DNA test for hypolactasia premature

I write in response to the article by Rasinperä and colleagues (Gut 2004;53:1371–6) in which a DNA test was proposed for "adult-type" hypolactasia.

The ability to digest the milk sugar lactose as an adult (lactase persistence) is a variable genetic trait in human populations, lactase persistence being the most frequent phenotype in Northern Europe, while lactase non-persistence or "adult-type hypolactasia" is more frequent in most other populations.1 In sub-Saharan Africa for example, lactase persistence is found only at low frequency in the majority of populations that have been tested, but in some populations, particularly pastoralist groups, it is significantly more frequent.

A CT polymorphism located 13.9 kb upstream of exon 1 of the lactase gene (LCT) was previously shown in a Finnish population to be tightly associated with the lactase persistence phenotype2 and it is this change that is proposed as a DNA test for both Europeans and Africans. We agree that presence of a T at this polymorphic site is indeed a fairly good predictor of lactase persistence in Northern Europeans,3 and that there is evidence that this nucleotide resides in a functional element.4 However, the presence of the alternative allele C at this site is not a good predictor of lactase non-persistence or "adult hypolactasia" in many non-Western populations.

I particularly draw readers’ attention to our recent study.5 We typed this polymorphism in 1671 individuals from seven African countries, which included 20 distinct cultural groups. In seven cases it was possible to match the groups tested with groups from the literature for whom phenotypic information was available. In five of these groups the published frequencies of lactase persistence were 72–97%. We found the T allele in Cameroon but it was so rare elsewhere that it cannot explain the frequency of the lactase persistence phenotype throughout Africa and we devised a statistical test to show that the two results were unlikely to have been obtained by chance.

Our ongoing results support this published information and we urge the community to refrain from using DNA tests on Africans and other non-Northern Europeans until an appropriate DNA change has been identified.

D M Swallow

Correspondence to: Professor D M Swallow, The Galton Laboratory, Biology Department, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK; dsmswallow@hgp.mrc.ac.uk

Conflict of interest: None declared.

References

Authors’ reply

Dr Swallow raises a question about another DNA variant underlying adult-type hypolactasia in sub-Saharan populations and does not recommend analysis of the C/T–13910 variant as a genetic test in African and non-Northern European populations. Although the studies performed by us1,2 and others3 do not support the existence of another variant, we agree with Dr Swallow that well conducted studies are needed to confirm this.

The significance of the C/T–13910 variant underlying adult-type hypolactasia was questioned in the article by Mulcare.4 Their doubt is based on several assumptions that make it difficult to evaluate the significance of the findings. These assumptions can be listed as follows:

(1) It is not known whether or not the study subjects presented with adult-type hypolactasia. Thus there is a risk of wrong conclusions being drawn. It is well documented that the clinical diagnosis of adult-type hypolactasia is difficult to assess due to inaccurate results in adult-type hypolactasia. There is evidence that the breath hydrogen test varies between 89% and 12% the clinical diagnosis of adult-type hypolactasia. When conducting phenotype-genotype correlation studies in lactase persistence/non-persistence, detailed clinical studies are essential. The studies are difficult as it is unethical to take an intestinal biopsy from a healthy subject that would give the most reliable diagnosis. Measurement of lactase activity from hospitalised patients with a clinical indication for intestinal biopsy may reflect a disease in the gut and the result obtained may not correlate with the genotype. These uncertainties should be taken into account when interpreting the genotyping results in adult-type hypolactasia.

K-L Kolho

Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland

I Järvelä

Department of Medical Genetics, University of Helsinki and Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland

Correspondence to: Dr K-L Kolho, Hospital for Children and Adolescents, Box 281, FIN-00029, Finland; kaija-leena.kolho@helsinki.fi

Conflict of interest: None declared.

References
3 Rasinperä H. A genetic test which can be used to diagnose adult-type hypolactasia in children. Gut 2004;53:1571–6.
5 Troelsen JT. An upstream polymorphism associated with lactase persistence has increased enhancer activity. Gastroenterology 2003;125:1586–94.
Conflict of interest: None declared.

was described by Mulcare and colleagues. Statistical method was developed, which hydrogen). To accomplish this, a novel diagnoses (for example, based on breath comparisons of "true" diagnoses (for exam-
tion) that level of error was. Instead, our uncer-
tainty about the true level of error was.

The first is their claim that with our statistical modelled in a Bayesian framework and
inaccurate diagnostic tests''. This would indeed be true had we applied a “naïve” test (for example, a \( \chi^2 \) test) in which we had assumed the diagnoses of hypolactasia to be without error. In fact, not only did we assume that diagnoses occurred with error, but we did not even presume to know exactly what level of error was. Instead, our uncer-
tainty about the true level of error was modelled in a Bayesian framework and (for example, based on breath hydrogen). To accomplish this, a novel statistical method was developed, which was described by Mulcare and colleagues. The fact that we incorporated these addi-
tional sources of error into our method means that the p values we obtained were not as low as they would have been had we applied a statistical naïve test such as \( \chi^2 \) test. Our remarkable finding was that, despite this, we still found significant departures in multiple sub-
Saharan African populations. This led us to reject the null hypothesis that the presence of the C-13910 variant alone, even with diagnostic error, could explain the published data on lactase persistence in Africa. Other reasons must be sought to explain our results, one of which is the possibility that additional genetic variants influence lactase persistence.

The second assertion by Kolbo and Järvelä was that there were “no statistics shown in the title of the paper, “Cannabinoid hyperemesis” is unduly presumptuous. Some of these cases appeared to improve with abstinence and then relapsed when patients were “rechallenged” with cannabis, but neither the patients nor the authors appear to have been blinded in the rechallenge. The proposed biological explanation is weak. We suggest that alternative explanations need to be sought for these cases. This syndrome should not be accepted as being caused by cannabis without additional reports and other evidence.

