PostScript

LETTERS

Hepatitis C virus clearance and gender

Bakr *et al* (*Gut* 2006;**55**:1183–7), in a large population-based cross-sectional survey on hepatitis C virus (HCV) infection in Egypt, found that HCV clearance rates (ie, positive HCV antibodies and negative HCV-RNA test results) were significantly higher in women (44%) compared with men (33.7%, adjusted odds ratio (OR) 1.77).[1] They conclude that these findings provide a strong evidence for a higher HCV clearance rate in women compared with men.

We have obtained different results in three published population-based surveys (not cited by Bakr in the references) performed in Southern Italian towns.[2–4] The overall prevalence of HCV antibody was 324/2561 (12.6%). The prevalence was slightly higher in women (13.7%) than in men (11.3%), a difference which is not statistically significant. None of the anti-HCV-positive patients had previously received antiviral treatment, none reported intravenous drug use, and none of them were HIV-positive. Of those with HCV antibodies (n = 324), 73.1% had chronic HCV infection (positive HCV antibody and positive HCV-RNA test results). Nearly identical proportions of men (35/127, 27.6%) and women (52/187, 26.4%) were HCV-RNA-negative, indicating cleared infection.

Regardless of the difference in results, we believe that the cross-sectional structure of all these studies (including that of Bakr) does not allow any valid conclusions in favour of or against the hypothesis of gender-related HCV clearance. The main problem in prevalence surveys is the potential for survival bias. Bakr and coworkers are aware of this bias, as they affirm that ''subjects with chronic infection will have increased mortality compared with those with cleared infection, resulting in overestimation of the population with cleared infection''. However, they failed to clarify that the survival bias could not have affected both genders similarly—that is, mortality might have been higher in women than in men with chronic HCV infection, resulting in a misleadingly higher proportion of women with presumably cleared infection.

We would like to suggest more caution before drawing any inference from prevalence surveys regarding the potential effect of gender on HCV clearance. Otherwise, conclusions may be biased.

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Authors' response

We have read with close attention the comments made by Stroffolini *et al* on our paper on sex-related hepatitis C virus (HCV) clearance rates published in a previous issue of this journal (*Gut* 2006;**55**:1183–7). We do agree with them that cross-sectional studies may be subject to biases in estimating clearance rates, and have extensively discussed potential biases and their effect in our paper. Stroffolini *et al* raise an interesting issue by stating that females chronically infected with HCV might have had higher mortality than males infected with HCV in the village of our study, thus leading to an under-representation of chronic infection and a falsely increased clearance rate in females compared with males. While considering this hypothesis, we were surprised by the postulate of higher mortality in infected females than in males. Indeed, the literature indicates rather the opposite—that is, a higher morbidity/mortality in infected males than in females.[1 2] In our study, chronically infected males were more likely to have increased alanine aminotransferase levels—that is, increased HCV-related morbidity, compared with infected females (75/158 (47.5%) vs 32/108 (29.6%), p = 0.004). Differential mortality by sex in our study is therefore more likely to be in the direction opposite to that suggested by Stroffolini *et al*, hence resulting in an underestimate of the true difference in clearance rates between females and males. This in turn would explain the absence of difference observed in studies in Italy, where the increased mortality in infected males compared with females would result in falsely increased clearance rates in males, hence masking the difference in clearance rates between females and males. Differential mortality by sex may be more pronounced in Italy than in Egypt, as it may be partly related to higher alcohol use in males than in females, a feature not seen in the Muslim communities of rural Egypt, where alcohol use is almost non-existent.

In conclusion, we agree with Stroffolini *et al* that differential mortality between chronically infected females and males may lead to biases in estimating differences in clearance rates in cross-sectional surveys. However, we believe that the mortality is higher among chronically infected males than among females, rather than the reverse, thus underestimating differences in clearance rates between females and males and not the opposite.

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Inadequate statistical power in Barrett's oesophagus ablation study

We found the article of Sharma *et al* (Gut 2006;**55**:1233–9) of great interest. They have looked into 2-year data comparing the efficacy of achieving complete reversal (endoscopic and histological) between multipolar electrocoagulation (MPEC) and argon plasma coagulation (APC) in patients with Barrett's oesophagus.

This was an important study if one accepts the hypothesis that the reversal between MPEC and APC may reduce the risk of adenocarcinoma of the oesophagus. However, we believe that the authors have misinterpreted their power calculation for the study and failed to address the potential limitations of both methods if they were to be adopted into standard clinical practice and shown to be of clinical benefit.

Firstly, there were no results on the practical aspects of both procedures for the readers to know whether they would be acceptable to the patients and feasible from the endoscopist point of view. We suspect that these frequent and time-consuming methods have resulted in difficulty to recruit patients for this study. Only 35 patients from two large hospitals had agreed to participate in the study over a 4-year study period. A participants' flowchart as recommended by the Consolidated Standards of Reporting Trials statement in 2001 would help to understand the acceptance by patients of these potential useful methods. The average duration for both procedures would also help the endoscopist to assess the feasibility in an already ''demand outstretching capacity'' endoscopy unit.

Secondly, we thought that the study was grossly underpowered and incorrectly accepted the null hypothesis (type II error)—that is, no difference in outcome between the two methods. Our calculation using Stata V.8 showed that 51 patients are needed to detect a significant difference with 80% power for each group. Therefore, the total number needed is 102, based on the assumption of 90% ablation of Barrett's oesophagus in the MPEC group and 65% ablation of Barrett's oesophagus in the APC group with 80% power and type I error of 5%.

We recommend that the authors accept the possibility for type II error in their results and conclusion.

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Competing interests: None.

Reference

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Authors' response

We thank Lim *et al* for their keen interest in our article (Gut 2006; **55**: 1233–9) on the long-term results of ablation in patients with Barrett's oesophagus (BO). In this prospective, randomised controlled trial, we compared the long-term efficacy of achieving complete reversal (endoscopic and histological) between multipolar electrocoagulation (MPEC) and argon plasma coagulation (APC) in patients with BO. Patients underwent stratified randomisation by BO length, using sealed opaque envelopes. Results of this study showed that complete reversal can be maintained in ~70% of patients, irrespective of the technique, with no predictors identified associated with achieving complete reversal.

In this study, we used stratified randomisation instead of simple randomisation, which allowed us to use the paired Fisher's test for determination and analyses of power and sample size. The paired Fisher's test finds whether there is a difference between two groups when the outcome is a response proportion and the difference is assessed by testing if the odds ratio (OR) is 1 or not. This works only when the subjects are stratified into groups before randomisation, as it is believed that the response proportions will vary across the strata, but that the OR is constant across the strata. In the case of our study, the strata are the BO lengths.[1] We believe that Lim *et al* have assumed a simple randomisation and hence used a non-paired Fisher's exact test for their sample size calculation.

Crucial demographic and endoscopic features of patients and the number of ablation sessions required in the two groups were reported in this study. We agree with Lim *et al* that information such as the duration of each procedure for the two techniques, information on patients with BO who refused participation, and so on was not provided in the manuscript. However, as we have highlighted in our article, we reiterate that techniques such as MPEC and APC are not ready for clinical application in ablation of non-dysplastic BO and should not be offered outside the research arena. The degree of long-term control of neoplasia is not as yet known. Persistence of underlying intestinal metaplasia after ablation (buried glands) carries the potential to progress to dysplasia and adenocarcinoma, a problem that cannot be ignored.[2] It has also been shown that recurrent and/or persistent BO after ablative therapy still contains genetic alterations associated with malignant progression to cancer.[3] At the present time, the available data do not support the application of ablative therapies for patients with non-dysplastic BO.

