Gluten does not induce gastrointestinal symptoms in healthy volunteers: the British Broadcasting Company Experiment

To the Editor,

While the gluten-free diet (GFD) is the best treatment for clinical gluten sensitivity (GS) [(e.g. coeliac disease (CD), non-celiac gluten sensitivity (NCGS)], scientific opinion supports that gluten is safe for the general population [1]. However, celebrity/athletic endorsement of the GFD has cultivated an image of gluten as "unhealthy" [2].

"Lifestylers", "free from" or "people who avoid gluten" are individuals who avoid gluten as a lifestyle choice. American market research [3] found that 44% of people buy gluten-free food for reasons other than GS, and that 65% believe that a GFD is generally healthier. This trend has driven the worldwide gluten-free industry from values of $1.7bn in 2011 to $3.5bn in 2016, and it is forecast to reach $4.7bn in 2020 [4].

The surge in gluten-free popularity has also encouraged an opposing belief that it is a "fad" diet [2]. This is unfortunate for people with CD/NCGS who express that they are not taken seriously in restaurants, and even face dismissive attitudes from non-specialist clinicians [5]. The drawing of a clear line between those who do and do not benefit from a GFD is needed to ground public and clinical perspective on these issues. For this reason, in conjunction with the British Broadcasting Company (BBC) we undertook a televised experiment. Our hypothesis was that giving gluten to healthy volunteers (via gluten-containing flour) should not cause any symptoms.

Participants (who received no financial incentives), recruited by BBC advertising, were ≥18 years, had no diagnosed gluten-related disorders and followed gluten-containing diets. The experiment aimed to recruit 30 subjects to divide into two groups; no previous data in healthy individuals is available but NCGS Double Blind Randomised Controlled Trials (DBRCT) have reported gastrointestinal symptom changes induced by gluten which would carry 89.2% power if observed within a group of N=15 [6]. Participants were point of care tested (IgA/IgG-deamidated gliadin peptide) to detect CD. Prof. Sanders sought ethical approval from the Yorkshire and Humber REC and was advised that as this was a BBC experiment being conducted out with a hospital on healthy volunteers and that these individuals were not taking an investigational medicinal product (IMP) then ethical approval was not required. This also means that this experiment was not eligible for Registration as a Clinical Trial https://www.hra.nhs.uk/planning-and-improving-research/research-planning/research-registration-research-project-identifiers/. Nevertheless, in conjunction with the BBC the experiment was conducted with the principles of the declaration of Helsinki in mind. To this effect a BBC participant information sheet, consent form and protocol were devised.

Participants attended face to face sessions at a community venue. Participants were educated by a dietitian about a GFD and asked (with support) to commence a GFD for two weeks (Biagi score [7] measured GFD adherence). Subjects completed Gastrointestinal Symptom Rating Scales (GSRS) [8] to measure baseline abdominal pain, reflux, indigestion, diarrhoea and constipation. A visual analogue scale measured "Global fatigue".

Subjects were randomised by a team member (double-blinded, parallel, 1:1 allocation in an "A-B-A-B" sequence) into two groups who received flour sachets labelled "A" or "B", to add to their diet twice daily for two weeks while otherwise continuing their GFD. Flours (provided by Dutch Organic International Trade) contained either organic gluten (Gluten group; 2x10g vital gluten sachets, daily, providing 14g gluten protein and 1.4 g starch carbohydrates), or a gluten-free blend (Control group; rice, potato, tapioca, maize and buckwheat flour blend, 2x10g sachets daily). Finally, participants re-completed symptom measures and exited the experiment.

Variables were inspected for normality to determine appropriate analyses. Key variables were compared between randomised groups by frequency-based/groupwise testing to ensure baseline homogeneity. Primary/secondary outcomes examined change in symptom scores (follow-up minus baseline), compared between Gluten and Control groups

by independent t-test. The primary outcome was change abdominal pain score. Post-hoc analyses compared symptom scores within groups using paired t-tests.

Consecutive recruitment commenced in December 2015 and closed in January 2016. 45 people made contact before 30 were recruited. Reasons for not taking part included an unwillingness to commit to the dietary requirements/being unable to attend pre-specified experiment meeting dates. Point of Care CD testing excluded two from the experiment. The remaining 28 participants were randomised into the Gluten (N=14) and Control (N=14). There were no participants drop-outs, and no harms were reported.