A Byrne, R Hallinan
Byrne Surgery, Sydney, Australia

A Wodak
St Vincent’s Hospital, Darlinghurst, New South Wales, Australia

Correspondence to: Dr A Byrne, Byrne Surgery, 75 Redfern St, Redfern, 2016, Sydney, Australia; ajbyrne@ozemail.com.au

Dr Wodak is President of the Australian Drug Law Reform Foundation which supports the taxation and regulation of cannabis.

Reference

Authors’ reply
We would like to thank Byrne et al for their interest in our paper (Gut 2004;53:1566–70). It should be noted that we undertook an observational study by necessity. Cannabis is an illegal drug and double blind control trials with illicit substances are prohibited and unethical. The assertion that cannabis has been “consumed for many centuries” needs to be tempered with the fact that cannabis has been grossly under-researched clinically and, as we have shown with this syndrome, nowhere near fully understood in its neuro-

“Cannabis hyperemesis” causation questioned
The authors describe a number of cases of a bizarre syndrome of severe vomiting, abdominal symptoms leading to dehydration, in combination with repetitive bathing behaviour (Gut 2004;53:1566–70). They have concluded that these symptoms are due to cannabis use.

Cannabis has been consumed for many centuries and is currently used by millions of people in many countries. It is hard to believe that a distinctive syndrome caused by can-

IL-1 gene cluster and TNF-α–307 polymorphisms in the risk of perforated duodenal ulcer
Helicobacter pylori virulence markers have been associated with duodenal ulcer (DU) but there are few studies evaluating host factors such as cytokine polymorphisms and, to the best of our knowledge, no study has evalu-
ated these polymorphisms as risk factors for perforated DU. We investigated associations among interleukin 1 (IL-1) cluster and tumour necrosis factor α (TNFA–307) poly-
morphisms, and DU and perforated DU in a non-

Conflict of interest: None declared.

Reference

IL-1 gene cluster and TNF-α–307 polymorphisms in the risk of perforated duodenal ulcer

H pylori realization markers have been associated with duodenal ulcer (DU) but there are few studies evaluating host factors such as cytokine polymorphisms and, to the best of our knowledge, no study has evalu-
ated these polymorphisms as risk factors for perforated DU. We investigated associations among interleukin 1 (IL-1) cluster and tumour necrosis factor α (TNFA–307) poly-
morphisms, and DU and perforated DU in a non-

Conflict of interest: None declared.

Reference

IL-1 gene cluster and TNF-α–307 polymorphisms in the risk of perforated duodenal ulcer

H pylori realization markers have been associated with duodenal ulcer (DU) but there are few studies evaluating host factors such as cytokine polymorphisms and, to the best of our knowledge, no study has evalu-
ated these polymorphisms as risk factors for perforated DU. We investigated associations among interleukin 1 (IL-1) cluster and tumour necrosis factor α (TNFA–307) poly-
morphisms, and DU and perforated DU in a non-

Conflict of interest: None declared.

Reference
regarding perforated DU, as measured by surgical interventions in emergency services.1 Knowing who, among all *H pylori* infected subjects, will develop a perforated DU is therefore an important issue in treatment.

Garcia-Gonzalez and colleagues1 and Zambron and colleagues2, evaluating Spanish and Italian populations, respectively, did not find associations between single IL-1 polymorphisms and DU. Conversely, Furuta and colleagues3 found that IL-1RN allele 2 and IL-1β−511T/T were protective factors for DU in a Japanese population.4

In this investigation, in accordance with previous studies,5 no role could be established for IL-1β−31T or IL-1β−31C alleles in non-complicated DU. However, IL-1β−31C and TNFA−307A carriage was negatively associated with perforated DU. Thus the same IL-1B and TNFA polymorphisms which were associated with atrophy and increased gastric carcinoma risk in Caucasian populations6 were found to be inversely associated with perforated DU.

The mechanism by which overproduction of IL-1B and TNFα due to IL-1β−31 and TNFA−307 polymorphisms protects from DU perforation may not differ from that associated with gastric carcinoma. The prevailing mechanism is probably inhibition of gastric acid production. Consequently, bacteria spread to the corpus where they accentuate the inflammation, lowering acid production, with the net effect of diminishing the risk of DU perforation. Even though our results are biologically plausible, several factors may contribute to geographical specificities, as already seen in studies on other gastrointestinal diseases.7

Also, we have previously demonstrated8 in our population that the distribution of the inflammatory alleles at IL-1 loci is intermediate between Asians and Caucasians.9

In conclusion, one of the questions that motivated the studies associating host cytokine polymorphisms with *H pylori* associated diseases was the possibility of explaining why some infected individuals develop gastric carcinoma, others peptic ulcer, and the majorit remain otherwise uncharacterised conditions. These polymorphisms may play a role in the genesis of *H pylori* associated diseases but are probably insufficient to completely answer this question. Our study demonstrated independent inverse associations between IL-1B−31C and TNFA−307A polymorphic alleles and perforated DU, but no association with non-complicated DU.

### Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Uni All patients</th>
<th>H pylori positive subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>cagA positive status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1</td>
<td>0.00</td>
<td>24.72 (3.29–185.66)</td>
</tr>
<tr>
<td>1/2 Ref</td>
<td>0.42</td>
<td>20.0–90</td>
</tr>
<tr>
<td>1/2 C carrier</td>
<td>0.04</td>
<td>0.20–90</td>
</tr>
<tr>
<td>1/2 Ref</td>
<td>0.42</td>
<td>20.0–90</td>
</tr>
<tr>
<td>2/1</td>
<td>0.60</td>
<td>0.70</td>
</tr>
<tr>
<td>2/2 Ref</td>
<td>0.03</td>
<td>0.05–0.88</td>
</tr>
<tr>
<td>2/2 A carrier</td>
<td>0.00</td>
<td>0.05–0.88</td>
</tr>
</tbody>
</table>

**References**


### Cryptic gluten intolerance in type 1 diabetes: identifying suitable candidates for a gluten free diet

Long term exposure to gluten in coeliacs,1 and coeliac disease (CD) for two or more years after 16 years of age2 may induce type 1 diabetes (T1D) and other autoimmune disorders. Increased prevalence of CD among diabetes and their relatives is well documented.3 Early introduction of gluten to children at high risk for T1D produces T1D associated auto-antibodies.4 Similarly, in the absence of overt clinical symptoms of T1D, some coeliac children produce diabetes autoantibodies in a gluten dependent manner due to the intestinal challenge with gluten produces mucosal recruitment of lymphocytes,5 similar to that in CD patients.6 In diabetes, however, there is no production of CD related anti-tissue transglutaminase antibodies (anti-tTGA).