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Development of a bioartificial new intestinal segment using an acellular matrix scaffold

Intestinal rehabilitation for short-bowel syndrome is an integral part of modern intestinal transplant programmes. The mortality of patients with short-bowel syndrome is most significant in individuals with a residual small bowel of <50 cm, as shown by a 5-year survival rate of 57%.[1] Total parenteral nutrition and intestinal transplantation are options to extend life but are still plagued by serious complications and, in the case of transplantation, immunosuppression. As an alternative, several bowel elongation procedures have been described,[2–4] but have had limited clinical success and new techniques are warranted. The minimum length of bowel required to allow sufficient absorption of nutrients has not been confirmed.[1 5] Elongation of even a few centimetres may allow these patients to receive nutritional rehabilitation and become independent from total parenteral nutrition, and possibly avoid transplantation. We hypothesised that an acellular dermal matrix (ADM, AlloDerm, LifeCell Corporation, Branchburg, New Jersey, USA) scaffold placed in continuity with defunctionalised jejunal limb allows mucosal growth and intestinal elongation. We evaluated the morphology of neoformed intestine in ACI (August × Copenhagen-Irish) rats at different time points using two different types of anastomosis. Tubular scaffolds with an intraluminal diameter of approximately 0.3 cm were constructed using rehydrated ADM segments of 1 cm^2 and 0.78–1.77 mm thickness, and oriented with a luminal basement membrane and a serosal dermal surface. In group A (n = 5), the ADM graft was interposed in continuity with the jejunum using an interrupted end-to-end single-layer anastomosis. In group B (n = 11), the grafts were placed as blind-ended pouches to the defunctionalised jejunal limb. Postoperatively, animals were maintained on a liquid diet for 48 h followed by solid-rat chow and killed at different time-points postoperatively. Tissue samples for histological examination were obtained across the anastomosis. Survival and body weight were evaluated in both groups. All animals in group A were killed in the first week as a result of peritonitis. All animals in group B survived

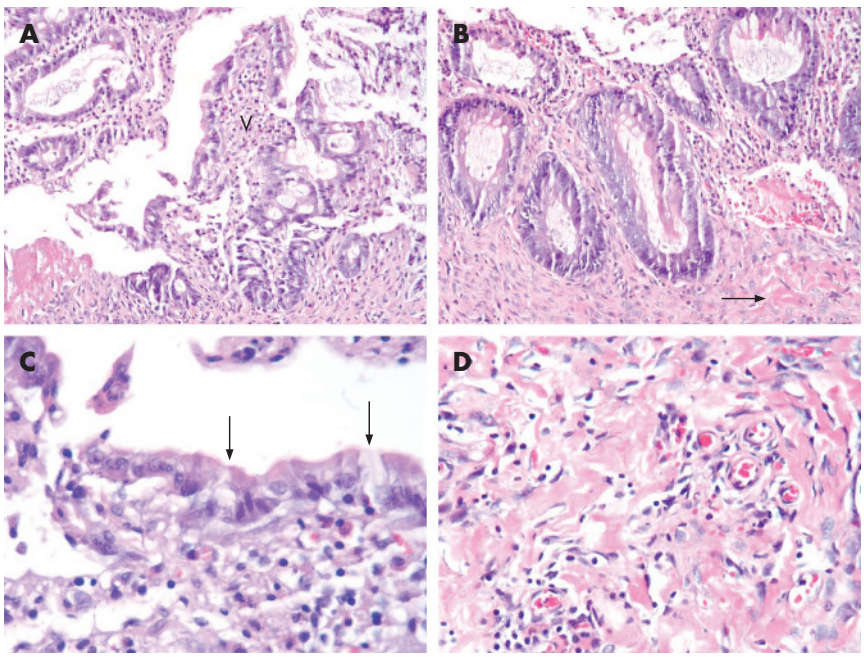

Figure 1 Photomicrographs of the anastomosis between the acellular dermal matrix (ADM) and small bowel at 4 months of transplantation. (A) Leading edge of regenerating epithelium with villus formation (V). Note a bit of remaining bare graft surface to the left of the villus, H&E ×20. (B) Regenerating crypts with goblet cells. Some residual collagen from the ADM can be seen nearby (arrow), H&E ×20. (C) Epithelium along the edge of the villus in (A) showing goblet cells (arrows) and absorptive cells with brush border, H&E ×60. (D) Vascularisation of the ADM just beneath the regenerating crypts, H&E ×40.

Table 1 Histology results of group B at different time points

Time after anastomosis	Status of anastomosis	Status of alloderm	Epithelial regeneration
2 days	Intact	Minimal acute inflammation	No
2 weeks	Intact but inflamed	Minimal acute inflammation. Proliferation of fibroblasts and endothelial cells	Early budding of crypts at bowel edge of anastomosis
4–5 weeks	Intact but inflamed	Less acute inflammation Full-thickness ingrowths of capillaries and myofibroblasts	Same as 2 weeks
4–6 months	Intact with granulation tissue around sutures	Full-thickness capillaries and fibroblasts and haemosiderin-laden macrophages	Regenerating crypts with goblet cells, and rudimentary villi with absorptive cells with brush border

and increased body weight appropriately. Tissue samples showed a progressive increase in the amount of cell infiltrate in the matrix (table 1, fig 1). After 2 weeks, acute inflammation was replaced by full-thickness ingrowths of capillaries and myofibroblasts. Epithelial regeneration into the anastomosis was first seen at 2 weeks, and well-formed branching crypts were seen at 4 weeks of transplantation. Goblet cells and absorptive cells with brush border were present at 4 months of transplantation. Morphologically intact regenerated mucosa extending across the anastomosis to the grafts was observed at 6 months of transplantation. To date, there is limited literature on the bioengineered intestine. Vacanti *et al*,[6–8] developed a cystic structure in which neomucosa forms in a biodegradable polymer in rodents. Once formed, the neointestinal cyst is anastomosed in continuity with the native bowel without causing feeding problems, but some animals had small bare areas of the cysts that lacked neomucosa.[7] We did not report such bare areas in our model; furthermore, there was progressive growth of the neomucosa in the ADM over time. It is possible that immediate contact of the ADM scaffold with the intestinal structures and with luminal content provided trophic stimuli for the new intestinal segment.[9] Another possible factor for the observed growth in our model may be the effects of small-bowel resection on the development of neointestine. It is well known that post resection gut mucosa growth factors have a stimulatory effect on intestinal regeneration.[10] In conclusion, we have demonstrated that ADM can be successfully used as a scaffold to generate a bioartificial new intestinal segment in vivo, and we propose this method as a basis for developing new intestinal elongation techniques.

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doi: 10.1136/gut.2006.116848

Competing interests: None.

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No evidence of persisting measles virus in the intestinal tissues of patients with inflammatory bowel disease

The hypothesis that measles virus (MV) is implicated in the pathogenesis of Crohn's disease (CD) was initiated by Wakefield *et al*[1] in the early 1990s. Several other reports soon followed, which suggested the presence of MV in CD samples using electron microscopy, immunohistochemical analysis, in situ hybridisation and nested PCR.[2–4] Controversy in response to this claim resulted in falling measles vaccination rates, mainly in the UK. Immediate concerns were raised regarding several aspects of these studies, including reagent specificity.[5] In addition, several groups were unable to replicate their findings using a range of PCR techniques.[6–9]

In 1998 and 2002, the initial investigators again raised concerns over MV persistence in bowel tissue, this time in children with autism.[10] These allegations further damaged confidence in MV vaccination programmes. As the primer pairs that had been used to implicate MV in inflammatory bowel disease (IBD; Kawashima)[4] and autism (Uhlmann)[10] were not included in the negative studies,[6–9] it could still be argued that the negative results arose due to the use of incorrect or suboptimal primers. We therefore used the Kawashima and Uhlmann primers in an attempt to detect MV nucleic acids in patients with IBD and control intestinal biopsy samples. Three real-time and two nested PCR assays targeting the MV nucleocapsid, fusion and haemagglutinin genes using the Kawashima and Uhlmann primers were performed, with detection by SYBR Green I. We also designed our own probe-based F gene assay. Positive samples were analysed by melting curve analysis, electrophoresis and sequencing. A total of 41 biopsy samples were available for this study (table 1). Full details of the primers and methods were published previously.[11]

We report that all samples yielded positive signals in the Uhlmann real-time PCR assays (fig 1A). Our study generated mean (SD) copy numbers that were very similar to those reported by Uhlmann *et al*—that is, between 5.08E3 ($\pm$6.32E3) and 2.94E5 ($\pm$5.75E5). However, only a proportion of the PCR products had melting peaks that overlapped with those of the positive control sample (fig 1A,B). Of these positive results, no sample in the Uhlmann fusion or haemagglutinin gene assays yielded an appropriate 150 bp band when analysed by electrophoresis. Only 2 of 17 CD samples produced appropriately-sized bands (150 bp) using the nucleocapsid gene assay. Repeated attempts to clone and sequence were successful for one of the two nucleocapsid gene amplicons. This amplicon had sequence homology to various mammalian genes, including ''*Homo sapiens* cDNA FLJ34053 fis, highly similar to histone H1'' (National Center for Biotechnology Information (NCBI) accession #AK091372).

In all, 10 samples (4 CD, 1 ulcerative colitis and 5 non-IBD) had the correct band size (181 bp) after amplification using the Kawashima fusion gene assay. Nine of ten amplicons successfully cloned and sequenced had homology to ''*Homo sapiens* mitochondrial DNA gene'' (NCBI accession #AY289053). The remaining amplicon did not match any genes from the NCBI database. No sample was positive by nested PCR using the Kawashima haemagglutinin gene primers or our own probe-based fusion gene assay.

Both the Kawashima and Uhlmann primers demonstrated significant cross-reactivity. Searches for sequence similarities revealed homology from 43% to 75% between the mammalian sequences that we amplified and the Kawashima and Uhlmann primers.