The overall group had a mean age of 38 years (range=19-63, SD=12), and was 75% female (N=21). Biagi score (which measured GFD adherence while participants otherwise consumed the experiment flours) was not different between groups (independent t-test p=0.834), while X2 and independent t-tests/Mann-Whitney U showed no significant differences in any baseline characteristic (Table I).

Descriptively, mean symptom scores ubiquitously decreased in the Gluten group (implying symptomatic improvement). Individually, only one Gluten Group participant reported a worsening of some symptoms without improvement in others. Independent t-tests between randomised groups showed no significant differences in change of any symptom (abdominal pain: treatment/control mean (SD)=-0.36(1.95)/-0.29(1.49), p=0.914, partial ETA^2=0.000, 95% confidence interval=-1.42:1.28) (Table I).

Post-hoc paired t-tests examining change in GSRS scores within-groups showed that the diarrhoea score significantly reduced in the Gluten group (baseline/follow-up mean(SD)= 2.71(1.94)/1.64(0.92), p=0.033); this does not survive Bonferroni correction. No other analyses were significant.

This is the first experiment to demonstrate that the consumption of gluten-containing flour does not generate symptoms in healthy volunteers. We measured how daily ingestion of the flour (containing 14 g of gluten) affected a range of symptoms over two weeks, none of which significantly changed between groups. Within-group analyses similarly produced no significant findings, other than one indication that symptoms of diarrhoea improved in the Gluten group (likely anomalous and in any case does not support that the flour caused symptoms).

Our results support the view that gluten does not appear to cause symptoms in individuals who do not have a physiological susceptibility to it (i.e. the majority of the population). As the GFD is not only thought to be no healthier than a "normal" diet, but has been suggested as overall sub-optimal [1], there is possibly clinical justification in actively discouraging people from starting it if they have no diagnosable sensitivity.

A potential experiment limitation is the relatively short duration of 2 weeks. A similar study in NCGS indicated that onset of symptoms can begin after one week [6]. Another consideration is that the experiment topic may have unintentionally attracted participants with NCGS/IBS; however the presence of these would likely bias the experiment towards positive findings so confidence in the null results should remain high.

In conclusion, patients who self-report symptoms related to gluten must have CD and NCGS excluded, but on the basis of

Table I. Summaries of key variables and analyses

Variable	Gluten Group (N=14)	Control Group (N=14)	p	Partial ETA2 (95% CI)
Sex; % female	78.6	71.4	0.663	-
Age; mean (SD)	38.79 (11.64)	37.57 (13.32)	0.799	-
Baseline (top) & Follow-up Abdominal Pain GSRS; mean (SD)	2.50 (1.40) 2.14 (1.70)	2.36 (1.34) 2.07 (1.00)	0.721	-
Baseline (top) & Follow-up Reflux GSRS; mean (SD)	1.71 (1.14) 1.64 (1.15)	2.50 (2.24) 2.57 (1.95)	0.667	-
Baseline (top) & Follow-up Indigestion GSRS; mean (SD)	2.14 (1.35) 2.07 (1.33)	2.14 (1.35) 1.79 (0.98)	0.946	-
Baseline (top) & Follow-up Diarrhoea GSRS; mean (SD)	2.71 (1.94) 1.64 (0.92)	1.85 (1.46) 1.64 (1.22)	0.210	-
Baseline (top) & Follow-up Constipation GSRS; mean (SD)	2.50 (1.83) 2.36 (1.78)	1.93 (1.54) 2.50 (1.65)	0.454	-
Baseline (top) & Follow-up Global Fatigue; mean (SD)	6.64 (2.37) 6.64 (2.79)	6.57 (2.44) 5.57 (2.21)	0.839	-
Change in Abdominal Pain; mean(SD)	-0.36 (1.95)	-0.29 (1.49)	0.914	0.000 (-1.42 : 1.28)
Change in Reflux; mean (SD)	-0.07 (0.73)	+0.07 (1.98)	0.802	0.002 (-1.30 : 1.02)
Change in Indigestion; mean (SD)	-0.07 (1.69)	-0.36 (1.34)	0.623	0.009 (-0.90 : 1.47)
Change in Diarrhoea; mean(SD)	-1.07 (1.69)	-0.21 (0.89)	0.105	0.098 (-1.91 : 0.19)
Change in Constipation; mean(SD)	-0.14 (2.45)	+0.57 (1.51)	0.360	0.032 (-2.29 : -0.86)
Change in Fatigue; mean(SD)	0.00 (3.74)	-1.00 (3.60)	0.477	0.02 (-1.85 : 3.85)

Tests above the bold line show Mann-Whitney U/independent t-test/X^2 analyses which compare experiment groups for homogeneity on baseline characteristics. Primary and secondary analyses are shown below the bold line, which compare change in symptom scores between experiment groups using independent t-tests. Partial ETA^2 and 95% confidence intervals (CI) accompany these analyses to demonstrate effect size and precision.

this new data perhaps the assertion by 'lifestylers' that a GFD is beneficial can also be challenged.