We have used a phage display assay7 to show that in CD patients, production of anti-tTG is limited to the intestine. Here, we monitored the effects of a gluten free diet (GFD) on anti-tTG antibody synthesis in the intestinal mucosa of a diabetic adult and a boy at high risk of diabetes, both carrying HLA DQ2/DQ8, but lacking serum anti-tTG. Intestinal specimens from both patients and samples of peripheral blood lymphocytes were used to make phage-antibody libraries8 to look for lymphocytes synthesising anti-tTG antibodies.

Patient No 1 was a 35 year old man who had TID for 20 years. During 1998–2001, serum anti-tTG responses were negative, and clinical control of TID was good (mean glycosylated haemoglobin 6.8% (range 8.1–7.3%)). During the period of GFD, the patient did not treated diabetic retinopathy and microalbuminuria, with an average albumin excretion rate (AER) of 230 µg/min, despite treatment with angiotensin converting enzyme inhibitors. In 2001, ‘burning’ epigastric pain appeared with abdominal distension. Duodenal biopsy and number of intraepithelial lymphocytes were normal.

Patient No 2 was a two year old boy at risk of CD and T1D (diabetic father and coeliac brother) who tested negative for GFD on two occasions. HLA T1DM specific genotypes (DR1*0301, DQA1*0501, DQB1*0201 and DRB1*0401, DQA1*0301, DQB1*0302). Tests for anti-tTG serum antibodies were negative while anti-islet cell antibodies (ICA) became positive at 20 months. Informed of the potential risks, the child’s parents consented to intestinal biopsy to detect possible silent CD. Duodenal biopsy and number of intraepithelial lymphocytes were normal.

In both subjects, positive tTG antibody clones (table 1) were isolated only from the intestinal lymphocyte libraries. Two control subjects aged 10 and 45 years, suffering from Helicobacter pylori gastritis and with no family history of CD or TID, tested negative for intestinal anti-tTG clones. The diabetic adult and the child’s parents agreed to a GFD for 12 months, after which laboratory tests and biopsy were repeated. The adult, control of diabetes was unchanged but AER was markedly improved (20 µg/min). The boy tested negative to ICA. In both subjects, biopsies were normal, and analysis of new phage antibody libraries showed complete elimination of anti-tTG clones in the adult and 90% reduction in both positivity and diversity in the child (table 1). Both patients remain on a gluten free diet.

C J B Guerra, G A Rocha, A M C Rocha, C M de Castro Mendes, I E B Saraiva, C A de Oliveira, D M M Queiroz

Laboratory of Research in Bacteriology, Faculty of Medicine, UFMG, Belo Horizonte, Brazil

Correspondence to: Professor D M M Queiroz, Laboratory of Research in Bacteriology, FM/UFMG Av. Alfredo Balena, 190/4026-30130-100, Belo Horizonte, Brazil; dqueiroz@medicina.ufmg.br

doi: 10.1136/gut.2005.077362

Conflict of interest: None declared.

GFD; AER is still normal in the diabetic adult and the child is still negative for ICA.

In the context of genetic predisposition to gluten intolerance, in line with Maci's data on the gradual development of CD in diabetics, we found a gluten dependent immunological response, apparently only in the intestine. In the boy, reduced response to tTG and elimination of ICA after GFD may have been due to very early intervention, indicating temporary protection from the disease. In the diabetic adult, reduction of antibody response to gluten is in line with Maki's data and confirm the benefits of GFD.

Similar larger scale studies are needed to clarify whether there is a difference between the development of type 1 diabetes in individuals with or without CD.

**Table 1** Clones isolated from intestinal biopsies and number of anti-transglutaminase positive clones before and after 12 months of a gluten free diet in the adult diabetic, in the at risk child, and in the controls

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (y)</th>
<th>HLA</th>
<th>ICA</th>
<th>% of tTG positive clones</th>
<th>Different antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>35</td>
<td>DQ2/8</td>
<td>–</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Adult during GFD</td>
<td>35</td>
<td>DQ2/8</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Child</td>
<td>2</td>
<td>DQ2/8</td>
<td>+</td>
<td>50.3</td>
<td>5</td>
</tr>
<tr>
<td>Child During GFD</td>
<td>2</td>
<td>DQ2/8</td>
<td>+</td>
<td>50.3</td>
<td>5</td>
</tr>
<tr>
<td>Control subject</td>
<td>10</td>
<td>DQ2/8</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control subject</td>
<td>10</td>
<td>DQ2/8</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

GFD, gluten free diet; ICA, in soluble-cell antibodies; anti-tTG, anti-transglutaminase antibodies; Different, number of different clones determined by sequencing.

**Acknowledgements**

The paper was founded in part by MURST COFIN 2004060237/3 and RFI 149/03 IRCCS “Burlo Garofolo”.

**References**


**Acquired factor V inhibitor associated with valproic acid use in a cirrhotic patient**

Acquisition of factor V inhibitor is a rare event. The inhibitor most frequently encountered in clinical practice is directed against factor VIII. In a review of the literature, Streiff and Ness found 126 published cases of factor V inhibitor. The inhibitor emerged after major surgery, haemostatic therapy with bovine thrombin, malignancies, autoimmune disorders, blood transfusion, antibiotic therapy, or for unknown reasons. We report the emergence of factor V inhibitor in a cirrhotic patient receiving valproic acid for seizure control.