In summary, by direct application of the Uhlmann and Kawashima primers, we also failed to demonstrate the presence of MV nucleic

Table 1 Biopsy samples available for this study

Sample type	Sample (n)	Mean age (years)	Age range (years)	Sex distribution (n, males)
Crohn's disease	18	24.0	5.4 to 47.5	9
Ulcerative colitis	6	34.5	13 to 77	4
Non-inflammatory	17	16.6	2.5 to 69	6

acids in intestinal biopsy samples from either patients with IBD or controls.[5–8] Our results suggest at least one plausible mechanism for what we believe to have been false-positive results reported in the Kawashima and Uhlmann studies. Homologies identified between the primers themselves and the mammalian-origin amplicons that we sequenced strongly suggest that non-specific amplification, contamination or both occurred in these studies.

Acknowledgements

This study was supported by a Research Grant from the Crohn's and Colitis Foundation of Canada (EGS, BJW) and by a Tier I Canada Research Chair award in Immune Mediated Gastrointestinal Disorders (EGS) from the Canadian Institutes of Health Research.

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Ethical approval: The study protocol and consent forms were approved by the ethics review committees of Ste-Justine and Royal Victoria Hospitals (Montreal, Quebec, Canada). Informed consent was obtained from all patients or their parents or guardians.

doi: 10.1136/gut.2006.119065

Competing interests: BJW has served on a number of Canadian and US government advisory committees addressing the issues of vaccine use and safety between 1994 and 2006. He has provided expert testimony for both the US and Quebec vaccine injury compensation programs. Dr Ward has also provided advice and teaching to Canadian government and industry groups in the area of vaccine immunology. He has conducted and participated in several studies of measles vaccine safety sponsored by Canadian and US government funding agencies. He has also conducted a small number of phase I and phase II industry-sponsored clinical trials of non-licensed vaccines for smaller biotechnology companies. He has conducted a single, company-sponsored, immunological study of a licensed, acellular pertussis vaccine. None of the other authors have conflicts of interest to declare.

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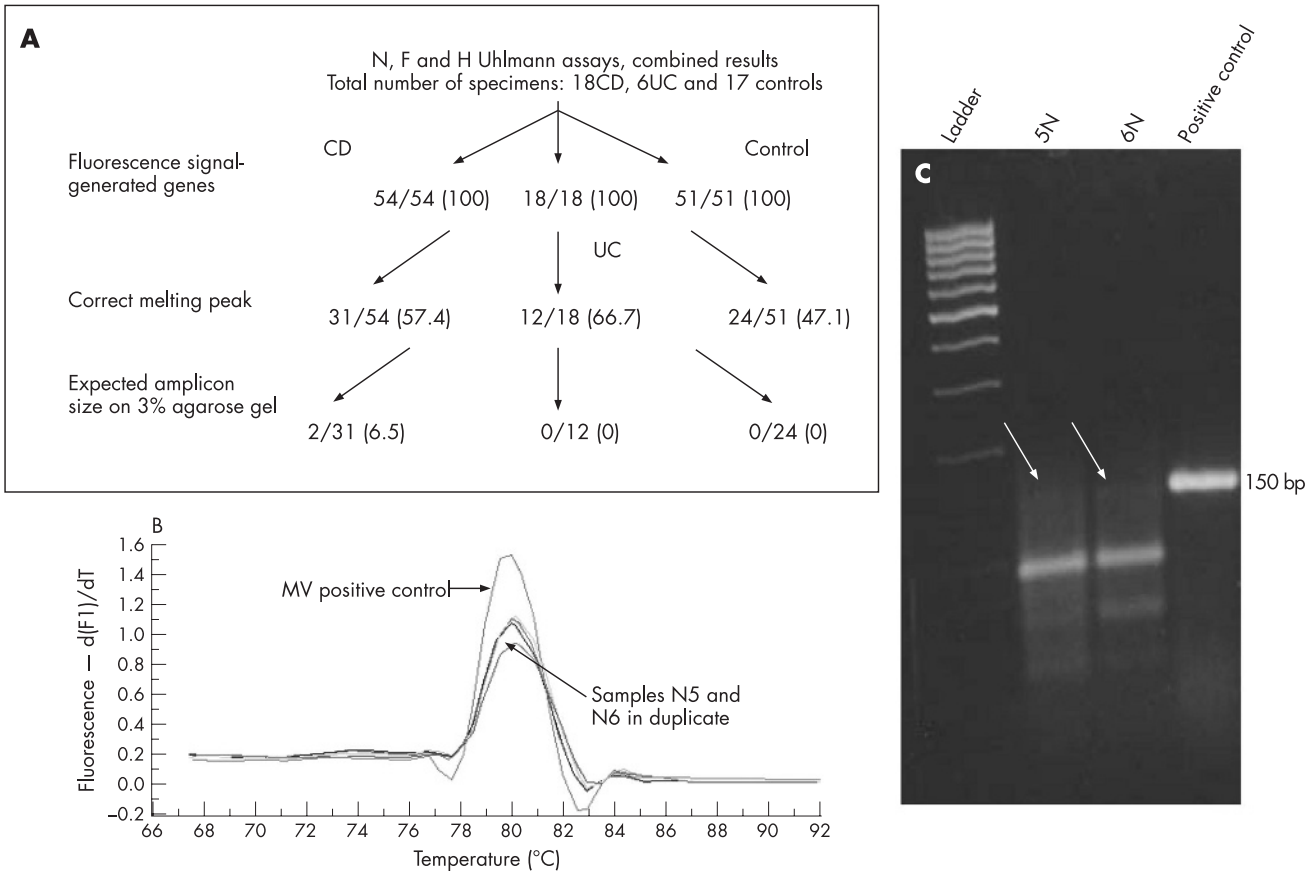

Figure 1 (A) Flow chart illustrating the algorithm and tests used for the handling of samples found to be positive by the Uhlmann nucleocapsid (N), haemagglutinin (H) and fusion (F) assays. Crohn's disease (CD) samples are shown on the left, ulcerative colitis (UC) samples in the middle, and controls on the right. Numbers in parentheses represent percentages. (B) Sample products (CD samples N5 and N6) amplified using the Uhlmann N assay. The samples were positive by melting curve analysis, as shown by the overlap of the melting curves for the samples (each run in duplicate) with the melting curve for the positive control. (C) The same amplicons from (B) were separated on a 3% agarose gel. Light bands of 150 bp are barely visible for these samples. Lane 4 is the positive control (150 bp).

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Clostridium difficile-associated diarrhoea: bovine anti-*Clostridium difficile* whey protein to help aid the prevention of relapses

Antibiotic treatment can usually help in symptomatic recovery in *Clostridium difficile*-associated diarrhoea (CDAD), but recurrent episodes of diarrhoea remain a major problem.[1–4] Besides increasing the dose or extending the course of antibiotics, and using alternating or pulsed regimens, there are no effective alternatives to prevent relapses.[5] Previously, we reported on anti-*Clostridium difficile* whey protein concentrate (anti-CD-WPC) made from the milk of cows immunised against *C difficile* and its toxins. Anti-CD-WPC is prepared using standard techniques used in the milk industry, and contains a high concentration of sIgA directed against *C difficile* and its toxins. It neutralises the cytotoxicity of *C difficile* toxins in vitro and protects hamsters against otherwise lethal *C difficile*-associated cecitis.[6]

The aim of this study was to assess the preliminary efficacy of anti-CD-WPC in aiding the prevention of relapses in patients with CDAD confirmed by positive faecal *C difficile* toxin assay and culture before enrolment.[7 8] After completion of at least 10 days of antibiotic treatment, patients received anti-CD-WPC for 2 weeks, with a follow-up period of 60 days. Patients provided written, informed consent.

Exclusion criteria were a history of milk intolerance, or inability to receive oral fluids. Patients received anti-CD-WPC 15 g/day, divided into 3 equal doses, for 14 days. Anti-CD-WPC was added to flat mineral water, stirred and taken in an empty stomach 1 h before each meal. Patients kept a diary to report stool frequency (stools/day) and consistency (scored as normal, semiformed or watery). A relapse of CDAD was declared if the patient reported looser stools compared with the day before (eg, change from normal to semiformed) and an increase in stool frequency for two consecutive days, or a single day with an increase of ⩾3 stools, or any day with passage of >6 stools/day.

Tolerability and safety were monitored by self-report diaries, laboratory monitoring and active surveillance by interview, and has been reported elsewhere.[9]

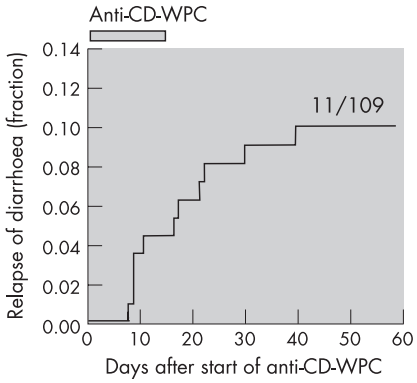

Figure 1 Kaplan–Meier plot showing the fraction of *Clostridium difficile*-associated diarrhoea (CDAD) episodes resulting in a relapse of CDAD during and after receiving anti-*C difficile* whey protein concentrate (anti-CD-WPC). The immune whey preparation anti-CD-WPC was taken for 2 weeks (indicated in graph), after completion of at least 10 days of standard antibiotic therapy.