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Utility of *Escherichia coli* Nissle in maintaining disease remission in inflammatory bowel disease

To the Editor,

I read with great interest in, and congratulate Bodini et al. [1] on their study entitled "Reduction of Fecal Calprotectin Levels Induced by a Short Course of *Escherichia Coli* Nissle is Associated with a Lower Likelihood of Disease Flares in Patients with Ulcerative Colitis in Clinical Remission". The authors reported a significant reduction in fecal calprotectin (FC) values with *Escherichia Coli* Nissle (EcN) treatment in inflammatory bowel disease (IBD) patients, which was also correlated to a reduced disease relapse rate in ulcerative colitis (UC) patients.

Escherichia coli Nissle has been investigated for over 25 years as a treatment modality in UC, and has been shown to be as effective as mesalazin therapy in maintaining remission in UC [2, 3]. Fecal calprotectin levels and their association with gut microbiota, both with EcN and pathogens, have revealed varying results [4, 5].

There are a few points in the study by Bodini et al. [1] in need of further explanation. In the abstract and methods section it is mentioned that the patients enrolled in the study were treated with EcN alone, but in the results section it is mentioned that the patients were supplemented with EcN. Also in Table I, there are reported to be no patients receiving concomitant steroids but in Table II, two patients with UC and four patients with Crohn's disease (CD) are reported to be on steroid therapy. If the patients were on EcN therapy alone during the course of study and concomitant medications were stopped, this would emphasize the strength of EcN therapy further. Conversely, taking concomitant medications with EcN therapy would raise the question whether the remission rates are solely attributable to EcN therapy or not. In this situation, a randomized study splitting patient population with and without concomitant therapy would yield better results.

In addition to the aforementioned questions, number of patients who relapsed are not given. Even considering the concomitant medications of the study population, there is a significant portion of patients taking no concomitant therapy. It would be interesting to compare the patient group with themselves in this particular scenario.

In conclusion, the article by Bodini et al. [1] contributes greatly on an important treatment modality which is often an overlooked therapeutic option. Monitoring this valuable study cohort to evaluate long-term results would be an intriguing and valuable addition to the literature.

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Reply,

To the Editor,

We appreciate the thoughtful comments and interest shown in our study titled "Reduction of Fecal Calprotectin Levels Induced by a Short Course of *Escherichia Coli* Nissle is Associated with a Lower Likelihood of Disease Flares in Patients with Ulcerative Colitis in Clinical Remission" [1]. We are grateful for the opportunity to address the points raised and clarify aspects of our study methodology and results. We agree on the significance of high specific biomarkers for monitoring disease activity in ulcerative colitis [2].

Regarding the use of *Escherichia Coli* Nissle (EcN) either as a sole treatment or as a supplement, we acknowledge an inconsistency in the terminology used throughout our manuscript. We included only those patients whose concomitant therapy was stable; we specifically excluded individuals who had modifications to their inflammatory bowel disease (IBD) therapy during the study, those experiencing disease flares treated with steroids, ulcerative colitis (UC) patients who had undergone proctocolectomy, those with recent (less than 6 months) gastrointestinal surgery, acute non-specific gastroenteritis, or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The term ‚supplementation' used in the results section might have led to confusion, and we are grateful for this issue being highlighted. We have also to report that about half of patients were concomitantly treated with a stable long-course biological therapy emphasizing the complexity of the cohort included [3].

Regarding the concomitant use of steroids, we recognize an inconsistency in the reporting between Table I and Table II. This issue arises because Table I reported the use of high-dose steroid therapy initiated less than six months prior the study period, indicated for the treatment of IBD. Conversely, Table II lists patients who were chronically treated with steroid therapy for the management of extraintestinal manifestations, receiving no more than 5 mg/day of prednisone for an extended period, with no changes during the study period.