A 50 year old man treated for alcoholic cirrhosis was admitted for epistaxis. He had no history of autoimmune disorders or blood transfusion. For three years he had been taking valproic acid 1 g/day orally for seizures, and propranolol 60 mg/day. On admission, prothrombin level was 5% of control, factor V was 1%, and factor II was 49%. Two months previously prothrombin level had been 83% of control on two occasions one month apart. Physical examination showed compensated cirrhosis. Epistaxis was linked to valproic acid and consequently was stopped and re-challenged with low dose valproic acid (34). Valproic acid was replaced by phenobarbital while propranolol was maintained.

Three months later prothrombin level was 33%, factor V 14%, factor II 72%, factor VII 70%, factor IX 92%, and factor X 65% of control. Factor VIII, XI, and XII levels were normal, as was thrombin time. He was screened for factor V inhibitor by measuring residual factor V after one hour of incubation at 37°C of equal parts of pooled normal plasma and patient plasma. Antibody titre was determined with the Bethesda method used to titre factor VIII inhibitor. Factor V inhibitor titre was 1.0 Bethesda units. Protein immunoelectrophoresis was normal, and tests for antinuclear, antinative DNA, anti-beta-2-glycoprotein 1, anticardiolipin, anti-thyroidperoxidase, antimitochondria, and anti-smooth muscle antibodies were negative. Aspartate aminotransferase was 73 U/l (normal <40 U/l), albuminaemia 35.2 g/l and α fetoprotein 9.6 ng/ml. Abdominal sonography and colonoscopy were normal. Gastroesophageal endoscopy showed grade I oesophageal varices. Eighteen months after admission, the patient was asymptomatic and epistaxis had not recurred. Factor V was 41% and weak factor V inhibitor activity persisted (0.6 Bethesda units).

Hypocoagulability due to factor V inhibitor is rare and can be difficult to diagnose in a patient with cirrhosis. Eighty seven of the 126 cases described by Streiff and Ness occurred during the last decade, and two thirds of cases followed bovine thrombin exposure. Antitibodies to factor V and antihuman factor V antibodies can interact, potentially inactivating human factor V in vivo. Other noteworthy causes are blood transfusion, cancer, treatment with beta lactam antibiotics or steroids, mycetoma, major surgery (usually in patients having received transfusions or betalactam agents), and autoimmune disorders (coeliac disease, bullous pemphigoid, Sjögren’s syndrome, Hashimoto thyroiditis) associated with congenital factor V deficiency. No cause was found in nearly 20% of cases. To date, no cases have been linked to dental extraction or other minor surgeries. Anti-factor V antibodies can appear at all ages but most frequently occur after age 65 years. The inhibitor was discovered fortuitously in nearly 40% of cases following an isolated increase in prothrombin time. Bleeding was the main presenting sign in 60% of cases, and was life threatening in 22%.

To our knowledge, this is the first reported case of factor V inhibitor associated with valproic acid use. The use of valproic acid inhibits fatty acid beta oxidation, potentially leading to life threatening microvesicular steatosis. However, our patient had no clinical or biological signs of hepatopathy. Rare cases of cutaneous vasculitis or lupus-like syndrome have been linked to valproic acid or its prodrug valproamide. Factor V inhibitors have occasionally been detected in patients with such syndromes, but our patient had no clinical or biological signs of an autoimmune process. Factor V inhibitor disappeared after three years of treatment with valproic acid, and prothrombin
level improved partially after drug withdrawal. In previously reported cases, the inhibitor disappeared in 88% of patients overall, after a mean of 10 weeks. In patients with no identified cause, the inhibitor only disappeared in 62% of cases after a mean of 23 weeks, although this did not affect outcome. Bleeding is difficult to treat in patients with factor V inhibitor. Various approaches have been tried, such as infusion of fresh frozen plasma or, better, platelet concentrates. Plasmapheresis has been used to lower antibody titre and high dose immunoglobulin to neutralise the antibodies. Steroids and immunosuppressants (azathioprine, cyclophosphamide), alone or in combination, have been used for long term inhibition of factor V inhibitor synthesis. However, the results are difficult to interpret as the series were small and included patients with heterogeneous manifestations. There is no consensus treatment.

In conclusion, the onset of hypoagglutinability linked to a decline in factor V level in a cirrhotic patient should not be systematically attributed to hepatic cellular insufficiency; in the absence of marked cytolysis, the presence an acquired factor V inhibitor and a possible drug related cause should be sought.

B Godart
Service d'Hépato-Gastroentérologie, CHU la Milétrie, Poitiers, France

C Bointet
Laboratoire d'Hématologie, CHU la Milétrie, Poitiers, France

C Remblay
Service de Pharmacologie Clinique, Pavillon Rans le Blaye Nord, Poitiers, France

A Hajar
Service de Médecine, Centre Hospitalier, Le Blanc, France

M Beauchant
Service d'Hépato-Gastroentérologie, CHU la Milétrie, Poitiers, France

Correspondence to: Dr B Godart, Service d'Hépato-Gastroentérologie, CHU la Milétrie, 86021 Poitiers cedex, France; b.godart@panline.fr
doi: 10.1136/gut.2005.076232
Conflict of interest: None declared.

References

Acylated ghrelin stimulates food intake in the fed and fasted states but desacylated ghrelin has no effect

We were interested to read the article of Asakawa et al (Gut 2005;54:18–24) which reported that intracerebroventricular and peripheral administration of desacylated ghrelin inhibited food intake in mice in the fasted state. Acylated ghrelin (AG) has a unique biological structure with an acyl side chain on the third amino acid residue. AG is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) and stimulates feeding and growth hormone release. In contrast, desacylated ghrelin (DAG), which does not have the acyl side chain, has no affinity for the GHS-R1a. As the authors suggest, their results might indicate the presence of an alternative receptor through which desacylated ghrelin acts.

