to signal (fig 1A, top left panel), the expression of EphB2 was reduced in colorectal carcinoma tissue (fig 1A, bottom left panel). Next, serial sections were stained to assess the expression of the hypoxia-specific marker carbonic anhydrase-IX (CA-IX). To this end, CA-IX was hardly detectable in the epithelia of normal colonic crypts (fig 1A, top right panel); but was highly, although heterogeneously, expressed in colorectal carcinomas (fig 1A, bottom right panel). Since the occurrence of hypoxia could vary within tumours, depending on the extent of vascularisation, one may expect a heterogeneity in CA-IX and EphB2 expression. Indeed, in some cancer tissue we observed a patchy staining pattern for both EphB2 and CA-IX with tumours containing areas that stained largely positive adjacent to areas with downregulated EphB or CA-IX (fig 1B). Remarkably, in these cases, the staining patterns of EphB2 and CA-IX were complementary

(ie, areas weakly positive for EphB2 had low CA-IX (fig 1B, red arrows), and regions with strong CA-IX staining had reduced levels of EphB2 (fig 1B, black arrows)). It is important to note that the heterogeneous staining of EphB2 was also observed by Batlle *et al.*⁶ Given that CA-IX is a hypoxia-specific marker, these findings suggest a possible role for hypoxia in silencing EphB2 receptor expression. To gain further insight into the potential role of hypoxia in the downregulation of EphB2 receptor expression, we obtained hypoxic large-bowel tissue from patients with mesenteric ischaemia to examine EphB2 and CA-IX expression. Interestingly, while the expression of CA-IX was markedly induced in hypoxic colon tissues indicating a state of hypoxia (fig 1C, bottom right panel), EphB2 receptor expression was lost from the bottom of the hypoxic colonic crypts (fig 1C; top right panel, green arrows), where it is normally expressed (fig 1C; top left panel, green arrow). Taken together, these findings suggest that hypoxia may reduce β -catenin/TCF-4 activity in vivo where it results in the downregulation of EphB2 expression from the bottom of hypoxic colonic crypts as well as the hypoxic regions in advanced colorectal carcinomas.

In light of this study, we provide a possible explanation for the loss of EphB receptor expression in advanced colorectal cancers, despite the constitutive accumulation of nuclear β -catenin. As we have previously demonstrated, the inhibition of TCF-4 activity during hypoxia occurs as a consequence of direct dynamic competition between TCF-4 and HIF-1 α for binding to nuclear β -catenin.⁹ The current in vivo findings suggest hypoxia as a possible silencing mechanism that may underlie, at least in part, the reduction in EphB2 receptor expression at the advanced stages of colorectal tumorigenesis. Hypoxia is thought to arise during tumorigenesis of solid

Figure 1 Expression of EphB2 and CA-IX in colonic tissue. Immunohistochemical analyses were performed on formalin-fixed paraffin-embedded sections. EphB2 staining was carried out using anti-EphB2 goat polyclonal antibody (R&D Systems) at a dilution of 1:1000. CA-IX was carried out using anti-CA-IX rabbit polyclonal antibody (Novus Biologicals) at a dilution of 1:1000. The staining procedure was described previously in reference 3. The dotted lines indicate the superimposing of the serial sections. Black arrows: EphB2 negative, CA-IX strongly positive; red arrows: EphB2 weakly positive, CA-IX negative. Green arrows: EphB2 staining; bars: 100 μ m.

tissues and it contributes to the tumorigenic process through the promotion of cell survival, stimulation of angiogenesis, and enhancement of invasion and metastasis.⁸⁻¹⁰ The current investigation highlights the critical role of the microenvironment in transcriptional regulation, whereby an extrinsic fine-tuning mechanism is instigated to favour those events that particularly promote tumour progression.

Acknowledgements

This work was funded by a programme grant from Cancer Research UK, a scholarship from the Algerian Government and the Citrina foundation. Tissues were obtained from the archives of the Department of Histopathology, Bristol Royal Infirmary, UK. This was approved by the local research ethics committee.

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doi: 10.1136/gut.2007.131540

Competing interests: None.

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Inflammatory bowel disease: reviewing an old study under a new perspective

We would like to comment on the paper by Professor S Shivananda and colleagues (*Gut* 1996;**39**:690-7), which examined whether there was a difference in the incidence of inflammatory bowel disease (IBD) between northern and southern Europe.

Shivananda *et al.* conducted an epidemiological study across Europe from 1991 to 1993, and concluded that IBD, which includes

ulcerative colitis (UC) and Crohn's disease (CD), is possibly not more frequent in the north than in the south.

Figure 1 Incidence of IBD across Europe. (A) Incidence of ulcerative colitis (UC). (B) Incidence of Crohn's disease (CD). The maps were designed with Philcarto 4.50 for Windows (<http://perso.club-internet.fr/philgeo>).

Although their results are solid and groundbreaking, they did not manage to provide an explanation of the geographic distribution of the disease. A possible reason is that they did not include countries from Eastern Europe in their study.