In our study, we tracked relapse rates and observed that 15 and 16 patients with ulcerative colitis relapsed at 3 and 6 months, respectively, while 10 patients with Crohn's disease relapsed at both 3 and 6 months.

Finally, we agree that a long-term follow-up study would provide valuable insights into the sustained efficacy and safety of EcN in UC management. Moreover, it is well-expected that a trial design to test the use of EcN therapy alone in UC patients could emphasize the strength of EcN therapy in this setting.

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COVID-19 vaccinations in patients with chronic liver disease

To the Editor,

We would like to comment on "COVID-19 vaccinations in patients with chronic liver disease - experience of a tertiary centre in Melbourne [1]." The research done on vaccination uptake and vaccine reluctance in patients with chronic liver disease offers important new information on what influences these people's choices about becoming vaccinated against coronavirus disease 2019 (COVID-19). The results show that this patient cohort has a higher vaccination rate than the whole population, but they also point to particular subgroups that have lower immunization rates, like those who have cirrhosis or come from particular ethnic origins. The reasons given for vaccine reluctance, such as worries about safety and efficacy, highlight how crucial it is to correct misinformation and offer specialized education to patients with chronic liver disease so they may make educated decisions.

A significant constraint of the research is its limited sample size and brief time span, which could have affected the applicability of the conclusions. To confirm the trends seen and establish other factors impacting vaccination uptake, future research should try to replicate these findings in larger and more diverse patient populations. Furthermore, in order to comprehend patients' general attitudes about immunization and create focused interventions to remove vaccination acceptance hurdles, it is crucial to investigate the degree of

vaccine hesitation among patients with chronic liver disease beyond the COVID-19 vaccination.

Additionally, there are still a few intriguing questions for this study. It is necessary to provide more information on the causes of the poor immunization rates seen in patients with hepatitis C and alcoholic liver disease. It's also intriguing to consider how to address the issue of low knowledge about at-risk status among cirrhotic patients and improve immunization rates in this population. Furthermore, considering the significant influence that ethnic background had on vaccination rates in this study, it is debatable if any recommendations exist for tailoring vaccination strategies to take into account the many sociocultural and cultural variables that exist across different patient populations.

According to Al-Dury and Kanberg [2], the development of customized vaccines that are catered to each person's own immunological profile offers new prospects, especially for people with long-term illnesses. Current recommendations state that in this situation, prompt vaccination and regular booster shots are required. The majority of vaccination-related side effects are moderate and comparable to those observed in the general population and the liver problem, as adverse effect, is very rare [3]. The study highlights the need for more education and support for this vulnerable demographic by focusing on cirrhotic patients and their lack of awareness of their elevated risk of COVID-19 related problems. It is recommended that healthcare professionals give priority to talking about vaccinations with patients who have cirrhosis, stressing the possible advantages of immunization in lessening the severity of viral infections. Future interventions could take the form of focused education and outreach programs designed to raise awareness and address issues unique to these patients, which would increase vaccination uptake and lower the likelihood of unfavorable outcomes.

The study concludes by emphasizing how critical it is to comprehend and treat vaccine reluctance in individuals with chronic liver illness, especially cirrhosis patients. Healthcare practitioners can increase immunization rates and eventually shield this vulnerable population from the complications of viral infections by identifying certain subgroups at higher risk of not receiving the vaccination and addressing their particular concerns and impediments. In order to improve overall immunization coverage in this patient population and to guide targeted interventions, future research should build on the characteristics that influence vaccination acceptance among patients with chronic liver disease.

To increase COVID-19 immunization rates in patients with chronic liver disease, particularly those with cirrhosis, more investigation and treatments are required. Healthcare providers can assist in shielding this susceptible group from the serious COVID-19 consequences by addressing vaccine hesitancy and offering focused education and support. To better understand the determinants impacting vaccination uptake in this patient population and to inform future interventions, more research with longer durations and larger sample sizes is required. Furthermore, studying broader perspectives on vaccination beyond the COVID-19 vaccine can yield important information for creating all-encompassing immunization plans for individuals with chronic liver disease. Prioritizing vaccination talks with cirrhotic patients and putting in place targeted initiatives to raise awareness and address issues unique to this population are crucial for enhancing immunization rates generally.