We were interested in investigating whether DAG would modulate feeding. We injected saline, 0.3 nmol/kg AG, and 0.3 nmol/kg DAG into C57Bl6 male mice intraperitoneally on two occasions, firstly in the fed state and secondly following a 20 hour fast, and measured food intake at 1, 2, 4, 6, and 24 hours post injection (fig 1). In the fasting experiment, we also injected 0.03 nmol/kg PYY3–36 as a positive control. All animal procedures were approved by the British Home Office Animals (Scientific Procedures) Act 1986 (project license No 70/5281). Results were analysed using a one way repeated measures ANOVA. As previously reported, AG stimulated feeding in the fed state. However, DAG had no significant effect on food intake in the fed state. In the fasting study, PYY3–36 significantly inhibited feeding. AG stimulated cumulative food intake in fasted mice for up to six hours post injection although the percentage increase compared with saline was less than in the fed state (per cent increase two hours following ghrelin injection: fed state 320%, fasted state 30%). In contrast with the findings of Asakawa et al, DAG had no effect on food intake at any time point examined. We used a higher dose of DAG than that administered by Asakawa et al (approximately 7.5 nmol x 3 nmol per mouse) and therefore the absence of a feeding effect associated with DAG is unlikely to be explained by differences in dosing.

In conclusion, we have observed that acylated ghrelin stimulated food intake in the fasting as well as in the fed state. In contrast with the findings of Asakawa et al, there was no alteration in feeding in either the fed or fasting state following desacylated ghrelin. Our results suggest that circulating acylated ghrelin stimulates feeding independently of desacylated ghrelin.

Acknowledgements
We thank the Wellcome Trust for programme grant support and for clinical training fellowships for NMN and MRD.

N M Neary, M R Druce, C J Small, S R Bloom
Department of Metabolic Medicine, Hammersmith Hospital, Imperial College London, London, UK

Correspondence to: Professor S Bloom, Department of Metabolic Medicine, Hammersmith Hospital, Imperial College London, 6th Floor Commonwealth Building, Du Cane Rd, London W12 ONN, UK; s.bloom@imperial.ac.uk

Conflict of interest: None declared.

Future use of the Glasgow alcoholic hepatitis score

We read with interest the findings of Forrest and colleagues (Gut 2005;54:1174–9) regarding their prognostic algorithm for alcoholic hepatitis, the Glasgow alcoholic hepatitis score (GAHS). The study uses robust clinical endpoints to develop an algorithm that has diagnostic advantages over the modified discriminant function score (DFS). We would like to discuss some of the future implications of this important study.

The overall death rate in the study was 23% at 28 days and the death rate of patients with a DFS >32 was 29% at 28 days in the derivation population. The latter figure is lower than the placebo arms of many of the standardised controlled trials of alcoholic hepatitis that range between 35% and 50%.

This difference compared with the published literature may be attributable to case definition. It is possible that there were a fewer number of patients in the derivation cohort for GAHS with true alcoholic hepatitis. Some of the previous studies of alcoholic hepatitis have required liver biopsy evidence of alcoholic hepatitis as part of the case definition. This was not the case for the GAHS study and the case definition was based solely on clinical and biochemical evidence of liver dysfunction in patients with heavy alcohol consumption. In the validation population there was biopsy evidence of alcoholic hepatitis in only 33%.

While this may invalidate the GAHS as a means of identifying cases of alcoholic hepatitis, it does not invalidate its use in identifying patients at risk of death when admitted to hospital with liver dysfunction on a background of heavy alcohol use. This makes it far more pragmatic than tests based on biopsies as many hospitals do not have access to specialised services to perform transjugular liver biopsies in the acute setting. Furthermore, there are published...
randomised controlled trials which have not required histological evidence of alcoholic hepatitis before allocating treatment. The corollary to this is that although alcoholic hepatitis often presents with clinical features of fever, leukocytosis, and hyperbilirubinemia, there remains a differential diagnosis which may require a biopsy to resolve.

It is important to differentiate between true alcoholic hepatitis and severe liver dysfunction in patients with heavy alcohol consumption because it will influence the choice of intervention. Randomised controlled trials that use GAHS to identify patients with alcoholic hepatitis might be greatly underpowered if the therapy (for example, steroids) is effective in alcoholic hepatitis but ineffective or harmful in other clinical conditions where abnormal clinical parameters might be associated with heavy alcohol consumption. Selection of risk stratification models should be determined by the severity of the adverse effects of the therapy under trial. Those with more severe adverse effects will warrant models with high specificity whereas drugs with minimal side effects will benefit from a model with a high sensitivity. Compared with the DFS, the GAHS has an increased specificity, decreased sensitivity, and improved accuracy, making it suited to the selection of subjects in studies using more toxic therapies.

The utility of the GAHS will depend on the effect of its use in the care of patients. We suggest that the next step in the evaluation of GAHS should be a clinical trial to see if patients randomised to risk stratification with GAHS followed by appropriate interventions have a better outcome than those managed conventionally.

We believe this is an excellent study using robust clinical end points. It is a practical model which can be used easily at the bedside to give valuable prognostic information. Success of future therapeutic trials in alcoholic hepatitis will not only depend on the efficacy of the drug but also the appropriate selection of patients by models and their respective cut off points.

References

A proof of concept study establishing Necator americanus in Crohn’s patients and reservoir donors
The emergence of autoimmunity, including Crohn’s disease (CD) where the immune relationship with commensal bacteria is corrupted, has been linked to hygiene. A gradual decline in endoparasites is but one argument that might explain this phenomenon. Weinstock and colleagues have successfully tested the pig whipworm, Trichuris suis, in patients with inflammatory bowel disease (IBD). However, repeated inoculation was required and concern has been raised that aberrant migration could occur. The haemopлагous hookworm, Necator americanus (NA), is proposed as an alternative. We have tested if CD patients tolerate hookworm infection, and the practical issues associated with establishing reservoir donors (RDs).