Recent data from this area give us the opportunity to examine this issue again, providing a possible explanation. In fig 1, we present the incidence of UC (fig 1A) and CD (fig 1B) across Europe, according to data mentioned in reviews by Lakatos and Lakatos,¹ and Loftus.² We note some characteristics that are the basis for the new explanation:

- Although Estonia is located in northern Europe, the incidence of IBD shows pronounced differences compared with the high incidence in Scandinavian countries.¹
- Estonia, Hungary, Romania, Croatia, the Czech Republic, Slovakia and Poland have incidence rates of UC that range from 0.5 to 5.9 per 100 000 population¹ while countries of Western Europe have an overall incidence for UC of (at least) 10.4 (*Gut* 1996;**39**:690–7). For CD, the incidence rates of the previously mentioned countries of Eastern Europe range from 0.1 to 5.7,¹ whereas countries of Western Europe have an overall incidence no less than 5.6 (*Gut* 1996;**39**:690–7).
- The countries of Eastern Europe are mainly less developed than the countries of Western Europe.³

Thus, is there a developed–developing gradient? The authors believe that it might be so. The development gradient is consistent with the aetiological cause of “westernisation” in the expression of IBD, since more developed countries are generally more westernised. Westernised lifestyle means increased consumption of refined sugar, fatty acids, fast food, cereals and bread and reduced consumption of fruit, vegetables and fibre. Furthermore, indoor and sedentary occupations as well as improved sanitation are common characteristics of westernised cultures. For all of these aspects of westernisation, there have been noted associations with IBD.^{4–5}

Therefore, we can see that IBD can be better distributed according to the development of countries, instead of the north–south gradient. The underlying cause for such a distribution could be westernisation, which coincidentally explains the increase of the incidence observed in some countries (eg, according to this hypothesis, we could say that the incidence of IBD has increased in Hungary over the last few years because it has become more westernised since its level of development has risen³). This possible association of a wealth factor, such as the development level, with the incidence of IBD, has been noted by Ekbom, who mentions a possible influence of wealth on the incidence of IBD: “The disappearance of the north–south gradient in Europe might be an illustration of what will happen when society gains affluence. It is therefore of extreme interest to follow the temporal trends for IBD in Eastern Europe.”⁶ Similar comments have been made by other reviewers.^{4–5} Finally, this theory is furthermore consistent with observations about other diseases such as type I diabetes and multiple sclerosis.⁷ Specifically, Patterson *et al.*⁸ note for type I diabetes that “the strikingly higher incidence in Finland compared to ethnically-similar sub-populations in Estonia as well as the apparent decrease in type I diabetes in

Germany could reflect wealth-related risk factors.”

All these observations together allow us to suppose such a distribution of IBD, an issue that would be an interesting subject of research.

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Colonic colonisation with *Giardia lamblia* in a patient receiving fibrates

We read with interest the article by Troeger *et al* exploring the epithelial barrier dysfunction related to duodenal *Giardia lamblia* infection (*Gut* 2007;**56**:328–35). Not only does this protozoan interact with the intestinal epithelium but also its life cycle depends on its host's bile metabolism.

We report here on a patient for whom treatment with fibrate was likely to facilitate *Giardia lamblia* colonic colonisation. A 72-year-old woman reported a 1-year history of diarrhoea. She had a past history of type 2 diabetes, hypertension and hypercholesterolaemia. She was treated with metformin, glimepiride, enalapril, furosemide and fenofibrate. She had had a 30 kg loss of weight in the past year (from 90 to 60 kg, 157 cm) but no haematochezia, vomiting or abdominal pain.

A physical examination was normal. Repeated glycaemic controls and haemoglobin A1c were deemed satisfactory. Further laboratory tests showed iron-deficiency anaemia and hypoaalbuminaemia with normal electrolytes. Human immunodeficiency virus testing was negative. Upper digestive endoscopy and biopsies showed an atrophic gastritis with

Helicobacter pylori and a massive duodenal *G lamblia* infection with no underlying mucosal lesion. Ileocolonoscopy was normal, but colonic biopsies showed numerous trophozoites on the surface of colonic epithelium (fig 1). These micro-organisms were pear-shaped and binucleate (some being leaf-shaped on profile, some lying on the colonic epithelial surface), similar to those found on duodenal biopsy specimens and morphologically consistent with *G lamblia*. Colonic mucosa was normal.

The patient received a 7-day course of *H pylori* and *G lamblia* eradication treatment with metronidazole (1500 mg/day), clarithromycin and pantoprazole. Oral iron supplementation was started. Complete remission of diarrhoea was achieved within 2 weeks. Six months after eradication treatment was completed, the patient had gained 12 kg, and haemoglobin, plasmatic iron and albumin rates were normal.