We enrolled 101 patients (51 female; median age 74 years) with a total of 109 CDAD episodes (table 1). Most patients had underlying conditions that made them susceptible to acquiring CDAD (ie, older age cohort, antibiotic usage, surgery, stay in the intensive care unit, immunosuppressive medication).[1–4] Eight patients did not complete anti-CD-WPC (1 died because of underlying disease, 4 stopped because of taste dislike, 1 relapsed and in 2 the attending doctor made a decision of no treatment). Of those who completed the course, five patients died within the 60-day follow-up because of underlying disease. Seven received anti-CD-WPC twice and one received it thrice.

In all, 11 (~10%) of 109 episodes were followed by a relapse of CDAD (table 1, fig 1). Faecal toxin assay and *C difficile* culture were positive in all cases. Patients with severe underlying disease were more likely (relative risk (RR) 11.7; 95% CI 1.5 to 96) to relapse than those without underlying condition, as were patients with *C difficile* PCR-ribotype 027 (RR 2.2, 95% CI 0.6 to 8.2).

The findings indicate that anti-CD-WPC, an immune whey protein concentrate directed against *C difficile* and its toxins, may aid the prevention of relapse in CDAD. This is based on the 10% relapse rate in those given anti-CD-WPC, compared with a 20–25% relapse rate in contemporary controls in the Dutch epidemic and published relapse rates from 20% to 47% in *C difficile* type O27 epidemics.[3–5] The effect of anti-CD-WPC is probably mediated by sIgA antibodies, and this has important advantages over repeated use of antibiotics, because of its suppression of resident microbial bowel flora and potential for inducing antibiotic resistance. In some relapsing subjects, the amount of anti-CD-WPC appeared insufficient to neutralise the *C difficile* toxins in the faeces, and there may

Table 1 Characteristics of 101 patients with *Clostridium difficile*-associated diarrhoea (CDAD; 109 episodes), who had or did not have relapse of CDAD after 2 weeks of the immune whey anti-*C difficile* whey protein concentrate given after standard antibiotic treatment with oral metronidazole or vancomycin

Characteristic	Relapse	No relapse	p Value
Sex (n)			>0.30
Male	5	49	
Female	6	49	
Age (median and IQR; years)*	76 (9)	70 (27)	>0.20
Chronic Health Scoring System†			<0.01
Score 0	1	46	
Score 2	0	8	
Score 5	10	44	
Episode of CDAD			>0.20
First	6	59	
Second	4	23	
⩾Third	1	16	
C difficile PCR-ribotype O27			>0.20
Yes	4	20	
No	7	75	
Not determined	0	3	
Antibiotic treatment of last episode			>0.30
Vancomycin	8	50	
Metronidazole	3	45	
In combination	0	3	

CDAD, *Clostridium difficile*-associated diarrhoea.
*IQR, interquartile range.
†Chronic Health Score in Acute Physiology, Age and Chronic Health Evaluation (APACHE) prognostic scoring system: angina pectoris New York Heart Association (NYHA) Class III–IV (n = 21); biopsy-proven cirrhosis and documented portal hypertension (n = 9); chronic obstructive pulmonary disease resulting in exercise restriction (n = 28); renal insufficiency necessitating (chronic) dialysis (n = 6); and immunocompromised status—for example, high-dose steroids, chemotherapy or an underlying haematological disease like chronic lymphatic leukaemia (n = 31), according to Knaus.[10]

be room for improvement by raising the dose. The findings merit clinical development of anti-CD-WPC, and should be confirmed in a prospective placebo-controlled randomised trial.

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doi: 10.1136/gut.2006.119016

Funding: This work was funded on a per-patient basis by MucoVax bv., Leiden, The Netherlands.

Competing interests: None.

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Is gliadin really safe for non-coeliac individuals? Production of interleukin 15 in biopsy culture from non-coeliac individuals challenged with gliadin peptides

Nowadays it is assumed that an innate immunity to gluten plays a key role in the development of coeliac disease (CD).[1] This innate response, mediated by interleukin (IL) 15 and elicited by ''toxic peptides'', like the 19-mer, through a DQ2-independent mechanism, induces epithelial stress and reprogrammes intraepithelial lymphocytes into natural killer (NK)-like cells[2] leading to enterocyte apoptosis and an increase in epithelium permeability. Thus, immunodominant peptides, like the 33-mer, can reach the lamina propria to trigger adaptive immunity. However, although an innate specific response in CD has been reported,[3] no differential factors between patients with and without CD have been described controlling the innate immune response. Thus, since the toxic 19-mer elicits its harmful effect through a DQ2-independent mechanism, we hypothesise that the innate response is common in patients with and without CD, whereas the adaptive response is exclusive of susceptible patients with CD.

Table 1 Gliadin-challenged patients without coeliac disease

Patient no	Duodenal mucosa	Age (years)	Symptoms	Diagnosis
1	Normal	51	Unfiliated ferropenia	Undiagnosed (non-coeliac)
2	Normal	63	Pyrosis	Hiatus hernia, GORD
3	Chronic inflammation	46	Colic abdominal pain	Chronic gastritis
4	Chronic inflammation	54	Epigastric pain	Chronic gastritis
5	Normal	16	Diarrhoea	Protracted diarrhoea
6	Normal	71	Dyspepsia	Polyps in stomach, Helicobacter

GORD, gastroesophageal reflux disease.
The final diagnosis of the patients was non-coeliac in all the cases. Those with chronic inflammation had normal villi as well as no intraepithelial lymphocytosis. All the patients were biopsy cultured in basal medium and challenged with both the 19- and the 33-mer gliadin peptides. Patients 1–3 were also challenged with gliadin.

To test the hypothesis, biopsy cultures were taken from at least three patients with CD who are on a gluten-free diet (GFD) and three patients without CD (table 1). Biopsy specimens were challenged with crude gliadin and the gliadin synthetic 19-mer and deaminated 33-mer peptides after discarding the presence of lipopolysaccharide in all the cases. This was carried out at 100 μg/ml for only 3 h to imitate what are considered the normal timing and concentration in the gut after a normal meal. All biopsy specimens were then washed and cultured for another 21 h in new clean culture medium to determine whether an innate stimulus is reflected by an adaptive response. Each sample cultured in basal medium constituted an internal control. Innate immune mediators IL15 and nitrites were determined by western blot in the biopsy protein extract and by a Griess reagent system in the 3 h supernatants respectively. mRNA levels of adaptive immunity mediators like signal transducers and activators of transcription (STAT) 1, STAT3, tumour necrosis factor α, interferon (IFN) γ, IL23 (p19), IL27 (p28) and IL12 (p35) were determined by real-time polymerase chain reaction using β actine levels as housekeeping.

All patients with and without CD on GFD who were challenged with the gliadin solution produced IL15 when compared with the basal culture (fig 1A). Moreover, the IL15-mediated response in patients without CD was also triggered by the toxic 19-mer gliadin peptide (three of six) and, especially, by the 33-mer gliadin peptide (five of six). Importantly, none of the basal cultures produced this cytokine and, although not expected, the ''non-toxic'' immunodominant 33-mer was also able to induce an innate response. Interestingly, this IL15 response was also confirmed by western blot in the supernantant of one GFD patient with CD and three patients without CD, who were on GFD (fig 1B), therefore, discarding an intracellular and non-biologically active IL15 response in patients without CD. We also found an increase in nitrite in the gliadin-challenged patients with CD who were on a GFD, although not in patients without CD. In a similar way, as expected, after the biopsy mRNA isolation, adaptive mediators (STAT1, STAT3, IFNγ) were only modified in GFD patients with CD. Finally, basal GFD-CD samples showed an 80-fold increase in IFNγ mRNA levels compared with non-CD basal samples (p value 0.002) and a slightly higher production of nitrites (p value 0.052).

We consider that, to our knowledge, this is the first time that an IL15-mediated innate response to gliadin and gliadin peptides is described in individuals without CD, as well as an IL15-mediated innate response to the ''non-toxic'' deaminated immunodominant 33-mer peptide.