Over 700 million people remain infected with hookworms. Infective larvae (L3i) are acquired through skin contact with contaminated soil. Auto-infection, direct person to person infection, aberrant migration, and hypobiosis do not occur. Adult worms live in the host small intestine for an average of five years. Infection can be easily terminated with an anthelmintic. Anaemia is the only disease of consequence but is an unusual outcome in properly nourished individuals. Using L3i originally obtained from Madang, Papua New Guinea, but maintained in a healthy researcher in the UK, five CD subjects with longstanding severe alcoholic hepatitis treated with oxandrolone and prednisolone.


Figure 1 Initial Crohn’s disease activity index (CDAI) score for each CD patient versus score at week 20 and at week 45 for the first five inoculated cases (mean 165 ± 5% confidence interval 145) v 64 (25), p = 0.132; mean 165 ± 75 (29), p = 0.246.

>150, occurred in two (CD4, CD5; table 1) after the doses of long term immune suppressive drugs had been reduced. The subject (CD3–7) driven trend was to reduce immune suppression as health improved, a strategy often associated with worsening of symptoms. The five CD subjects first inoculated were in remission at week 45 (fig 1).

Our pilot study has established a potential for NA, already a fact of life for many millions, as a candidate parasite to inoculate those with autoimmune disease. The natural advantages are lifecycle and migration predictability, ability to control the size of and eliminate a colony, and the parasite’s longevity. Inoculation proved safe, even in immune suppressed patients. Our hope that NA would suppress autoreactivity sufficiently to allow immune suppressive therapy to be stopped was unrealistic. Recent and compelling evidence has shown that IBD is self sustaining. It may be that after remission is achieved, endoparasites will offer an alternative or adjunct to immune suppressive therapy, a priority for some people with CD.

Conflict of interest: None declared.
can be cured by Helicobacter pylori eradication. It would be very useful to identify, at the time of diagnosis, the 25% of cases of gastric MALT lymphoma that will not respond to H pylori eradication. Although, lymphomas at stage I, II, or above do not respond to H pylori eradication. However, the prognostic value of staging in stage I cases is very limited, although tumours that involve the muscularis propria or serosa (stage IIE) show a higher failure rate than those restricted to the mucosa and submucosa (stage I).Paradoxically, the majority of gastric MALT lymphomas at diagnosis are at stage I, but 20% of these cases will not respond to H pylori eradication. In a previous study, we have examined the value of t(11;18)(q21;q21) in prediction of the response of gastric MALT lymphoma to H pylori eradication. Among the 11 cases of gastric MALT lymphomas studied, t(11;18)(q21;q21) was present in 42/63 (67%) non-responsive cases, including 26/43 (60%) at stage I. In contrast, translocation was detected in only 2/40 responsive cases and the two translocation positive cases showed a temporary response to H pylori eradication. Based on the same series of cases, we examined the value of t(11;14)(p22;q32)/IGH-BCL10 in prediction of the response of gastric MALT lymphomas to H pylori eradication. Of the 111 cases examined, 75 including 35 from the complete response group and 40 from the non-responsive group, had adequate tissue specimens for evaluation of BCL10 staining. Two cases showed strong BCL10 nuclear staining in virtually all tumour cells (Fig 1). In contrast, the remaining cases displayed either weak cytoplasmic or weak nuclear staining. Two cases showed strong BCL10 nuclear staining. Both cases failed to show BCL10/IGH translocation. To further investigate these cases, we performed real time quantitative reverse transcription-polymerase chain reaction (RT-PCR) to detect BCL10 mRNA. Unfortunately, adequate tissue materials were available only in case No 2. The level (ΔCt = 3.4) of BCL10 mRNA expression in this case was compatible with that in MALT lymphoma with t(1;14)(p22;q32) (mean 1.60 (SD 2.37)), well above that in those without the translocation (6.94 (2.37)). To further assess the impact of t(1;14)(p22;q32) on the clinical behaviour of MALT lymphoma, we retrospectively reviewed the clinical presentation of 11 cases, including six from the stomach with known BCL10 involved translocation (Table 1). Of these cases, nine including all those from the stomach, were at stage II, or above. Although clinical presentation and follow up data were not available in each case, three cases (Nos 1, 2 and 7) presented unusual wide dissemination, and 7) presented unusual wide dissemination.

### References


### Table 1

<table>
<thead>
<tr>
<th>Initial inoculation trial</th>
<th>Reinoculation trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID, age (y), sex</td>
<td>Time (weeks)</td>
</tr>
<tr>
<td>CD1.55 M</td>
<td>0–4</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>25 L3i</td>
</tr>
<tr>
<td>CDAI</td>
<td>79</td>
</tr>
<tr>
<td>CD2.46 M</td>
<td>5–8</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>25 L3i</td>
</tr>
<tr>
<td>CDAI</td>
<td>38</td>
</tr>
<tr>
<td>CD3.41F</td>
<td>9–12</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>25 L3i P5 M15</td>
</tr>
<tr>
<td>CDAI</td>
<td>47</td>
</tr>
<tr>
<td>CD4.44F</td>
<td>13–16</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>50 L3i P38 M30</td>
</tr>
<tr>
<td>CDAI</td>
<td>260</td>
</tr>
<tr>
<td>CD5.21F</td>
<td>17–20</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>50 L3i P10 M20</td>
</tr>
<tr>
<td>CDAI</td>
<td>144</td>
</tr>
<tr>
<td>CD6.33F</td>
<td>0–4</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>50 L3i P15 M20</td>
</tr>
<tr>
<td>CDAI</td>
<td>49</td>
</tr>
<tr>
<td>CD7.33M</td>
<td>5–8</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>50 L3i A150</td>
</tr>
<tr>
<td>CDAI</td>
<td>260</td>
</tr>
<tr>
<td>CD8.46F</td>
<td>9–12</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>50 L3i M20</td>
</tr>
<tr>
<td>CDAI</td>
<td>145</td>
</tr>
<tr>
<td>CD9.44F</td>
<td>13–16</td>
</tr>
<tr>
<td>Inoculum therapy</td>
<td>100 L3i P5 M20</td>
</tr>
<tr>
<td>CDAI</td>
<td>173</td>
</tr>
</tbody>
</table>

| Inoculum therapy         | 410                 | 180           |
| CDAI                     | 122                 | 60            |

L3i, n 3rd stage N americanus larvae inoculated percutaneously; P, prednisone n mg/day; A, azathioprine n mg/day; M, methotrexate n mg/week.