G lamblia is a worldwide, highly prevalent, flagellated enteric protozoan. Orally transmitted as a cyst, it may turn into a trophozoite and adhere to the bowel wall. *G lamblia* intestinal infection is not usually associated with mucosal lesion (mild villous shortening and mild non-specific inflammation of the lamina propria have been reported in <5% of patients with duodenal infection).¹ The study of Troeger *et al* provides a better understanding of its pathogenic pathways, suggesting that *G lamblia* duodenal colonisation may be responsible for acute and chronic malabsorptive and secretory diarrhoea. Encystation of *G lamblia* trophozoites occurs in the small bowel. Cysts are then excreted with stools. It is therefore not common to see trophozoites in the colon¹ or in stools.²

In a series of 567 patients diagnosed with *G lamblia*, the site of colonisation was the small bowel in most cases (97%), but rarely the colon (two patients (0.4%), but the number of patients with colonic biopsies is not given in this study).¹ In another series of 124 patients diagnosed with *G lamblia* positive stools, 120 had only cyst (97%), four had trophozoites and cysts (3%) and none had trophozoites only.² Our patient had chronic malabsorption, which resolved after metronidazole treatment, and we

Figure 1 *Giardia lamblia* trophozoites on the surface of colonic epithelium.

therefore believe that most symptoms were related to the duodenal giardiasis, and that finding *G lamblia* trophozoites in the colon was likely to be an epiphenomenon to the upstream small bowel infection.

Colonic giardiasis has been rarely reported in immunocompetent hosts^{1,2} and its contributing factors are speculative. Cholesterol deprivation and exposure to bile acids³ are demonstrated in vitro as two major stimuli of *G lamblia* encystation. Similarly, in vivo, cholestasis and diet supplemented with cholestyramine have also been shown to dramatically decrease the fecal excretion of *G muris* cysts in mice.⁴ We suggest that fibrates treatment, which increases biliary cholesterol excretion and decreases the excretion of bile acids, hampered giardia encystations in our patient, subsequently facilitating *G lamblia* colonic colonisation of trophozoites.

A search through national adverse event databases (Canadian Adverse Drug Reaction Monitoring Program, United Kingdom Medicines and Healthcare products Regulatory Agency: Drug Analysis Prints, United States Food and Drug Administration Adverse Event Reporting System) failed to find any similar report. Changes in bile metabolism induced by hypolipidaemic drugs (not only fibrates but also niacin and statins) or bile acid resins (cholestyramine) may influence the presentation and spreading of *G lamblia*.

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Bone marrow stem cells and liver disease

The review article by Kallies *et al* focuses on the various relationships between bone marrow stem cells (BMSC) and liver diseases (*Gut* 2007;**56**:716–24). It discusses experimental

studies with animal models and critically examines the possible role of BMSC in liver repair through the delivery of growth factors, fibrosis resolution and new blood vessel formation.

In addition, the authors examine the development of BMSC treatment of liver diseases and describe three uncontrolled phase I clinical trials in humans published up to December 2006. The main end point of these trials was to evaluate the feasibility and safety of BMSC treatment in patients with chronic liver disease. In one trial, human CD34+ stem/progenitor cell populations mobilised into the blood by granulocyte colony-stimulating factor were injected directly into the portal vein or hepatic artery.¹ The study from Terai *et al* transplanted autologous BMSC that were prepared from a large amount (400 ml) of iliac crest aspirate and infused into the peripheral vein of the patients.² Feasibility and safety was observed in all studies. Patients' follow-up showed improvement of serum albumin levels and Child–Pugh score.

Our research group has recently published data on 10 patients on a waiting list for a liver transplant who had end-stage chronic liver disease and were enrolled to receive infusion of autologous BMSC.³ About 50 ml of bone marrow was aspirated under mild sedation and prepared by centrifugation in a Ficoll–Hypaque gradient. A minimum of 100 millions of mononuclear-enriched bone marrow cells were infused into the hepatic artery of the patients by the routine technique used for arterial chemoembolisation of liver tumours. Our results showed that infusion of BMSC into the hepatic artery of patients with advanced chronic liver disease is safe and feasible. In addition, we also observed a decrease of serum bilirubin and an increase in albumin levels.

It seems clear that trials of BMSC treatment in patients with liver disease are still at a preliminary stage and a better understanding of the physiology and mechanism of action of BMSC in animal models of liver disease is needed. Nevertheless, the high death rate of patients on a waiting list for a liver transplant, especially in some areas of the world, and the fact that autologous BMSC treatment is feasible, safe and seems to lead to improvement of the Child–Pugh score and serum albumin levels suggests that randomised controlled studies should be carried out to evaluate the efficacy of BMSC treatment in patients with advanced liver disease.

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Authors' reply

We are grateful to Lyra *et al* for their recently published study showing the apparent safety of bone marrow (BM)-derived mononuclear cell injection for advanced liver cirrhosis.¹ We agree that there is an urgent need to develop alternative treatments for liver cirrhosis given the worsening mismatch between the number of patients requiring a liver transplant and the number of organs available for transplantation.

Stem cell therapy has the potential to benefit this group of patients. However, unlike a defined pharmacological treatment there is additional complexity in selecting BM cells or other stem cells for therapy. There is a wide heterogeneity of cell phenotypes within the BM, and indeed BM stem cells have a wide differentiation potential. Thus different cell isolation and sorting protocols may result in differing cell populations engrafting the liver, with widely varying effects. Initial optimistic reports that BM cells readily differentiated into hepatocytes in vivo have not been substantiated.² The case for the clinical use of BM cells in cirrhosis treatment is therefore based on alternative mechanisms of action such as collagen degradation, enhanced vascularisation or secretion of cytokines and growth factors to stimulate liver regeneration.

