Figure 1 Platelet count since diagnosis of Crohn's disease. ↓, infusion of infliximab; ⇓, infusion of adalimumab.

with persistently low platelet counts for many months. Moreover, the second episode of thrombocytopenia 1 week after the adalimumab infusion is consistent with the typical picture of onset of thrombocytopenia after rechallenge.

Monoclonal antibodies against TNF α are used for the treatment of patients with autoimmune disorders who have had an inadequate response to conventional treatment.^{2,3} In patients with Crohn's disease, TNF α is a key proinflammatory cytokine and infliximab is an established treatment for patients resistant to conventional therapy. Infliximab is a chimeric monoclonal antibody because it is manufactured with mouse binding VK and VH domains and human constant Fc domains. In contrast, adalimumab is constructed from a fully human monoclonal antibody but shares the same Fc domain with infliximab. Both antibodies bind to the same region of TNF α , preventing it from activating TNF receptors and inducing down-regulation of inflammatory reactions associated with autoimmune diseases.

Some autoimmune side effects have been described with infliximab.³⁻⁶ Although the mechanisms underlying them are unknown, autoantibodies produced during apoptosis could play a role in the pathogenesis of this phenomenon. We hypothesise the possible contribution of such a mechanism, or even the involvement of antibodies against infliximab that later recognise the Fc domain of adalimumab. The temporal relation between the initiation of anti-TNF α monoclonal antibodies and the onset of thrombocytopenia, in addition to the effects of the discontinuation and resumption of anti-TNF α monoclonal antibodies, are definite evidence of anti-TNF α -induced thrombocytopenia in our case. In patients with a suspicion of immune side effects after infliximab treatment, the use of other antibodies against TNF α should therefore be discouraged.

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References

- 1 George JN, Raskob GE, Shah SR, *et al*. Drug-induced thrombocytopenia: a systematic review of published case reports. *Ann Intern Med* 1998;**129**:886-90.
- 2 Van Dulleman HM, van Deventer SJH, Hommes DW, *et al*. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* 1995;**109**:129-35.
- 3 Travis SP, Stange EF, Lemann M, *et al*. European evidence based consensus on the diagnosis and management of Crohn's disease: current management. *Gut* 2006;**55**(Suppl 1):i16-35.
- 4 Vidal F, Fontova R, Richart C. Severe neutropenia and thrombocytopenia associated with infliximab. *Ann Intern Med* 2003;**139**:E238-39.
- 5 Hamaguchi M, Kawahito Y, Yoshida M, *et al*. A case report of tumor necrosis factor-alpha antibody-induced thrombocytopenia associated with emerging IgM anticardiolipin antibody in patients

with scleroderma overlap/rheumatoid arthritis. *Clin Rheumatol* 2007;**26**:988-90.

- 6 Marotte H, Charrin JE, Miossec P. Infliximab-induced aseptic meningitis. *Lancet* 2001;**358**:1784.

Non-alcoholic fatty liver disease in patients with HCV genotype 4

Hepatitis C infection is considered the leading cause of chronic liver disease in Egypt. Hepatitis C virus (HCV) genotype 4 is the predominant genotype in the Middle East including Egypt.¹

A striking feature of HCV infection is its association with fat accumulation within hepatocytes,² which is defined by the presence of hepatic steatosis with or without steatohepatitis.³ Hepatic steatosis has been reported in 30-70% of patients with HCV infection, even when the most common causes of steatosis are excluded.⁴ A causal relationship between infection with HCV genotype 3 and the incidence of hepatic steatosis has been elicited.⁵

In Egypt the true prevalence of non-alcoholic fatty liver disease is unknown among patients with HCV who are commonly infected with genotype 4. Our aim was to determine the prevalence of steatosis in patients infected with HCV genotype 4 and to correlate steatosis with risk factors such as obesity, type 2 diabetes mellitus, hypertriglyceridaemia and the impact of steatosis on necroinflammatory injury and fibrosis score.