Lastly, in relation to vaccine hesitancy, the patient's access to information should be prioritized. Information must originate from and be under the control of an unofficial, unfavorable source. Currently, there are a number of websites publishing content that may or may not encourage the use of the COVID-19 vaccination. Since a website is a type of business, in a more complicated scenario, the proprietor of the website opposing the COVID-19 vaccination may attempt to compose a paper for publication in a paid journal that may not meet standards and raise questions about the authors' qualifications and the article itself [4]. Allowing that kind of item to serve as a source of information could put patients at increased risk of hesitation.

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Gastrointestinal mucormycosis presenting as melena in a patient with severe COVID-19

To the Editor,

A 79-year-old male patient presented with complaints of fever, difficulty in breathing and loose stools for last 15 days in April 2021 for which he was admitted to a local hospital where he tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). His SpO2 was 88% at the time of

the admission. There was no history of coronavirus disease 2019 (COVID-19) vaccination. During the hospital stay, he received moist oxygen, broad-spectrum antibiotics, dexamethasone intravenous (i.v.) 6 mg 12 hourly, and pantaprazole 40 mg i.v. once daily. On the 16th day of symptoms, he had a black tarry stool with worsening of breathlessness. He was referred to our tertiary care hospital.

At the time of admission, the patient complained of diffuse abdominal pain with severe breathlessness. He did not have any prior history of hematemesis or bleeding from other sites. On examination, his Glasgow coma scale (GCS) was E4V5M6, blood pressure 104/60 mmHg, pulse rate 118/min, respiratory rate 26/min, SpO_2 80% on room air. He had severe pallor with bilateral fine basal crepitations on chest auscultation. The baseline investigations revealed hemoglobin 4.3g/dl, platelets 50,000/mm^3, albumin 2.6g/dl, total protein 4.3 g/dl and C reactive protein 13 mg/dl.

Oxygen therapy via reservoir bag was initiated. Five units of packed RBC were transfused. Steroid was tapered. Upper gastrointestinal (GI) endoscopy could not be done as patient was oxygen-dependent. Contrast enhanced computed tomography (CECT) of the abdomen showed asymmetrical wall thickening in the gastric cardia and gastroesophageal junction with relatively maintained wall stratification and non-enhancing muscularis layer, suggestive of GI mucormycosis (Fig 1a). In addition, high resolution CT thorax showed a SARS-CoV-2 CT severity score of 17/25. However, CT scan of the peripheral nasal sinus was normal.

In view of the above findings, gastric aspirate was done. On the Potassium hydroxide (KOH) test mount of aspirate, sparsely septate branched hyaline hyphae were observed (Fig 1b). The aspirate was inoculated on Saborauds dextrose agar and potato dextrose agar and incubated at 25°C; however, there was no growth even after 28 days of incubation. Injection of liposomal amphotericin B (5 mg/kg body weight) was started, but the patient died due to shock within 48 hours of admission.

Isolated GI mucormycosis is a rare entity accounting for 7% of the mucormycosis cases [1]. It is transmitted by consumption of food contaminated with spores, germinate causing tissue and angioinvasion especially in old age and malnourished children. The stomach is the predominantly affected site followed by colon, ileum and esophagus [2]. It usually presents as abdominal pain, gastrointestinal bleed and features of perforation due to bowel infarction.

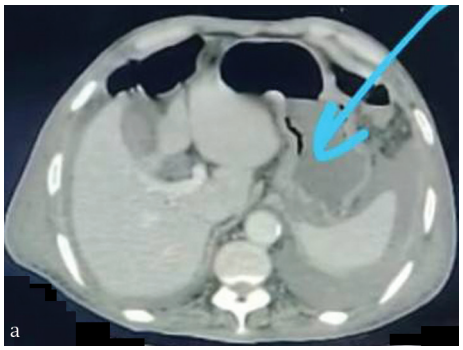

Fig. 1a. CECT abdomen of the patient showing asymmmetrical bowel wall thickening in gastric cardia and gastroesophaegeal junction with relatively maintained wall stratification and non enhancing muscularis layer-suggestive of infective etiology (arrow).

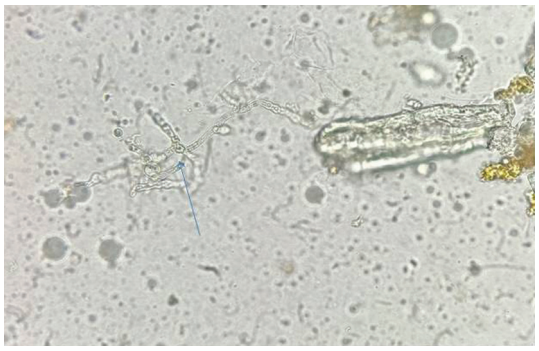

Fig. 1b. KOH wet mount of gastric lavage revealing the sparsely septate branched hyaline hyphae of Mucomycosis, 400x (arrow).