We agree that given the growing problem of deaths of patients on the liver transplant waiting list and the apparent safety of the use of BM stem cells reported by Lyra *et al*¹ and by other authors,³ it is ethically justifiable to perform a randomised trial of mononuclear cell therapy for liver cirrhosis. However, many questions remain to be answered such as: Which are the optimal cells to select, and are these cells actually beneficial? If so, what phenotype do the cells eventually adopt in the liver and how do they exert a therapeutic effect? How long does this benefit last, and would you get a longer lasting benefit by injecting more primitive stem cells than more differentiated cells? If a randomised trial was negative should more or different cells have been tried? Of course, the answers to these questions are likely to be found through a combination of preclinical and translational clinical studies that will elucidate mechanism, and randomised trials that will hopefully define a clinical effect. A word of caution should be added though, a population of BM-derived stem cells has been shown to have a fibrotic potential in the liver.^{4,5} Furthermore, macrophages, although "antifibrotic" in the recovery phase after liver injury, can also be "profibrotic" during continuing liver damage.⁶ These factors should be borne in mind when planning and implementing new stem cell trials for cirrhosis.

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Inactivation of digestive proteases by deconjugated bilirubin: the possible evolutionary driving force for bilirubin or biliverdin predominance in animals

In humans and some other animals, bilirubin is excreted as the end product of haem originating from haemoglobin and other haemoproteins, and it has been the cause of jaundice and kernicterus.^{1, 2} The conversion of haem to bilirubin is a two-step process.¹ First, haem is converted to biliverdin by haem oxygenase. Then biliverdin is converted to bilirubin by biliverdin reductase. Interestingly, many animals take biliverdin as the end

product of haem, without converting it further to bilirubin.² Biliverdin is water soluble and readily excreted through bile or urine.

Why the energy-consuming conversion in some animals of the innocuous biliverdin to the water-insoluble, potentially toxic bilirubin, which needs more resources for transportation and excretion, takes place has been a great puzzle.^{1, 2} Discovery of the antioxidant property and other properties of bilirubin has suggested that the change of biliverdin to bilirubin might be an advance in evolution.¹ However, this notion is challenged by the fact that biliverdin- or bilirubin-predominant species exist in animals at different stages of evolution, from fishes to mammals.² The discrepancy in bile pigments exists even in closely related species. For instance, nutria is a rodent like rats and mice, but the bile of nutria is predominantly biliverdin, in contrast to the predominant bilirubin in rats and mice.² Change of biliverdin to bilirubin needs only one enzyme—biliverdin reductase.¹ This enzyme exists in the oldest bacteria like cyanobacteria,¹ far before eukaryotic cells appeared on earth. So what has caused the bilirubin or biliverdin predominance in animals? Here I suggest that the inactivation of digestive proteases is the evolutionary driving force for bilirubin or biliverdin predominance.

Table 1 shows that trypsin and chymotrypsin are significantly inhibited by free bilirubin, but not conjugated bilirubin or biliverdin. The nature of this inhibition and its physiological relevance were further explored for trypsin. Figure 1 shows a Lineweaver–Burk plot of the inhibition of trypsin by free bilirubin. It indicates that the inhibition is non-competitive, suggesting that free bilirubin can inactivate the enzyme.

The effect of free bilirubin on protein digestion by trypsin was further investigated using chymotrypsinogen as the substrate. Bilirubin (10 µmol/l) showed 61% inhibition for the proteolytic activation of α-chymotrypsinogen A to chymotrypsin in a system containing 1 µg/ml trypsin and 1 µg/ml chymotrypsinogen for 30 min. Digestive proteases are inhibited by free bilirubin but not conjugated bilirubin. As bilirubin is secreted from the bile to the lumen mainly in the conjugated form,² the digestion of dietary

Figure 1 Lineweaver-Burk plot of the inhibition of trypsin by free bilirubin. Trypsin activity was measured using different concentrations of TAME as the substrate with (●) or without (○) 2.5 µM bilirubin. TAME, N_α-p-tosyl-L-arginine methyl ester.

proteins in the upper small intestine would proceed smoothly. Deconjugation of bilirubin by β-glucuronidase from the mucosal cells³ would form a protective layer on the surface of the gut. A more dramatic deconjugation of bilirubin by the high amounts of β-glucuronidase from gut bacteria³ would further cause a prompt and effective inactivation of these digestive proteases in the lower intestine. Here we can see the wonderful design of nature that turns a waste byproduct into a precious treasure. The amount of digestive proteases secreted by the pancreas largely depends on the amount of protein in the diet.⁴ This would provide an explanation for the observation that bilirubin-predominant species tend to be carnivores or omnivores, while biliverdin-predominant species tend to be herbivores.² Large amounts of bilirubin exist in the bile of cats, dogs, opossums, armadillos, alligators, African clawed toads, bullfrogs, mudpuppies, sharks (spiny dogfish), small skates, trout, goosefish, and perch, while biliverdin is the main bile pigment of rabbits, nutrias (rodents that eat water plants), sloths (leaf eaters), birds and tilapia (fish that eat