The study was conducted at Cairo Liver Center and included all patients who underwent liver biopsy during the period from January 2003 to May 2005. One hundred and twenty-four patients were diagnosed with chronic HCV genotype 4 (group 1) and 48 subjects with non-HCV chronic liver disease matched for age and sex were included for comparison (group 2). The histopathological, clinical and biochemical features of all patients were reviewed. Associated comorbid conditions such as overweight/obesity (body mass index ≥ 25 kg/m²), type 2 diabetes mellitus and hypertriglyceridaemia were studied for their correlation with steatosis. Further assessment of the biopsy specimens with steatosis was carried out to identify steatohepatitis. The paraffin sections were retrieved and new 4 μ m thick sections were obtained and immunohistochemical staining for cytokeratin 8/18 was applied to detect Mallory hyaline bodies (fig 1).

Steatosis was mainly macrovesicular in all specimens and was detected in 50 (40.3%) patients in group 1 and 16 (33.3%) patients in group 2 ($p = 0.550$). Steatohepatitis was identified in four patients in group 1, which was

Figure 1 Immunohistochemical staining for cytokeratin 8/18 and detection of Mallory hyaline bodies (peroxidase $\times 400$).

Table 1 Pathological features of the two study groups

Parameter	Chronic hepatitis C (group 1) (n = 124)	Non-C chronic liver disease (group 2) (n = 48)	p Value
Steatosis	50 (40.3%)	16 (33.3%)	0.550
Mild	36 (29.0%)	8 (16.7%)	
Moderate	10 (8.1%)	6 (12.5%)	
Severe	4 (3.2%)	2 (4.2%)	
Steatohepatitis	7 (5.7%)	1 (2.1%)	0.445
Modified HAI†	6.7 (2.7), 0–13	4.8 (3.4), 0–13	0.010*
Fibrosis score†	3.3 (1.9), 0–6	1.8 (1.7), 0–6	0.002*
Haemosiderosis	4 (3.2%)	0 (0.0%)	0.487

HAI, Histology Activity Index.

*Significant difference.

†Values are mean (SD), range.

increased to seven (5.6%) in group 1 and one (2.1%) in group 2 ($p = 0.445$) after applying the immunohistochemical staining for detection of Mallory hyaline bodies. Pericellular fibrosis was identified in all steatohepatitis sections, but neutrophil inflammatory reaction was only identified in three specimens. Necroinflammatory injury, as reflected by modified Histology Activity Index (HAI), was significantly higher in group 1 than in group 2 (6.7 (2.7) vs 4.8 (3.4), $p = 0.01$). The fibrosis score was significantly higher in group 1 than in group 2 (3.3 (1.9) vs 1.8 (1.7), $p = 0.002$). Overweight/obesity (≥ 25 kg/m²) was reported in 80.6% of patients in group 1 and 62.5% in group 2 ($p = 0.097$), type 2 diabetes mellitus (fasting blood sugar >126 mg/dl and postprandial blood sugar 200 mg/dl on more than one occasion) was encountered in 27.4% of group 1 and 12.5% in group 2 ($p = 0.038$), while hypertriglyceridaemia (>200 mg/dl) was found in 6.5% of group 1 and 8.3% of group 2 ($p = 0.759$, table 1).

While steatosis was not associated with HCV ($p = 0.555$), it was significantly correlated with overweight/obesity ($p = 0.001$), diabetes mellitus ($p = 0.001$) and hypertriglyceridaemia ($p = 0.019$).

By multivariate analysis, only overweight/obesity and diabetes mellitus were found to be significantly associated with steatosis. Neither necroinflammation nor fibrosis was correlated with steatosis in patients with HCV genotype 4 ($p = 0.953$ and 0.463). This lack of correlation may be explained by the discrepancy between a high frequency of steatosis (40%) and a low frequency of steatohepatitis (5.6%) in group 1.

We conclude that hepatic steatosis is prevalent in nearly 40% of patients infected with HCV genotype 4, which is more highly attributed to associated metabolic factors. It seems that there is no correlation between necroinflammation or fibrosis and steatosis in patients with HCV genotype 4.