The data obtained in this pilot study support the hypothesis that gluten elicits its harmful effect, throughout an IL15 innate immune response, on all the individuals. This innate response is found in both patients with and without CD, although the triggering of an adaptive response is CD specific. We propose that somehow patients with CD need to be DQ2 and also have a lower threshold for triggering an adaptive TH1 response. This lower threshold could be mediated by the higher basal levels of immune mediators, like IFNγ mRNA, found in patients with CD, a defect in the CD permeability or even a higher IL15-sensitive response under the same stimulus, which might be mediated by a higher density of IL15 receptor in patients with CD.[4]

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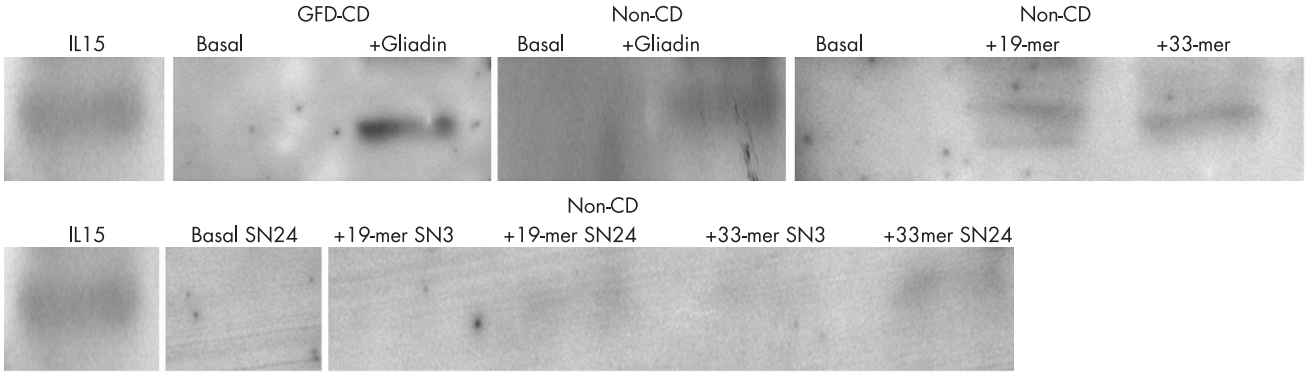

Figure 1 Interleukin (IL) 15 production after gluten challenge. Representative IL15 western blot of (A) whole biopsy protein extract of a responding patient with coeliac disease (CD) on gluten-free diet (GFD) challenged with gliadin compared with basal culture, and two patients without CD challenged with gliadin and the synthetic gliadin peptides 19-mer and deaminated 33-mer, respectively, compared with the basal culture. Interestingly, none of the basal cultures produced this cytokine. (B) 24 h culture supernatant (SN) of a patient without CD basal culture and the 3 and 24 h culture supernatants of the same patient after challenge with the 19-mer and deaminated 33-mer, respectively. IL15, positive control lane.

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SR and LF-S are the gastroenterologists who performed the follow-up of the patients as well as provided the duodenal biopsy samples. DB performed the biopsy cultures and the western blot analysis, and JAG performed the qPCR and statistical analyses. The study design was carried out by EA.

doi: 10.1136/gut.2006.118265

Funding: This work has been partially funded by the Spanish Ministry of Education (FPU, AP2002-2696), the Spanish Ministry of Health (FIS, PI020895; 02/3068), Junta de Castilla y Leon (SAN1052-VA02/05 VA057/04), Phadia (Sweden Diagnostics affiliated to Pharmacia Diagnostics) and the Coeliac Disease Association of Madrid (Spain).

Competing interests: None.

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The value of endothelin 1 in the early diagnosis of severe intestinal strangulation

In patients with intestinal strangulation, early diagnosis and prompt intervention can play a key role in the final outcome. Physical signs and routine laboratory findings only raise the level of suspicion to the presence of intestinal ischaemia. Several markers such as alkaline phosphtase,[1] creatinine phosphokinase,[2] and lactate dehydrogenase[3] and procalcitonin (Pct) serum levels have been proposed for the early diagnosis of intestinal ischaemia.[4]

Plasma levels of endothelin 1 (ET1) have been recently associated with cardiac ischaemia.[5] It has also been reported that ET1 plays a central role in the pathophysiology of intestinal ischaemic injury.[6] Wang *et al*[7] recently reported that ligation of mesenteric vessels caused an increase in serum ET1 levels in rats. On the other hand, other researchers reported that in spite of increased release of ET1 from the strangulated loop, no statistically significant increase of ET1 in peripheral blood samples can be established.[8] These controversial results may be attributed to the occlusion of the local venous vessels.

In an attempt to further study these controversial data, we performed concomitant ligation of the arterial and venous vessels that correspond to the occluded loop. It is also noted that this approach may better reflect the sequence of events that follow the onset of severe strangulation.

In 11 New Zealand rabbits, the intestinal lumen and the corresponding mesenteric vessels were occluded using silk sutures, one at 30 cm from the duodenum and one 15 cm distal to the first ligation. This model reproduces the phenomenon of the obstruction of intestinal wall perfusion due to severe strangulation and the pathophysiological processes at the onset of ischaemia that complicates the natural history of the disease. For the six controls, the steps of the operation were exactly the same, except the strangulation of the jejunum was not performed.

Blood samples were drawn preoperatively and consequently at 30, 60, 180 and 360 min after strangulation. At the same time intervals, a transmural biopsy specimen was obtained from the strangulated loop. Tissue samples were embedded in paraffin wax and submitted for H&E staining. Ischaemic injury of the intestine was scaled in four degrees of severity.[9]

The plasma concentration of ET1 was estimated with a commercial immunoassay kit (Assay Designs, Ann Arbor, Michigan, USA).

The comparison of ET1 values between the two groups revealed higher concentrations in the ischaemia group, at every time interval, which were statistically significant for all postoperative samples ($p<0.05$; fig 1). ET1 levels in plasma increased as early as 30 min after intestinal strangulation, and continued to increase with time up to 10-fold in the next 360 min. By contrast, in the control group no statistically significant alteration of ET1 levels, within the measured samples, was observed.

According to the analysis of histopathological grading of ischaemic injury, the ischaemia group samples were graded as follows: at 30 min, 2 samples of grade I; at 60 min, 8 of grade II and 2 of grade I; at 180 min, 3 of grade II and 8 of grade III; and, finally, at 360 min 1 of grade I and 10 of grade IV. No evidence of ischaemic injury was detected in specimens derived from the control group. ET1 plasma levels correlated with histopathological damage. Mild increase in ET1 levels reflected mild, mainly mucosal intestinal ischaemic injury graded in this study as grade I and II, whereas a twofold or higher increase in circulating ET1 is consistent with extensive epithelial injury affecting the mucosa and the submucosa, and the muscle layers of the intestinal wall classified as grade III and IV.

It is evident that plasma levels ET1 rise at 30 min of strangulation before any histological

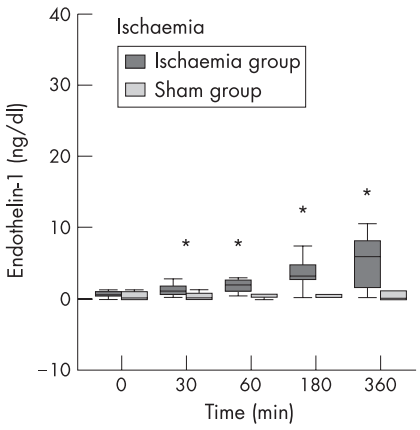

Figure 1 Variation of endothelin 1 levels in peripheral blood samples (*$p<0.05$).

damage is observed. This makes ET1 an additional useful marker for severe intestinal strangulation than other hitherto reported markers including procalcitonin.[4] According to these results, measurement of plasma ET1 in a clinical setting is justified to further evaluate its value in the early diagnosis of intestinal ischaemia due to strangulation.

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doi: 10.1136/gut.2006.118448

Competing interests: None.

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The RANTES −28 g polymorphism is associated with primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic cholestatic disorder commonly associated with inflammatory bowel disease (IBD). The pathogenesis of PSC remains unknown, but bacterial translocation from the gut to the portal circulation, resulting in activation of an immune cascade and subsequent bile duct injury, is believed to be one of the triggering factors. In a previous study,[1] we found a significantly lower frequency of the Δ32 deletion in the CC-type chemokine receptor 5 (CCR5) gene in patients with PSC compared with patients with IBD and healthy controls (HC), suggesting a protective effect of this mutation on the development of PSC. Given this association in our population, we further investigated the role of RANTES (*Regulated on Activation Normal T Expressed and Secreted*, also known as CC-motif chemokine ligand 5 (CCL5)), one of the ligands for the CCR5 receptor.

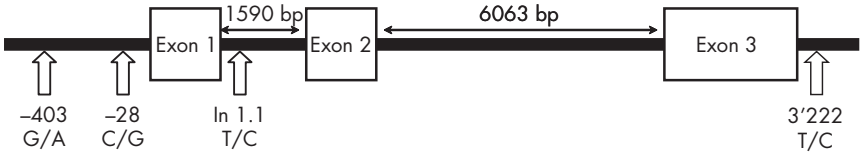

Figure 1 The RANTES (*Regulated on Activation Normal T Expressed and Secreted*) gene and the position of the different studied polymorphisms.