Table 1 Effect of free bilirubin, biliverdin and conjugated bilirubin on trypsin and chymotrypsin activity

	Control (µmol/l)	Free bilirubin (µmol/l)				Biliverdin (µmol/l)	Conjugated bilirubin (µmol/l)
	0	1.25	2.5	5	10	10	10
Trypsin							
Increase of OD per min	0.0367 (0.0023)	0.0255 (0.0013)†	0.0197 (0.0018)†	0.0142 (0.0033)†	0.0102 (0.0006)†	0.0459 (0.0025)†	0.0461 (0.0007)†
Test/control	1.00	0.70	0.54	0.39	0.28	1.25	1.26
Chymotrypsin							
Increase of OD per min	0.0218 (0.0030)	0.0166 (0.0025)*	0.0121 (0.0008)†	0.0061 (0.0017)†	0.0002 (0.0006)†	0.0271 (0.0023)*	0.0237 (0.0016)
Test/control	1.00	0.76	0.56	0.28	0.01	1.24	1.09

Data are expressed as mean (SD) of quadruplicate measurements.
*p<0.05; †p<0.001 versus control.

Trypsin and chymotrypsin activities were measured using N_α-p-tosyl-L-arginine methyl ester (TAME) or N-benzoyl-L-tyrosine ethyl ester (BTEE) as the substrate, respectively, by a method modified from that described by Hummel.⁵ Free bilirubin (3 µl 50 X; Sigma, St Louis, Missouri, USA), biliverdin (Frontier Scientific, Logan, Utah, USA) or conjugated bilirubin (Frontier Scientific) was added to a 96-well ultraviolet-transparent microplate. The final volume of the reaction solution was 150 µl, giving the final concentrations indicated in table 1. For trypsin assay, 72 µl buffer (0.046 M Tris/HCl, 0.0115 M CaCl₂, pH 8.1) containing 150 ng trypsin was added to the microplate by multiple pipette, followed by 75 µl of buffer containing 150 nmol TAME to start the reaction. The plate was then read at 247 nm on a microplate reader. For chymotrypsin assay, BTEE was dissolved in dimethylsulphoxide (DMSO) at 4.054 mg/ml, and then added with nine volumes of water to obtain a concentration of 1.07 mmol/l. DMSO was used instead of methanol (which was used in the original paper) because methanol greatly reduces the solubility of free bilirubin. After adding 77 µl buffer (0.080 M Tris/HCl, 0.1 M CaCl₂, pH 7.8) containing 150 ng chymotrypsin, the reaction was started by adding 70 µl BTEE solution. Then the plate was read at 256 nm on a microplate reader. Data were collected at 30-second intervals for 5 minutes and expressed as the increase in optical density (OD) per minute in the linear range (the first 2–3 minutes).

algae).² Although cattle and sheep are herbivores and their bile consists mainly of bilirubin, the activity of their hepatic biliverdin reductase was just 4–5% of that of the rats,⁵ and their bile indeed contains certain amounts of biliverdin,² suggesting that they may be in an intermediary state of transition.

Studies have also shown that bile pigments change from bilirubin to biliverdin in fishes after starvation.⁶ This further suggests bilirubin is only produced as necessary, which depends in some way on the feeding activities. On the other hand, the energy-consuming conversion of biliverdin to bilirubin suggests that bilirubin may have an important role for the body. An impairment in inactivation of digestive proteases by deconjugated bilirubin may have a causative role in diseases such as inflammatory bowel disease.⁷ It would also provide a possible explanation as to why chronic blockage of bile flow in diseases such as primary sclerosing cholangitis is often accompanied by gut damage like inflammatory bowel disease.⁸ These areas require further investigation.

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doi: 10.1136/gut.2007.132076

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E/A ratio alone cannot reliably diagnose diastolic dysfunction in the assessment before and after TIPS

We congratulate Cazzaniga *et al* on their study focusing on the heart (*Gut* 2007;**56**:869–75), an often neglected organ when assessing a patient with cirrhosis. The cardiovascular changes in cirrhosis are complex and measurement of the ratio between the early maximal ventricular filling velocity and the late filling velocity (E/A ratio) after the insertion of a transjugular intrahepatic portosystemic shunt (TIPS) may

provide a powerful tool for identifying a poorly responding subgroup of patients.

However, we have certain observations about the study and its conclusions. Specifically, whether it is possible to diagnose diastolic dysfunction (DD) using the E/A ratio alone, given its dependence on loading conditions and age.

The use of echocardiography is the cornerstone of a comprehensive evaluation of DD. However, it is operator dependent and prone to interobserver variation and bias. Lack of an independent observer to verify the measured calculations weakens this study.

Loading conditions are of critical importance when interpreting diastolic function based on the E/A ratio. Any increase in preload will cause an increase in left atrial pressure and hence the early velocity of ventricular filling.