Despite the large number of cases of mucormycosis reported after COVID-19 , besides our case only few cases of COVID-19 associated GI mucormycosis have been reported in literature [3-7]. Similar to our case, a small number of patients among them did not have the traditional risk factor like type 2 diabetes mellitus but had history of steroid intake. In a multicentric study done in South India, which included 217 COVID-19 associated mucormycosis patients, only 3% cases have GI mucormycosis [8].

Radiological imaging helps in the early presumption of GI mucormycosis where CECT imaging reveals the gastric pneumatosis , reduced gastric wall enhancement, focal discontinuity of gastric wall and focal or diffuse thickening of the gastric wall [9]. The bowel enhancement is decreased secondary to varying degrees of arterial occlusion with or without reperfusion or venous occlusion. Involvement may be segmental, patchy or multifocal.

Endoscopic examination in gastric mucormycosis usually shows large ulcer with necrosis, eventually presenting an adherent, thick, green exudate [10]. However, due to active COVID-19 disease with severe lung involvement it could not be performed in our case. We did a gastric lavage in which sparsely septate branched hyaline hyphae, highly suggestive for mucormycosis, were observed on KOH wet mount which indicates the germination of the ingested spores, which has angioinvasive property.

Mucormycosis affecting the bowel has a high mortality rate. Treatment includes surgical debridement, management of comorbidity and systemic antifungals. Most mortality is due to bowel perforation, peritonitis, sepsis and massive gastrointestinal haemorrhage [1]. Our patient was elderly, had severe pulmonary involvement due to COVID-19 and presented with severe gastrointestinal bleeding due to mucormycosis, all of which could have contributed to his early mortality.

To conclude, isolated COVID-19 associated GI mucormycosis should be considered in a patient of COVID-19 with abdominal pain and melaena and prompt endoscopy with biopsy or in the circumstances where it is not possible gastric aspirate examination should be done to establish diagnosis and start treatment.

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Differential expression of NFκB p65 contributes to the fate of liver cells in acute liver failure

To the Editor,

Acute liver failure (ALF) is defined as a cute liver injury with development of hepatic encephalopathy and synthetic impairment (INR≥1.5) in a patient without preexisting cirrhosis or liver injury [1]. It is a clinical syndrome that results from many inciting agents included viruses, toxins, ischemia, and idiosyncratic drug reactions whose common endpoint is massive hepatic necrosis [2]. Although the inciting agent may directly damage hepatocytes, recent evidence has suggested that inflammatory cells and their products [tumour necrosis factor (TNF-α), interleukin (IL)1α, IL-1β, IL-6] may contribute to hepatic cell necrosis [3-4]. Different types of stimuli such as endotoxins, lipopolysaccharide, TNF- α, ILs can trigger rapid activation of cellular transcription, nuclear factor *kappa* B (NFκB) and subsequent modification of its target genes and proteins.

This study was designed to evaluate the expression and characterize the role of Nfkb (p50 and p65 subunit) in patients of acute liver failure and to correlate it with the biochemical profile and disease outcome.

The study comprised of 60 subjects (25 cases & 35 controls). The study group also included 12 pregnant women presenting with ALF. The control group included 20 healthy blood donors, 5 patients who underwent abdominal surgeries for non- liver related illnesses and 10 pregnant healthy females. Liver tissue was extracted intraoperatively from 5 controls and compared with liver tissue from 10 cases. NFκB p50 and 65 protein expression was compared in pregnant ALF patients with healthy pregnant females.

Serological tests were performed using commercially available ELISA kits for IgM anti-hepatitis A virus, Hepatitis B surface antigen, IgM anti-HBe, anti-hepatitis C virus, IgM anti-hepatitis E virus. All patients who were positive for hepatitis B and hepatitis C virus related serological markers were screened for HBV DNA and HCV RNA by PCR and RT-PCR respectively to confirm the diagnosis.

The expression of NFκB transcription factor p50 &p65 was investigated through nuclear protein extraction, gel electrophoresis and Immunoblotting.