### Strong BCL10 nuclear expression identifies gastric MALT lymphomas that do not respond to H pylori eradication

Approximately 75% of gastric mucosa associated lymphoid tissue (MALT) lymphomas associated lymphoid tissue (MALT) lymphomas that do not respond to H pylori eradication. Approximately 75% of gastric mucosa associated lymphoid tissue (MALT) lymphomas associated lymphoid tissue (MALT) lymphomas that do not respond to H pylori eradication.
including pleural effusion, and blood and bone marrow involvement (table 1).

Taken together, our results suggest that gastric MALT lymphomas with strong BCL10 nuclear expression or t(1;14)(p22;q32) are mostly likely resistant to *H pylori* eradication.

**Acknowledgements**

This study was supported by research grants from Leukaemia Research Fund, UK and Deutsche Krebshilfe. The authors thank J Audouin, L Bedenne, O Bouché, Marie-Christine Copin, Y Bouhnik, J Fournet, A De Mascarel, Ph Moreau, J Lafon, A Pariente, and F Piard of the Groupe d’Etude des Lymphomes Digestifs (GELD), France; T. Thomas and Pl. Zinzani of Università degli Studi di Bologna, Italy; and M Stolte of Institut fur Pathologie, Klinikum Bayreuth, Germany, for contribution of part of the specimens used for this study.

**Table 1** Clinical feature of mucosa associated lymphoid tissue (MALT) lymphoma with t(1;14)(p22;q32) or variants

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age</th>
<th>Sex</th>
<th>Primary site</th>
<th>Genetic investigations</th>
<th>BCL10 involved chromosomal translocation</th>
<th>BCL10 IHC</th>
<th>Staging*</th>
<th>Dissemination</th>
<th>Clinical follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71</td>
<td>M</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>Strong nuclear staining</td>
<td>IIE</td>
<td>IVE</td>
<td>Perigastric lymph nodes, omentum, spleen, pleural effusion, blood</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>F</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>n/a</td>
<td>IIE</td>
<td>Perigastric and splenic hilar lymph nodes, pleural involvement, bone marrow involvement</td>
<td>4 cycles of MCP chemotherapy, partial remission, alive at two year follow up</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>M</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(q21;q22)</td>
<td>Strong nuclear staining</td>
<td>IIE</td>
<td>IVE</td>
<td>Perigastric lymph nodes and spleen</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>F</td>
<td>Stomach</td>
<td>Interphase FISH</td>
<td>n/a</td>
<td>Strong nuclear staining</td>
<td>IIE</td>
<td>IVE</td>
<td>Perigastric and mesenteric lymph nodes</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>M</td>
<td>Stomach</td>
<td>Interphase FISH</td>
<td>n/a</td>
<td>Strong nuclear staining</td>
<td>IIE</td>
<td>IVE</td>
<td>Lung</td>
</tr>
<tr>
<td>6</td>
<td>n/a</td>
<td>n/a</td>
<td>Stomach</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>Strong nuclear staining</td>
<td>IIE</td>
<td>IVE</td>
<td>Blood, bilateral pulmonary involvement, pleural and ascitic effusions, retropertitoneal lymph node</td>
</tr>
<tr>
<td>7</td>
<td>63</td>
<td>F</td>
<td>Lung</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>n/a</td>
<td>IIE</td>
<td>n/a</td>
<td>8 year low grade B cell lymphoma, then presented an aggressive clinical course presenting with lymphocytosis, pleural and ascitic effusions, partially response to chemotherapy, died of disease</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>F</td>
<td>Lung</td>
<td>Interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>IGH-BCL10 fusion</td>
<td>n/a</td>
<td>IIE</td>
<td>No clinical evidence</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>F</td>
<td>Lung</td>
<td>Interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>IGH-BCL10 fusion</td>
<td>n/a</td>
<td>IIE</td>
<td>Anirial lymph nodes</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>F</td>
<td>Breast</td>
<td>Karotyping, interphase FISH</td>
<td>t(1;14)(p22;q32)</td>
<td>n/a</td>
<td>IIE</td>
<td>n/a</td>
<td>No clinical evidence</td>
</tr>
<tr>
<td>11</td>
<td>75</td>
<td>F</td>
<td>Breast</td>
<td>Interphase FISH</td>
<td>IGH-BCL10 fusion</td>
<td>Strong BCL10 nuclear staining</td>
<td>IIE</td>
<td>IVE</td>
<td>No clinical evidence</td>
</tr>
</tbody>
</table>

* Ann Arbor-Musshoff staging system for extranodal lymphoma; the clinical stage was likely to have been underestimated as appropriate staging was unlikely to be carried out in each of these archival cases.

IHC, immunohistochemistry; FISH, fluorescence in situ hybridisation; n/a, not available.
Conflict of interest: None declared.

References

Interferon-β plus ribavirin for patients with hepatitis C virus genotype 1: a randomised pilot trial

The rate of sustained eradication of hepatitis C virus (HCV) in response to a combination of interferon-β and ribavirin remains unsatisfactory in patients with genotype 1 infection.1 No effective alternative treatment is currently available for non-responders. Interferon-β is also a type 1 interferon commonly used to treat chronic HCV infection in Japan. A previous study showed that a 24 week course of therapy with interferon-β plus ribavirin resulted in sustained loss of HCV in three of nine patients with chronic hepatitis C.2 However, the efficacy and safety of interferon-β combined with ribavirin has yet to be fully evaluated.