In the above study, all patients showed an increase in E/A ratio, corroborating the view that the E/A ratio changes with an increase in preload (left ventricular end-diastolic volume). Even in the subgroup whose E/A ratio was <1 at 28 days (group 1), the E/A ratio rose to 0.97 (11), which is probably normal and expected for that age group (65 years).

With increasing age there is a physiological decrease in E/A ratio.¹ Although the difference between the groups' ages (65 vs 59) was not statistically significant, this might have been owing to the small sample size.

More relevant to the outcome, may have been the drop in the left ventricular ejection fraction in the group with the poor outcome, despite the increased preload. Hence, rather than DD we could interpret the data as unmasking a deterioration in systolic function.

The assertions about DD might have been made clearer had more "load-independent" methods for assessing DD been used.

Colour M-mode echocardiography using the early diastolic flow propagation velocity as the blood flows from the mitral valve to the apex,² or mitral annular Doppler tissue imaging measurements of the myocardium during the cardiac cycle³ are two load-independent methods that can be used, in conjunction with the E/A ratio, to better characterise DD.

In summary, the importance of cardiac dysfunction in patients undergoing TIPS is significant. Although Cazzaniga *et al* may show that the E/A ratio after TIPS is possibly a powerful indicator of prognosis, it cannot be reliably used to diagnose diastolic dysfunction on its own. Load-independent methods such as colour M-mode echocardiography, or Doppler tissue imaging, may help to clarify the contribution of diastolic dysfunction in cirrhosis and may improve patient selection for TIPS.

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Authors' reply

We thank Dr Abeles and colleagues for their comments on our recent paper on the predictive value of E/A ratio in cirrhotic patients with a transjugular intrahepatic portosystemic shunt (TIPS), which gives us the opportunity to extend the discussion on some particular aspects.

We agree that echocardiographic measurement of the E/A ratio can be an imperfect tool for diagnosing diastolic dysfunction compared with new more sophisticated methods such as Doppler tissue imaging. However, when we started our study this new methodology was not available in our centre. Nevertheless, almost all previous studies on cirrhotic cardiomyopathy have used E/A ratio, deceleration time and/or isovolumic relaxation time to diagnose diastolic dysfunction.^{1–3} Moreover, Abeles observes that echocardiographic calculations were not verified by an independent observer to avoid operator bias. However, this risk was limited in our study because there was just one echocardiographic operator who was unaware of the clinical conditions of the patients.

The most important concern of Abeles and colleagues is that interpretation of the E/A ratio is affected by two important variables: loading conditions and age. For the first variable, one should take into account that patients with advanced cirrhosis have central hypovolaemia owing to a redistribution of the blood volume from the central to the splanchnic bed, and that TIPS insertion markedly increases the blood volume return to the heart and often corrects central hypovolaemia.⁴ This is confirmed by our data, which show a significant increase of the average value of the left ventricular end diastolic volume (LVEDV) after TIPS in both groups of patients. Therefore, measuring the E/A ratio in patients with TIPS may lead to a wrong diagnosis of normal diastolic function (pseudonormalisation effect) but is less likely to lead to a wrong diagnosis of diastolic dysfunction. Abeles and colleagues affirm that the E/A ratio increased after TIPS in all patients, but this is incorrect as the E/A ratio decreased or remained unchanged in 11 of 32 patients. Indeed, we have already reported that TIPS in cirrhotic patients may be able to distinguish between patients who have a true diastolic dysfunction due to ventricular stiffness (those whose E/A ratio did not rise or decrease after TIPS) and those who have an E/A ratio <1 because of circulatory underfilling and an excessively low preload (those whose E/A ratio increases after TIPS).

We also agree that age must be taken into account when interpreting E/A ratios, because they tend to reduce with aging. In our study, however, the average age was not significantly different between the two groups of patients with low or normal E/A ratio after TIPS insertion. In addition, although we observed a marginal inverse correlation between the E/A ratio and age ($p = 0.1$), age was not associated with death at univariate analysis, making it unlikely that the predictive value of the E/A ratio was dependent on aging.

The hypothesis that a deterioration of systolic function rather than of diastolic

dysfunction was responsible for the poor outcome in our cirrhotic patients is attractive but not sufficiently supported by our data. Indeed, the statistical analysis excluded the possibility that a drop in the ejection fraction (EF) or any other echocardiographic variable, except E/A ratio, was associated with death. Moreover, none of the patients had cardiac symptoms and no death was caused by evident cardiac failure. Nevertheless, we hope that this subject will be investigated further in a larger group of patients.

We agree with Abeles and colleagues that the most important message of our study is that cardiac function should be carefully assessed in critically ill patients with cirrhosis. This concept has recently been corroborated by other investigations, which showed that a relatively insufficient cardiac output was associated with the development of renal failure in cirrhotic patients with spontaneous bacterial peritonitis.³ The prevalence of diastolic dysfunction in cirrhosis should be re-evaluated with more accurate methods such as colour M-mode echocardiography and Doppler tissue imaging, which are independent of the load conditions.