RANTES, localised to the cytokine cluster on chromosome 17q11.2–q12, is a plausible candidate gene for PSC, given its proinflammatory properties both independent of and as a ligand for the CCR5 receptor. A higher expression of RANTES has been reported in the gut epithelium and lymphocytes of patients with IBD.[2] Moreover, in an animal model of colitis, treatment with RANTES antagonists resulted in decreased bacterial translocation, as measured by portal blood endotoxin levels.[3]

Two promotor polymorphisms in RANTES (−28G and −403A) result in increased transcription of the gene, whereas a single-nucleotide polymorphism (SNP) in the first intron (In1.1C) results in reduced transcription of RANTES.[4] We studied these three SNPs together with a fourth known SNP of as yet unknown function in the 3′UTR (3′222), for their role in susceptibility to PSC, and behaviour and progression of PSC (fig 1).

We genotyped 124 patients with PSC (63 without IBD, 25 with Crohn's disease (CD), 31 with ulcerative colitis (UC) and 5 with indeterminate colitis (IC)) for the −403G/A, −28C/G, In1.1T.C and 3′222T/C variants in the RANTES gene. Patients with PSC and IBD were recruited from the hepatology and gastroenterology departments of the same tertiary care referral centre. Table 1 summarises and compares the disease characteristics of IBD in patients with and without PSC. Genotype and allele frequencies were compared with 595 patients with IBD without PSC (418 CD, 160 UC, 17 IC), and with 362 HC. SPSS V.14.0 software was used for statistical analysis, Haploview for calculation of linkage disequilibrium (LD)[5] and fastPHASE 1.1.4 for haplotype estimation.[6]

Allele frequencies for the studied polymorphisms are summarised in table 2. All studied polymorphisms were in Hardy–Weinberg equilibrium. The −28G allele was significantly more frequent in patients with PSC (5.9%) compared with those with IBD (3.4%, $p = 0.05$) and HC (2.2%, $p = 0.005$). Furthermore, in patients with CD, the −28G allele was significantly more frequent in those patients who developed PSC (10.4%) compared with those who did not develop PSC (3%, $p = 0.005$), with an odds ratio (OR) of 4.16 (95% CI 1.4 to 12). The allele frequency for the RANTES 3′222 allele was <1% in our cohort. The –403, –28 and in1.1 SNPs were all in strong LD (D′ 0.838 for –403/–28, 0.946 for –403/in1.1 and 0.78 for –28/in1.1). Haplotype analysis could not identify differences between the studied groups. There was no association between any of the studied RANTES SNPs and location and behaviour of IBD or PSC in the different subgroups, nor with the CCR5Δ32 mutation.

In conclusion, this is the first report of an association between a functional promotor polymorphism in the RANTES gene, with an OR of 4.16 for the development of PSC in patients with CD. This –28G promotor polymorphism is known to result in increased RANTES transcription and expression. Given its known proinflammatory properties, and taking the data on the action of RANTES antagonists in experimental colitis into account,[3] a possible mechanism to explain the

Table 1 Characteristics of patients with IBD and of patients with PSC with concomitant IBD

	Crohn's disease		Ulcerative colitis	
	CD with PSC	CD without PSC	UC with PSC	UC without PSC
	n = 25 (%)	n = 418 (%)	n = 31 (%)	n = 160 (%)
Localisation of CD				
Ileal	1/25 (4)	107/396 (27)	—	—
Colonic	8/25 (32)	65/396 (16)		
Ileocolonic	15/25 (60)	177/396 (45)		
Upper	1/25 (4)	47/396 (12)		
Anal involvement	8/25 (35)	132/392 (34)		
Behaviour of CD				
Inflammatory	16/25 (64)	144/386 (37)	—	—
Stricturing	3/25 (12)	86/386 (22)		
Penetrating	6/25 (24)	156/386 (40)		
Localisation of UC				
Proctitis	—	—	3/31 (10)	33/146 (23)
Left-sided colitis			10/31 (32)	66/146 (45)
Extensive colitis			18/31 (58)	47/146 (32)

CD, Crohn's disease; PSC, primary sclerosing cholangitis; UC, ulcerative colitis.

Table 2 Allele frequencies for the −403, −28, in1.1 and 3′222 RANTES polymorphism in primary sclerosing cholangitis, inflammatory bowel disease and healthy controls

	PSC total n = 124 (%)	PSC without IBD n = 63 (%)	PSC CD n = 25 (%)	PSC UC n = 31 (%)	IBD total n = 595 (%)	CD without PSC n = 418 (%)	UC without PSC n = 160 v(%)	HC n = 362 (%)
RANTES-403A	18.1	14.9	25.0	20.0	18.6	19.1	17.9	19.7
RANTES-28G	5.9	3.4	10.4	6.5	3.3	3.0	4.2	2.2
RANTES in1.1C	15.3	12.9	22.9	14.5	13.8	15.0	10.6	14.6
RANTES 3′222C	0.0	0.0	0.0	0.0	0.7	0.9	0.3	0.0

CD, Crohn's disease; IBD, inflammatory bowel disease; PSC, primary sclerosing cholangitis; RANTES, Regulated on Activation Normal T Expressed and Secreted; UC, ulcerative colitis.

association between the RANTES −28G allele and PSC could be that an increased inflammatory response in the gut predisposes to bacterial translocation through the bowel wall.

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doi: 10.1136/gut.2006.115329

Competing interests: None.

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Increased risk of colorectal neoplasia in asymptomatic liver-transplant recipients

Liver-transplant recipients (LTRs) are known to carry an increased risk of cancer, including tumours of the skin and lymphoproliferative disorders.[1] Whether the risk of colorectal cancer (CRC) is increased after orthotopic liver transplantation (OLT) is controversial.[2] There is also no consensus whether screening for colorectal neoplasia in asymptomatic LTRs is warranted, except for those with primary sclerosing cholangitis (PSC) with associated ulcerative colitis (UC).[2] In a previous study, we found an increased risk of CRC in patients after OLT compared with the general population.[3] In this report, we examined the prevalence of colorectal adenomas and carcinomas in asymptomatic LTRs.

Between 1979 and 2001, 381 adult patients underwent OLT at the University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. For this retrospective study, all patients were included in which a colonoscopy had been performed for screening purposes, at least 5 years after OLT. Patients with symptoms suggestive of colorectal neoplasia and patients with previous colorectal neoplasia were excluded. Only complete colonoscopy reports including caecal inspection were included. Data on the presence, location and number of colorectal neoplastic lesions were collected. Patients with more than one lesion were categorised according to the most advanced lesion. All pathological specimens were reviewed by the study pathologist for assessment of the degree of dysplasia and the presence of villous architecture according to the World Health Organization criteria.[4] Advanced neoplasia was defined as an adenoma of at least 1 cm in size, and/or (tubulo) villous architecture, and/or high-grade dysplasia, or cancer, in accordance with the literature.

In all, 92 asymptomatic patients, including 20 with PSC, had undergone colonoscopy at a median (range) age of 53.5 (35.8–71.2) years, after a median (range) interval of 11.1 (5.0–23.2) years after OLT. One or more adenomas were found in 20 of 92 (21.7%) patients (9 men, 11 women). Of these 20 patients, 4 had PSC, including 1 with concomitant UC. In 9 of 20 patients, one or more adenomas were located in the proximal colon. Advanced adenomas were found in 8 of 92 (8.7%) patients. Cancer was found in none of the patients.

These prevalence data in patients with OLT were compared with six asymptomatic cohorts from the literature (table 1). Although the prevalence of all neoplasia was comparable with other cohorts, advanced neoplasia was more often found in our population than in most studies. In addition, the relative proportion of advanced neoplasia, expressed as a ratio of all neoplasia, was 0.40 in our study, which is higher than in any other report. As the prevalence of colorectal neoplasia increases with age, data from our population were compared with data from the literature stratified in three groups (<50, 50–59 and >60 years). For patients aged <50 years and 50–59 years, the prevalence of advanced neoplasia was higher in our study population than in any other study (table 1). Our data are probably best compared with the only other available Dutch study that was performed in a relatively young asymptomatic population.[5] In patients aged <50 years, the relative risk in the population which underwent OLT was 3.6 ($p<0.05$) for overall neoplasia and 8.9 ($p<0.01$) for advanced neoplasia. Taken together, our data suggest that LTRs have a higher risk of colorectal neoplasia, particularly advanced neoplasia, compared with average-risk populations. The absence of a gender- and age-matched control group limits the strength of this retrospective study. We suggest designing larger and prospective studies including adequate control groups to better assess the risk of colorectal neoplasia in LTRs.

Currently, CRC screening is recommended in many countries worldwide for all patients aged ⩾50 years. It was recently recommended that for the asymptomatic liver transplant population, CRC screening should be offered to those aged >50 years and those with PSC associated UC.[2] The results from our preliminary study suggest that CRC screening could be considered in all adult LTRs.

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doi: 10.1136/gut.2007.120121

Competing interests: None.