In our study the expression of NFκB p50 and p65 protein has been arbitrarily categorized into the following: ++++ high; +++ moderate; ++ normal; + low; - Null. On analyzing the expression profile of p50 and p65 by immunoblotting, we observed that all ALF cases showed moderate to high level of expression of p50 component of NFκB. On the other hand, there was a complete absence of p65 expression in most of the cases and either low or negligible in rest of the cases. A similar pattern was also observed in the post-mortem live biopsy tissue of ALF cases who died during hospital stay.

The control group showed normal expression of both p50 & p65 in most cases. Thus, many test samples showing high expression along with low expression of p65 suggest homodimerization of p50 subunit of NFκB in ALF patients. We can say that the expression/presence of p65 protein is essential for recovery and survival in ALF patients.

It is interesting to note that all the recovered patients (n=6), showed moderate expression of p50 suggesting it as being the major component, while p65 component showed low to moderate expression which may be indicative of increasing heterodimerization of p50 and p65 leading to functional signalling for better treatment outcome.

Our previous study also showed that p65 expression is suppressed in pregnant women with hepatotropic virus induced ALF and it was correlated with more severe liver damage, higher mortality, and hence poor prognosis [5].

We believe that selective suppression of p65 and an increased homodimerization of p50 subunits leads to disruption of normal NFκB complex and its function of preventing apoptosis and allowing cellular growth which in turn affects the regeneration of hepatocytes, contributing to poor prognosis in ALF.

This hypothesis gains further support from the upregulated expression of NFKB in 6 recovered patients of ALF. Therefore, we conclude that the lack of p65 could be responsible for severe liver damage and mortality in ALF and therefore could be used as a prognostic tool.

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Progression of segmental colitis associated with diverticulosis to ulcerative colitis

To the Editor,

Segmental colitis associated with diverticular disease (SCAD), also known as diverticular colitis, is a distinct clinical entity defined as chronic colitis affecting the diverticular segment with endoscopic and histologic sparing of the rectum [1]. The prevalence of SCAD is 1.99% of 2,215 patients with diverticulosis in a recent 3-year prospective international cohort [1]. The progression of SCAD to ulcerative colitis (UC) has been focused on, suggesting a certain pathogenic relationship. More than 30 cases of such progression have been reported, but detailed clinical information can be evaluated in only eight patients by PubMed search [2-7]. The clinical data of these cases are shown in Table I. There were no gender differences, and the mean age of SCAD was 63 years. The interval period from the diagnosis of SCAD to that of UC was as short as 11 months. Refractory SCAD cases requiring surgery (sigmoidectomy) were more likely to progress to UC. Postoperative conditions, such as ischemia and changes in bacterial flora, may accelerate the progression. In addition, such UC cases became aggressive forms requiring surgery (colectomy). Subtype classification of SCAD may be helpful for careful surveillance and prediction of natural history [1].

Whether SCAD exists as a distinct entity, or it is a subtype of inflammatory bowel diseases remains controversial. Due to the heterogeneity of clinical manifestations and endoscopic and histopathologic features, diagnosis of SCAD and evaluation of progression from SCAD to UC may be challenging in clinical practice [8]. First, because SCAD by definition does not involve the rectum, although eight cases with progression have been rigorously evaluated, not all cases of SCAD in several large-scale studies have not received histopathologic evaluation of the rectum to differentiate UC. A proper protocol of biopsy sampling and better collaboration between endoscopists and pathologists may improve the establishment of the diagnosis. Second, since most SCAD patients are over middle-aged with colonic diverticulum and receiving medications such as non-steroidal anti-inflammatory drugs, antihypertensive drugs, or laxatives that may potentially injure the colon, accurate differentiation of drug-induced colitis should be difficult. Careful evaluation of clinical information is critical in differentiating other colitis. In conclusion, although most cases of SCAD respond well to medical therapy, severe forms of SCAD may have the potential to progress to UC, which also has aggressive disease behavior. Prospective multicenter collaborative studies are needed to elucidate the pathogenic mechanism of SCAD and the disease progression to UC and the optimal treatments.

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Table I. Clinical data of eight cases with the progression from SCAD to UC

Gender	Age at diagnosis of SCAD	Interval from SCAD to UC	Treatment of SCAD	Treatment of UC
Male (n=4) Female (n=4)	63 years (45-77)	11 months (4 m-2 y)	surgery 5 medical 1 none 2	surgery 5 medical 3

SCAD: segmental colitis associated with diverticulosis; UC: ulcerative colitis.

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