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Hypnotherapy for non-cardiac chest pain: long-term follow-up

We have been researching the effects of hypnotherapy in gastroenterology for many years and recently reported in *Gut*¹ that a course of 12 sessions of hypnotherapy seems to be beneficial in the treatment of non-cardiac chest pain. In a study on 28 patients comparing the effects of either hypnotherapy or a similar duration of supportive listening combined with placebo medication (control group), the active treatment resulted in a much better outcome. Of the 15 patients randomised to hypnotherapy, 12 (80%) responded to treatment, as judged by either a complete or moderate improvement in their chest pain, compared with 3/13 (23%) controls ($p = 0.008$). A similar trend was observed for general wellbeing, which was regarded as a marker of quality of life, where 11 (73%) of the hypnotherapy

Figure 1 Percentage of patients reporting a global improvement in chest pain with either hypnotherapy ($n = 15$) or supportive therapy ($n = 13$).

Figure 2 Percentage of patients reporting an improvement in quality of life with either hypnotherapy ($n = 15$) or supportive therapy ($n = 13$).

patients improved compared with 3 (23%) controls ($p = 0.023$).

Unfortunately, it is impossible to undertake a double blind trial of a treatment such as hypnotherapy and the choice of control treatment is difficult as it is not possible to provide “sham” hypnotherapy. The only realistic alternative is to use some form of supportive treatment for a similar length of time and we have found that this, combined with placebo medication, provides a reasonably robust alternative. Obviously, this does not overcome any differences in expectation between the two treatments in the short term, but if the beneficial effects were sustained after hypnotherapy this would suggest that any observed improvement is indeed genuine. Furthermore, hypnotherapy would only be a viable option for more widespread use if the

patients remained well over a long period of time without the need for continuing sessions

In our non-cardiac chest pain study efficacy was only reported at the end of 17 weeks’ treatment. We have now had the opportunity to follow up all members of this cohort of patients for at least 2 years (mean 2.8 years) after stopping treatment and would like to report these data. In the 15 patients who received hypnotherapy there has been further improvement, with 14 (93%) now classified as responders compared with 3/13 (23%) controls ($p = 0.001$) (fig 1). The quality of life has also improved in 14 (93%) of the hypnotherapy group compared with 3 (23%) controls ($p = 0.001$) (fig 2).

Thus not only do the beneficial effects of hypnotherapy for non-cardiac chest pain persist for at least 2 years, but patients do seem to carry on improving once treatment has been completed. This outcome is in accord with our previous observations in irritable bowel syndrome² and functional dyspepsia,³ where we have also reported long-term benefit and a tendency for continued improvement after stopping treatment. All these results suggest that incorporating hypnotherapy into the provision of care for functional gastrointestinal disorders is worthy of serious consideration.⁴

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doi: 10.1136/gut.2007.132621

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BOOK REVIEWS

Gastroenterology and Liver Disease

Edited by Richard G Long, Brian B Scott. St Louis: Elsevier Mosby, 2005. £38.99 (softcover), pp 350. ISBN 0-72343252-X

I have to be honest and say up front that I have often wondered whether there is a continuing role for a traditional textbook in gastroenterology. Surely most junior doctors access all the information they require via web-based learning? Who has time to sit down and browse a textbook in between shifts, particularly given that the information contained within it will gradually be going out of date from before the time of publication? Traditional texts are often written by “experts” and therefore out of touch with the needs of a trainee. Finally, modern

educational theory promotes the value of case-based learning and problem-solving as opposed to digesting long tracts of text.

However, having read *Gastroenterology and liver disease*, edited by Richard Long and Brian Scott, I have changed my mind! This comprehensive book is organised into easy-to-use sections on areas of gastroenterology and liver disease, with individual chapters on key topics such as inflammatory bowel disease, nutrition and the management of gastrointestinal bleeding. Although it is designed for doctors starting their training in gastroenterology, it would also be particularly valuable for specialist nurses. It is clearly written by those who practise gastroenterology rather than preach about it, and contains a wealth of well-presented and pertinent information in both the text and the many tables. In addition to clear descriptions of all common GI diseases, it has a chapter on the organisation of gastroenterology services that is essential reading for those about to start a consultant post and also for current consultants who feel the need to reorganise their service! A particular highlight for me was the section on statistics and epidemiology for the gastroenterologist. The use of relevant examples manages to simplify the basis of the common study designs and statistical analyses used in the gastroenterological literature, which will be invaluable in deciphering the increasingly complicated trial and epidemiological literature.

Each self-contained chapter is packed with useful guidelines. Some, such as the guidelines on managing diabetics during endoscopy, appear to be designed locally, whereas others are based upon British Society of Gastroenterology guidelines, such as the polyp surveillance intervals. Its joy is that it is comprehensive, but not so overstuffed with information as to make it impractical to use on a day-to-day basis. By definition, it does not contain the latest data on novel therapies or a comprehensive description of all gastrointestinal conditions – I guess if you need more information, it's time to log on and read about it online!

So in summary, this is an excellent and well put together guide to the practice of gastroenterology and would make an excellent introduction to the subject for any interested professional.