We report the results of a randomised pilot trial comparing interferon-β plus ribavirin with interferon-α plus ribavirin in patients with HCV genotype 1 who poorly responded to interferon-α plus ribavirin. A total of 28 patients with HCV genotype 1 were given 6 MU of recombinant interferon-2b (Schering-Plough, Kenilworth, New Jersey, USA) by intramuscular injection daily for four weeks. Twenty seven patients (16 men and 11 women; mean age 47 (± 8) years) in whom HCV RNA was detected in serum on polymerase chain reaction at week 2 were included in this study and randomly assigned to receive one of two regimens from week 3. Fifteen patients continued to receive 6 MU interferon-2b intramuscularly, given daily from week 5 to week 8, and three times weekly from week 9 to week 24 (interferon-α group). The other 12 patients were assigned to 6 MU natural interferon-β (Toray Industries Inc, Tokyo, Japan), given by intravenous injection daily from week 5 to week 8, and three times weekly from week 9 to week 24 (interferon-β group). Ribavirin (Schering-Plough) was concurrently administered at a daily dose of 600 mg to patients who weighed 60 kg or less and 800 mg to those who weighed more than 60 kg. At the time of this study, a 24 week course of interferon-α plus ribavirin was commonly used in Japan. The data were analysed according to intention to treat.

Baseline characteristics of the patients in the treatment groups were similar. At week 4 of therapy, when treatment was randomly assigned, the proportion of patients without detectable HCV RNA in serum did not differ between the interferon-α group and interferon-β group (table 1). The proportion of patients without HCV RNA in serum was higher in the interferon-β group than in the interferon-α group at week 12, but did not differ between the groups at the end of treatment (week 24). However, 24 weeks later (week 48), the proportion of patients with a sustained virological response was significantly higher in the interferon-β group than in the interferon-α group. During treatment, neutralising antibodies to interferon were detected in two patients in the interferon-α group and in no patients in the interferon-β group (table 1). The proportion of patients with HCV RNA in serum was higher in the interferon-β group than in the interferon-α group at week 12, but did not differ between the groups at the end of treatment (week 24). However, 24 weeks later (week 48), the proportion of patients with a sustained virological response was significantly higher in the interferon-β group than in the interferon-α group. During treat-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Proportions of patients without detectable hepatitis C virus RNA in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interferon-α group</strong></td>
<td><strong>Interferon-β group</strong></td>
</tr>
<tr>
<td>(n = 15)</td>
<td>(n = 12)</td>
</tr>
<tr>
<td><strong>Week 4</strong></td>
<td>4 (27%)</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
<td>7 (47%)</td>
</tr>
<tr>
<td><strong>Week 24 (end of therapy)</strong></td>
<td>10 (67%)</td>
</tr>
<tr>
<td><strong>Week 48</strong></td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

doi: 10.1136/gut.2005.081117
patients given recombinant interferon-α, can cause resistance to therapy. Both interferon-α and -β bind to a common type I interferon receptor but utilise different regions of the receptor subunits for specific signalling pathways,1 potentially leading to distinct biological responses. An oligonucleotide array study has shown that some interferon stimulated genes are preferentially induced by interferon-β, but not by interferon-α.2 We thus believe that interferon-β might be beneficial for some patients who are resistant to interferon-α. A large randomised trial of peginterferon-α plus ribavirin versus interferon-β plus ribavirin for 48 weeks is being conducted in patients with HCV genotype 1 who do not have a virological response3 to 12 weeks of treatment with peginterferon-α and ribavirin.

In summary, a combination of interferon-β and ribavirin produced a significantly better sustained virological response than a combination of interferon-α and ribavirin in patients with HCV genotype 1 who were resistant to interferon-α plus ribavirin. Although the overall safety profiles of the two regimens were similar, the rates of treatment discontinuation and of reduction in the dose of ribavirin were lower in patients receiving interferon-β and ribavirin than in those receiving interferon-α and ribavirin.

Acknowledgement
Grant support was received from Ministry of Health, Labour, and Welfare, Japan.

M Enomoto, A Tamori, N Kawada, H Jomura
Department of Hepatology, Osaka City University Medical School, Osaka, Japan

S Nishiguchi
Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan

T Saibara, S Onishi
Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi, Japan

S Mochida, K Fujimura
Division of Gastroenterology and Hepatology, Internal Medicine, Saitama Medical School, Saitama, Japan

Correspondence to: Dr S Nishiguchi, Division of Hepatology and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, Hyogo 663-8501, Japan; nishiguc@s-hyom-ed.ac.jp
doi: 10.1136/gut.2005.081935
Conflict of interest: None declared.

References

5 Davis GL. Monitoring of viral levels during therapy of hepatitis C. Hepatology 2002;36(suppl 1):S45–S51.

EDITOR’S QUIZ: GI SNAPSHOT

Answer

From question on page 122

At explorative laparotomy, the pancreatic tumour involving the head and proximal body of the pancreas was judged to be resectable. Pylorus preserving proximal pancreaticoduodenectomy was performed. Histology of the tumour was consistent with a diagnosis of renal cell cancer (RCC) metastasis to the pancreas (Fig 2). Metastases were not detected in peri pancreatic lymph nodes. The patient did not receive any further adjuvant therapy and was discharged from hospital without any serious perioperative morbidity.

The vast majority of pancreatic carcinomas are primary, and among these, more than 90% are of ductal origin. Solitary pancreatic masses can be classified as secondary tumours to the pancreas in only 2% of all cases.1 In the latter group, RCC seems to be the most common cancer. Within the last three years, 43 new cases of RCC metastases to the pancreas have been reported (Medline review). Median interval from nephrectomy to diagnosis of pancreatic metastases is 83 months, but time intervals as long as 10–20 years were also reported.2 Complete resection of pancreatic metastases from RCC are associated with long term survival, particularly in cases of single tumours and/or a long disease free interval.3

References


Figure 2 2 Histomorphological appearance of the pancreatic tumour (haematoxylin-eosin, ×40). From the lower left to the upper right corner, normal pancreatic glandular tissue, desmoplastic capsule, and clear cell carcinoma are visible.