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References

- 1 Nguyen MH, Keefe EB. Prevalence and treatment of hepatitis C virus genotypes 4, 5, and 6. *Clin Gastroenterol Hepatol* 2005;3:S97–S101.
- 2 Leonardo A, Adinolfi LE, Loria P, et al. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. *Gastroenterology* 2004;126:586–597.
- 3 Contos MJ, Sanyal AJ. The clinicopathologic spectrum and management of nonalcoholic fatty liver disease. *Adv Anat Pathol* 2002;9:37–51.
- 4 Adinolfi LE, Gambardella M, Andreana A, et al. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001;33:1358–1364.
- 5 Rubbia-Brandt L, Quadri R, Abid K, et al. Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. *J Hepatol* 2000;33:106–115.

Association of the T300A non-synonymous variant of the ATG16L1 gene with susceptibility to paediatric Crohn's disease

The hunt for genetic variants conferring risk to inflammatory bowel disease (IBD) has been ongoing for more than 10 years, with a number of susceptibility genes implicated through various approaches. Earlier this year, through the genotyping of 16 360 non-synonymous single nucleotide polymorphisms (SNPs), Hampe *et al*¹ made an important addition to this repertoire by reporting a highly significant association between Crohn's disease and the autophagy-related 16-like 1 (*ATG16L1*) gene. Specifically, a common coding variant, rs2241880 (T300A), was shown to confer a strong risk for the disease, and this association was replicated in the same study in separate

cohorts of patients with Crohn's disease but not with ulcerative colitis. Although these findings are compelling, there are continuing concerns regarding the performance of association studies in complex traits.² Many errors and biases can blight any individual study, whereas independent replication ensures that the original findings are indeed robust and provide a more accurate estimate of the likely effect size.^{3,4} Such independent replication efforts are now considered mandatory in the study of complex traits.²

We have an ongoing genome-wide association study of children with Crohn's disease using the Illumina Infinium II HumanHap500 BeadChip.^{5,6} The probe for rs2241880 is present on this platform, so we were able to query our current dataset for its association with Crohn's disease as a single test. All analyses were carried out using the software package "plink" (<http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>). In order to avoid potential bias deriving from population stratification, controls were genetically matched to Caucasian patients with Crohn's disease by clustering of the pairwise identity-by-state distances. Complete linkage agglomerative clustering was used with restrictions based on a pairwise population concordance test so that one case was matched with two controls. Controls were matched with cases based on their pairwise identity-by-state as long as a pairwise population concordance test had a p value of >0.001 . In some limited cases, only one genetically matched control could be found based on the restrictions. Our association analysis included 142 out of 143 unrelated Caucasian children with Crohn's disease (71 boys, age of onset 1–17 years, mean 10.9 years) who had consented to the study and 281 matched controls (140 boys) recruited from the greater Philadelphia area from 2000 to 2006 at the Children's Hospital of Philadelphia. One patient with Crohn's disease was missing genotypes and was excluded from the analysis.

The diagnosis of Crohn's disease was based on standard criteria.⁷ Determination of disease site and extent was performed in each patient. The anatomical distribution of the disease was determined by involvement of regions of Crohn's disease as upper tract (oesophageal, gastric, duodenal), small bowel (jejunal, ileal), colonic and perianal. With the exception of jejunal and perianal Crohn's disease, all other areas of anatomical involvement were confirmed by microscopic evidence of chronic inflammation. Patients were categorised further into one of five groups based on the maximal extent of the disease:

- ileal, including gross involvement of the ileum without colonic disease but could include upper tract involvement ($n = 12$, 8.3%);
- ileocolonic, including gross involvement of the ileum and colon but could include any upper tract involvement ($n = 123$, 86%);
- colonic, including gross involvement of the colon without small bowel involvement ($n = 8$, 5.6%);
- perianal disease, including perianal fistulas, perianal abscesses, but not perianal fissures or perianal tags (this was considered a separate category and could occur with another site of involvement ($n = 46$, 32%); and
- upper gastrointestinal tract involvement with evidence of gross or microscopic involvement of the upper gastrointestinal