James Lindsay

Atlas of video capsule endoscopy

Edited by Martin Keuchel, Friedrich Hagenmüller, David E. Fleischer. Heidelberg: Springer Medizin Verlag, 2006, £130.50 (hardback), pp 296. ISBN 3-540-23128-5

Let me make a confession. When I was first asked to review this book, I jumped at the opportunity because about 3 months ago I bought my own copy and have found it extremely valuable, but I have felt guilty about keeping it to myself. I know that if I let the

trainees have access to it, it will very quickly disappear, for me never to see it again. Now I have two copies, so trainees can benefit.

The book title leads you to believe falsely that it is simply a series of pictures that allows you to pattern-match what you see on examinations of your patients with examples in the atlas. It is much more than that. Its 60 or so contributors deal with absolutely everything you could want to know about capsule endoscopy, from history to future developments. Indeed, the future developments chapter right at the end is particularly entertaining and was written by Paul Swain. Certainly, it will become dated but it is real "Star Trek" stuff at present. Applications, indications, technical and procedural routine, evaluation and reporting technique are all there; every known aspect of capsule endoscopy is dealt with, some in extremely ingenious ways. To complement the capsule endoscopy chapters of the book, there are supportive chapters dealing with other endoscopic and non-endoscopic imaging. These put capsule endoscopy in context and allow its role of therapeutic guidance to be discussed.

My only gripe is that the modern-day radiological techniques of CT and MR scanning are scarcely discussed – perhaps hardly surprising, as there is not a single radiologist amongst the contributors. Nonetheless, this is a pretty spectacular book from a technical and clinical endoscopic standpoint. The range of images of common, unusual and frankly rare conditions is virtually complete. There are very few conditions discussed in the text for which there is no capsule endoscopy image. The image in the section on coeliac disease of the capsule immersed in steatorrhoea in the colon is particularly inspiring. You want worms and parasites? They are here in profusion, as is everything else from angiodysplasia to tumours, coeliac disease to Crohn's disease. I think I'd probably go so far as to say that if you were considering setting up a capsule endoscopy service then you would be putting yourself at a very significant disadvantage if you didn't first go out and buy a copy of this book and read it thoroughly before starting.

Derrick F Martin

CORRECTION

doi: 10.1136/gut.2006.113456corr1

J Baillie, P-A Testoni. Are we meeting the standards set for ERCP? (*Gut* 2007;56:744–6). Both the editors and authors are grateful to the authors of the BSG audit of endoscopic retrograde cholangio-pancreatography (ERCP) study (EJ Williams, S Taylor, P Fairclough, *et al.* Are we meeting the standards set for endoscopy? Results of a large-scale prospective

study of endoscopic retrograde cholangio-pancreatography practice (*Gut* 2007;56:821–9)) for pointing out that both their article and the accompanying commentary by Baillie and Testoni refer to two sets of data, which the commentary failed to clearly distinguish. The 2003 NCEPOD study (NCEPOD. Scoping our practice. The 2004 Report of the National Confidential Enquiry into Patient Outcome and Death. London: NCEPOD, 2004, <http://www.ncepod.org.uk/2004.htm> (accessed 28 Mar 2007)) was a small, retrospective study that looked at all endoscopy-related deaths within 30 days for in-patients only over a period of several months. The 2004 BSG survey was a much larger prospective, practice-based audit of all ERCPs in five selected regions of England, which relied on self-reporting. Despite the different methodologies, there was broad agreement in both studies regarding mortality rates. The BSG audit looked at cannulation and success rates in addition, and found that at least some elements of practice (eg selection, preparation, pre-investigation, monitoring, antibiotics and consent) were not as bad in reality as the picture that had been painted by the NCEPOD report, which focussed solely on patients who died. It was the judgment of the NCEPOD panel, looking at retrospective data on mortality, that two-thirds of deaths in the ASA 3–5 group occurred following procedures that were futile. The subsequent BSG survey found that while one-third of all the deaths were in the ASA 3–5 group, 90% of the the same group survived their procedures. Although 60% of patients in ASA category 5 died, these were only 13/4561 (0.28%) patients. Possibly 100% of these unfortunate individuals would have died without ERCP.

Table 1 of the paper by Williams *et al* summarises the findings and recommendations of the NCEPOD that the authors consider relevant to ERCP. It refers to all deaths occurring in hospital within 30 days of therapeutic gastrointestinal endoscopy between 1 April 2002 and 31 March 2003. This is the source of concern expressed regarding lack of resuscitation equipment on site (19%), deaths of patients who were confused (16%) but had nonetheless provided written consent for their procedures, lack of any written consent (21%), lack of clotting tests (80%), inadequate or absent monitoring during sedation, and so on. The BSG audit yielded a large amount of data, not all of which could be included in the final paper. However, there is clear evidence from the BSG data that the current practice of ERCP is better than the gloomy picture painted by the NCEPOD study. For example, during the prospective study, prothrombin time was documented to have been performed within one week of ERCP in 86% of patients, which represents very good compliance with practice guidelines.

The authors wish to apologise for this confusion between the two distinctly separate studies referred to within their commentary, which created a falsely gloomy picture of current British ERCP practice, which they trust this notice will correct.