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Table 1 Prevalence of colorectal neoplasia in liver-transplant recipients, compared with selected studies from the literature in average-risk populations

	LTR	de Jong *et al*[5]	Strul *et al*[6]	Imperiale *et al*[7]	Soon *et al*[8]	Lieberman *et al*[9]	Schoenfeld *et al*[10]
Number of patients	92	342	1177	906	4859	3121	1463
All neoplasia (%)	21.7	6.1	20.9	11.0	18.9	37.5	20.4
Advanced neoplasia (%)	8.7	2.0	6.3	3.5	4.6	10.5	4.9
Ratio advanced/all neoplasia	0.40	0.33	0.30	0.32	0.24	0.28	0.24
Advanced neoplasia (%)							
<50 years	8.6	1.0	1.0	3.5	3.0	—	—
50–59 years	12.9	8.3	2.9	—	3.8	5.7	3.3

LTR, liver transplant recipients.

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Visceral leishmaniasis causes fever and decompensation in patients with cirrhosis

Infections and fever due to bacterial infections are well-known complications of liver cirrhosis, and often trigger decompensation and death.[1] Visceral leishmaniasis (VL), a protozoan infection endemic in the Mediterranean area, causes a febrile disease, the clinical and laboratory features (splenomegaly, pancytopenia, reduced serum albumin and increased γ-globulin concentrations) of which largely overlap with those of cirrhosis.[2 3] Although the areas where VL is endemic coincide with areas where the prevalence of cirrhosis is high, no study has described VL in patients with cirrhosis.

During a 12-year period, all patients with cirrhosis admitted for decompensation and fever lasting more than 1 week, unresponsive to broad-spectrum antibiotics, underwent diagnostic procedures for VL. For each case diagnosed as VL, three consecutive cases of bacterial infection observed in the same period were enrolled. Cirrhosis was defined by liver histology or clinical, laboratory, ultrasonographic and endoscopic findings. VL was diagnosed by the immunofluorescent-specific antibody test (IFAT) and confirmed by the identification of *Leishmania* from Giemsa-stained smears of bone marrow or spleen aspirates.[4] Bacterial infections were diagnosed by standard clinical procedures. All patients were followed up for 6 months after diagnosis.

In total, 11 patients with VL and 33 with bacterial infections were enrolled. Table 1 summarises the main clinical and laboratory data at presentation.

Patients with VL received standard-dose liposomal-amphotericin B (l-AmB; nine cases)[5] or meglumine antimoniate (two cases). Fever resolved within 72 h in patients receiving l-AmB and after 7 days in those receiving antimonials. No severe drug-related adverse reaction was recorded. Improvement in haematological parameters and reduction in liver enzymes, spleen diameter and anti-*Leishmania* antibody titre were recorded during the follow-up period in all patients with VL. The Child–Pugh class rapidly and stably improved in patients with VL (fig 1). The mortality was 17% for patients with bacterial infections and 0% for patients with VL.

VL was a troublesome diagnosis in patients with cirrhosis, as shown by the time from the onset of symptoms and diagnosis. This delay resulted from failure to consider this unusual diagnosis and was responsible for a progressive worsening in liver function. Indeed, once VL was cured, cirrhosis reverted to Child class A in all but one patient.

Apart from a long-lasting fever, there were no clear-cut clues to suspect VL. Some significant differences in the presentation data between VL and bacterial infections were found (table 1), but their clinical use is hampered by a substantial overlap of the laboratory values between VL and bacterial infection cases. Only white blood cell and γ-globulin data are of potential value.

IFAT serology is a sensitive and specific test for the diagnosis of VL in patients with cirrhosis; notably, IFAT was negative in all patients with bacterial infections and in 51 additional patients with cirrhosis without fever (table 1). In a series including 16 patients with cirrhosis, a strip test using *Leishmania* K39 antigen was sensitive and specific.[6] The diagnostic flow-chart for patients with cirrhosis with unexplained fever should include the anti-VL antibody test, thus avoiding the routine use of invasive diagnostic techniques. Once the diagnosis is established, l-AmB is a safe and effective treatment.

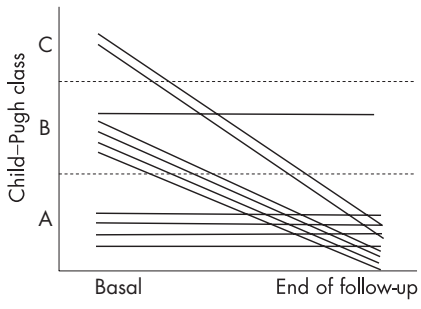

Figure 1 Child–Pugh class before and after treatment for visceral leishmaniasis.

The study was not designed to assess whether the patients with cirrhosis are at a higher risk of acquiring VL than those without cirrhosis. Assuming that there are 10 000–20 000 patients with cirrhosis per year in our area, the annual incidence of VL among patients with cirrhosis was 0.5–1/10 000—that is, 8–17-fold higher than the incidence (0.06/10 000) among the adult population in the same area ($p<0.001$).[7–9]

Overall, the study underlines the need to consider this unusual cause of fever and decompensation in patients with cirrhosis when they are living in or have travelled through an area endemic with VL.

Acknowledgements

The study is part of a research project supported by Ministero Università e Ricerca Scientifica e Tecnologica (MURST), Rome, Italy.

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doi: 10.1136/gut.2007.119495

Table 1 Main demographic, clinical and laboratory features at admission in patients with cirrhosis with visceral leishmaniasis or bacterial infections

Parameter	VL cases (n = 11)	Bacterial infections (n = 33)	p Value
Age (years)	60 (51 to 62)	67 (64 to 71)	0.002
Time from onset of symptoms (days)	60 (47 to 120)	4 (2.5 to 5)	<0.001
Fever (˚C)	38.5 (38 to 39)	37.2 (36.5 to 37.8)	0.004
Spleen size* (cm)	17 (15 to 19)	15 (13.8 to 16.6)	0.04
Child to Pugh C (%)	2 (18)	6 (18)	NS
Child to Pugh B (%)	5 (45)	14 (42)	NS
Creatinine (mg/dl)	0.9 (0.85 to 1.05)	0.89 (0.70 to 1.2)	NS
AST (UI/l)	100 (35.5 to 183.5)	65 (39 to 120)	NS
Bilirubin (mg/dl)	1.2 (0.84 to 2.01)	2.49 (1.57 to 3.58)	0.004
Haemoglobin (g/dl)	10.3 (9.2 to 10.9)	12.4 (10.8 to 13.6)	0.003
White blood cells ($\times 10^3$)	2.9 (2.10 to 3.45	5.8 (4.2 to 8.0)	0.001
Platelets ($\times 10^3$)	88 (80 to 96.5)	78 (56 to 105)	NS
γ-Globulin concentration (g/dl)	3.5 (3.2 to 4.4)	1.93 (1.61 to 2.52)	<0.001
Erythrocyte sedimentation rate (mm/h)	105 (94 to 110)	28 (20 to 49)	0.005
Number positive for anti-Leishmania antibodies†	11	0.00	<0.001

Quantitative data are expressed as median (interquartile range) and compared using the Mann to Whitney U test. Fisher's exact test was used to compare qualitative variables.
*As measured by ultrasonographic examination.
†Anti-*Leishmania* antibodies were negative in 51 additional consecutive patients with cirrhosis without infections and γ-globulins above 2.5 g/dl.

Competing interests: None.

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Magnifying videoendoscopic findings of Peyer's patches in the terminal ileum of Crohn's disease

It has been reported that Crohn's disease initially occurs as tiny aphthoid lesions at the sites of lymphoid follicles in the gastrointestinal tract.[1–3] The follicle-associated epithelium (FAE) of the gut-associated lymphoid tissues such as Peyer's patches (PPs)[3 4] is a single layer of epithelial cells covering each follicle and forms a dome between the surrounding villi.[3 4] Endoscopic observation of PPs in patients with Crohn's disease has rarely been performed in clinical settings.[1–3 5 6]

A total of seven patients with active Crohn's disease and 19 age- and sex-matched healthy controls were enrolled. Chromoendoscopy was carried out with crystal violet and/or indigo carmine to identify PPs. The FAE on the domes of PPs was examined by magnifying endoscopy. The macroscopic appearance of PPs was classified into two categories, a nodular or convolute elevation pattern (E type, fig 1A) and a flat pattern (F type, fig 1B), corresponding to lymphoid follicle and lymphocyte aggregation types, respectively, as described by Fujikura *et al*.[7] E-type PPs are associated with definite lymphoid follicles and abundant lymphoid hyperplasia, whereas F-type PPs consist of aggregated lymphocytes and reticulum cells, which were loosely mixed together.[7] Two endoscopic biopsy specimens taken from the domes of PPs were subjected to histopathological analysis and scanning electron microscopy.[3] All patients gave their written informed consent after approval by the university ethics committee.

The seven patients with Crohn's disease (four men and three women) had a mean age of 26 years (range 16–42 years). Six patients had minimal lesions including small ulcers and erosions within the PPs. There were 11 E-type and 8 F-type PPs in the control group, whereas all the PPs in patients with Crohn's disease were classified as F type. On magnifying chromoscopy, most of the domes within PPs in controls rose into a little mound and were surrounded by dense villi (fig 2A). However, flat, distorted domes, surrounded by scattered villi (fig 2B), were identified in six patients with Crohn's disease. Repeat endoscopy in remission stage after total parental nutrition or enteral feeding with an elemental diet showed that the irregularity of domes improved in four of the six patients with Crohn's disease, accompanied by densely surrounding villi, albeit the appearance of the PPs remained of E type. A non-caseous epithelioid granuloma was histopathologically seen in six of seven patients. On electron microscopy, M cells were seen on the domes in all cases.

PPs of Crohn's disease were exclusively categorised as F type, whereas E type was predominant in the age- and sex-matched controls. On observation by magnifying endoscopy, most patients with Crohn's disease had irregularly even domes surrounded by sparse villi. Initial lesions of Crohn's disease are postulated to be aphthous erosions of the domes in PPs,[6] and we identified some erosions on the domes' FAE. Taken together, the F-type PPs in Crohn's disease can reflect the irregularly affected domes with few covering villi. Thus, the FAE of patients with active Crohn's

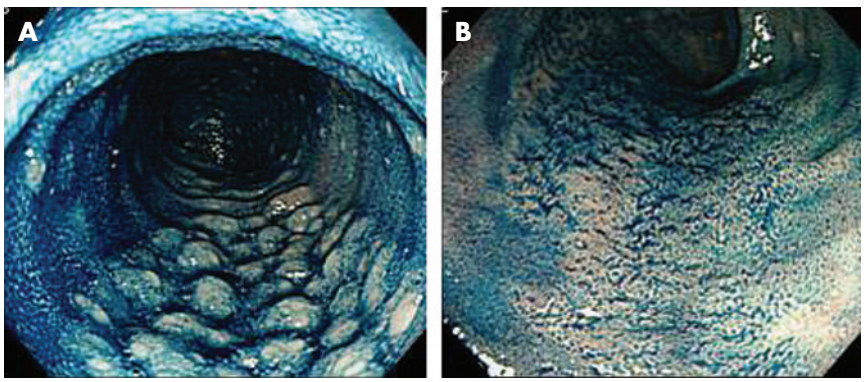

Figure 1 Chromoendoscopic view with a magnifying videocolonoscope (Olympus CF-240ZI, indigo carmine and crystal violet for (A) and (B), respectively). Nodular elevation of Peyer's patches (E type) and flat Peyer's patches (F type) for (A) and (B), respectively.

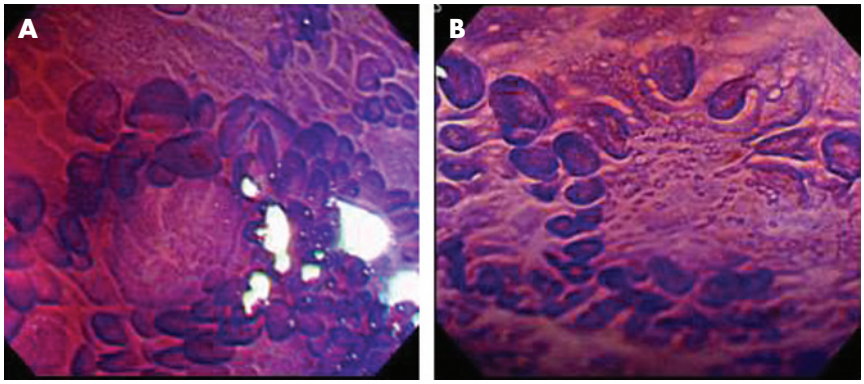

Figure 2 Magnifying chromoscopic view. (A) Domes in the Peyer's patches of a control subject, surrounded by dense villi. (B) Irregularly even domes in the Peyer's patches of a patient with Crohn's disease, surrounded by scattered villi.

disease is likely to be more exposed to the luminal antigens and to be in closer contact with the immune system.[4] Notably, the non-caseous epithelioid granuloma was frequently identified in the biopsy specimens taken from PPs. Such minimal Crohn's disease lesions may originate from PPs and may be related to gut-associated lymphoid tissues underlying the FAE. It has been postulated that M cells are related to the pathogenesis of Crohn's disease through their function as a portal of entry for potentially pathogenic agents.[4] In fact, M cells were detected exclusively in the FAE on electron microscopy. It is difficult to obtain M cells by conventional endoscopic biopsy, because they are located on the domes but not in the villi of PPs.[3 4] Therefore, magnifying endoscopy is useful to clearly recognise the FAE of the domes.

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Internationalisation of high-impact gastroenterology journals, 1970–2005

Recent decades have seen an increase in publications in English from the international community in basic science journals, as well as in general and specialty medical journals.[1–6] However, to date, no one has examined the international publishing trends in gastroenterology and hepatology journals. We examined the extent of internationalisation in this field with regard to high-quality research publications over the period 1970–2005. Additionally, earlier studies discussing internationalisation of biomedical literature did not deal with the impact of multinational collaborations (articles involving authors from two or more countries). Thus, our secondary aim was to describe changes in multinational research publications during this period.

We reviewed the three highest-ranked gastroenterology journals based on journal impact factor and total literature citations for 2005: *Gastroenterology*, *Hepatology* and *Gut*.[7] We collected data for every fifth year over the period 1970–2005. All issues (January through December) of the journals were retrospectively reviewed for each of the selected years, and all basic and clinical research articles involving original investigation were analysed. The nation of origin of each article was assigned based on the affiliation of its investigators. Additionally, articles were classified as national or international, with national articles defined as those published by authors from the country of publication for the journal (*Gastroenterology* and *Hepatology*—USA; *Gut*—UK); international articles were those published by authors from all other countries.

A total of 3769 research articles published in the two US-based journals and 1589 articles published in *Gut* were analysed. Figure 1A shows the proportion of national and international publications for the three journals collectively. The proportion of international articles in the journals increased significantly from 31.0% of all articles in 1970 to 67.6% in 2005. The countries most responsible for the increases in international publications were Germany (0.3% of all articles in 1970, rising to 9.9% in 2005), Japan (0.3 to 9.8%), France (0.7 to 6.1%) and Italy (0.3 to 5.3%). When journals were considered individually, the internationalisation of *Gut* was the most dramatic, with international articles representing 34.4% of all articles in 1970 and 83.4% in 2005.

A significant increase in the number of multinational collaborative publications occurred during the study period. For example, in 1970, collaborations comprised 0.7% of all research articles in *Gut*, compared with 6.8% in 1990 and 24.6% in 2005. Similar trends were seen in *Gastroenterology* and *Hepatology* (fig 1B).

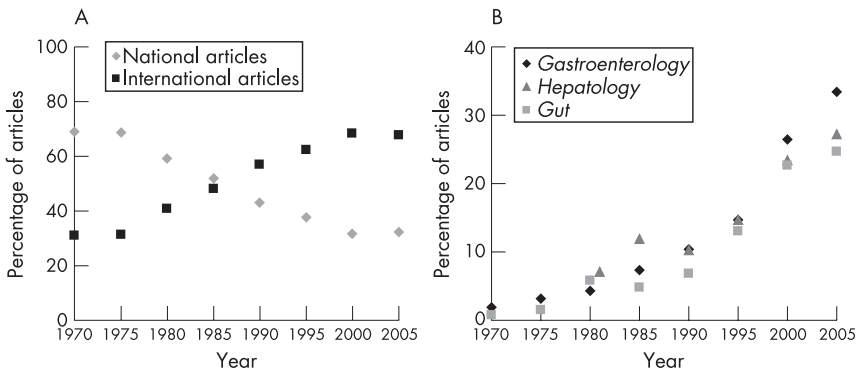

Figure 1 Proportion of (A) national and international research articles for the three journals collectively and of (B) multinational collaborative publications in the three top-rated gastrointestinal journals.

Our findings demonstrate the dramatic internationalisation of authorship of research articles in the leading gastroenterology journals since 1970. Additionally, they raise the question, ''What are the reasons for the decline in American and British dominance of gastrointestinal research?''

Potential explanations abound and are primarily speculative; however, editors of the American journals have described a definitive increase in international manuscript submissions and declining share from the US since 1995.[8–10] Possible causes for changes in the number of international submissions, and subsequent publications, include incentives to publish findings in prestigious English-language journals, increased English proficiency of international authors and the advent of electronic submissions. Although electronic submissions make it faster and easier for all authors to submit their work for publication, the impact on international authors is probably greater, helping them overcome barriers of time, distance and money. Furthermore, electronic submissions have made multinational collaborations much easier by providing the ability to incorporate investigators from multiple countries, which offers numerous advantages.

One major concern raised by our data is that the observed changes reflect a decline in American and British gastroenterology research. It is imperative that future studies be directed at identifying and correcting deficiencies hindering the growth of American and British gastroenterological science.

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doi: 10.1136/gut.2007.120899

Competing interests: None declared.

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