Original article

Elevated liver enzymes in inflammatory bowel disease: the role and safety of infliximab

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Abstract

Background: Abnormal liver enzymes are frequently encountered in inflammatory bowel disease (IBD) patients. Infliximab has been implicated in inducing drug-induced liver injury, autoimmune hepatitis or reactivation of HBV. We aimed to clarify the role of infliximab to liver impairment in an IBD cohort.

Study: 305 patients with IBD, without evidence of chronic liver disease, were included in the study and retrospectively evaluated. Laboratory and clinical data were retrieved from a prospectively acquired database. 176 consecutive patients treated with infliximab during the last 5 years were compared to a matched population of 129 patients that did not receive any anti-TNF treatment.

Results: Elevation of ALT was frequent in the whole population (36.4%) and it was not significantly associated with the use of infliximab (P=0.284). Elevations more than 3ULN were observed in 7.9% and these spontaneously resolved in 83%. The use of immunomodulators was the only factor that was significantly associated with liver enzyme abnormalities in multivariate analysis (OR 2.666, 95% CI 1.576-4.511, p<0.005). 39% of patients on infliximab had elevated liver enzymes and this was associated with increased ALT before starting infliximab (OR 3.854, 95% CI 1.800-8.251, P=0.001) and with longer duration of infliximab treatment (OR 1.030, 95% CI 1.013-1.047, P=0.001).

Conclusion: Elevated liver enzymes are frequently found in IBD patients and they usually spontaneously resolve. The use of immunomodulators was independently associated with increased ALT. Infliximab is relatively safe in terms of liver impairment and discontinuation of treatment is rarely required in the setting of modest elevations of ALT.

Key words: infliximab, IBD, hepatotoxicity, elevated liver enzymes

Introduction

Inflammatory bowel diseases (IBD), consisting mainly of Crohn's disease (CD) and ulcerative colitis (UC) are complex immune-mediated multifactorial gastrointestinal disorders [1]. Intestinal inflammation is a result of aberrant activation of T-cell response to commensal enteric flora in genetically susceptible hosts [2]. This chronic inflammatory process is characterized by periods of relapse and remission.

IBD therapy aims to induce and sustain remission as long as possible. Multiple therapeutic agents have been used for this purpose. Aminosalicylates, corticosteroids, thiopurines, methotrexate, cyclosporine and more recently tumor necrosis factor (TNFa) antibodies have been proven efficient in inducing and maintaining clinical remission in patients with active CD or UC [3].

However, most of these agents have been linked to some extent to hepatotoxicity. Almost all immunosuppressive regimens used in IBD have been reported to increase hepatic enzymes, sometimes necessitating drug discontinuation [4].

Infliximab is a chimeric monoclonal antibody that binds avidly to tumor necrosis factor α, resulting in its inactivation. It is currently indicated for CD, UC and several rheumatologic disorders[5]. Infliximab was considered to be a safe medication in terms of liver impairment even though it is known to result in asymptomatic elevation of liver enzymes[6-8]. Post-marketing reports however have shown that severe liver injury can be induced by this agent resulting even in liver transplantation [9].

The aim of this study was to define the true incidence of liver impairment related to infliximab in a real life IBD cohort and to compare it to an IBD control group not receiving this medication. As a secondary endpoint we tried to correlate the ALT levels with clinical events that could lead to potential discontinuation or change of treatment.

Materials and Methods

Study population

All consecutive IBD patients treated with infliximab from 2008-2013 in the Department of Gastroenterology, Royal Free Hospital, London, were retrospectively evaluated. The diagnoses of CD and UC were established by standard clinical, radiological, histological, and endoscopic criteria [10, 11]. Patients diagnosed with known liver related disease, such as primary sclerosing cholangitis (PSC) or chronic viral hepatitis, were excluded from the study. Laboratory parameters were obtained the day before infliximab induction as baseline and all subsequent follow up blood tests (full blood count and liver enzymes) were recorded. Elevation of transaminases was categorized as mild, moderate or severe (ALT ≤2ULN, ALT= 2-3ULN, ALT≥3ULN respectively). In those patients with raised liver enzymes post infliximab, we retrospectively checked their liver biochemistry up to one year before baseline and we recorded any other events of liver impairment during that time. Data were retrieved from medical records regarding concomitant medications, especially those potentially related to hepatotoxicity (immunomodulators, aminosalicylates, antibiotics). Liver enzymes were recorded until each patient's last follow-up.

The same parameters were obtained from a control group of IBD patients, who did not receive any anti-TNF agent and were matched by gender, type of IBD and duration of follow-up to the study group.

Definitions

Abnormality or increase of liver enzymes was defined as any elevation in alanine transaminase (ALT upper limit of normal (ULN) 33 U/l), aspartate transaminase (AST ULN 31 U/l), alkaline phosphatase (ALP ULN 129 U/l), γ-glutamyl transferase (gGT ULN 36 U/l) and/or bilirubin (Bil ULN 21 μmol/l). Elevation of transaminases was defined as mild in ALT ≤ 2ULN, moderate in ALT x2-3ULN and significant in ALT≥3ULN. The R value was calculated as the ratio [ALT/ALT ULN : ALP/ALP

ULN]. R≥5 corresponds to a hepatocellular pattern of drug-induced liver injury (DILI), R≤2 to a cholestatic pattern and R between 2 and 5 is defined as a mixed type of DILI[12].

Treatment

All patients treated with Infliximab were taking the standard regimen dose of 5mg/kg at weeks 0, 2, 6 and every 8 weeks thereafter [13]. Total duration of treatment in months was recorded. The use of azathioprine, 6-mercaptopurine, methotrexate, aminosalicylates and steroids was recorded when they were prescribed during the period of the study. Full blood count and liver biochemistry were monitored every 8 weeks for patients on infliximab and every 3-6 months for the rest of the population.

Statistical analysis

Data were expressed as mean and standard deviation (SD) for continuous and normally distributed variables, median and range for continuous variables without normal distribution, or frequencies (percentage) for categorical variables. For comparisons the chi-square test was used for categorical variables; the Student's t - test and the Mann-Whitney test for continuous variables with or without normal distribution, respectively. For comparisons between more than two groups, the Kruskal-Wallis test was applied. Univariate analysis was used to identify parameters associated with abnormal ALT. Multiple logistic regression was used for multivariate analysis. The level of statistical significance was set at $p \leq 0.05$. Statistical analysis was performed using the Statistical Package for Social Sciences, version 22 (SPSS, IL, Chicago).

Results

Patients' characteristics

One hundred and seventy six IBD patients were treated with infliximab between 2008 and 2013 and met the inclusion criteria for the study. As a control group, one hundred and twenty nine IBD patients (129) out of the total IBD population treated in the Gastroenterology Department were selected. In total, 305 patients with IBD were included in the study. Thirteen patients were evaluated and excluded due to other liver related diseases: 5 with concomitant PSC, 3 with HCV, 1 with established cirrhosis and 4 with active CMV viremia.

Mean age was 40 ± 14.7 years and 164 were males (53.8%). 223/305 (73%) had Crohn's disease and 82 had ulcerative colitis. Mean duration of infliximab treatment was 23.1±21.7 months.

Cumulative percentages for use of each drug during the 5 year follow up were: infliximab 57.7%, azathioprine 40.7%, 6-mercaptopurine 13.1%, aminosalicylates 21% and steroids 7.2%. These are summarized in Table 1.

Abnormal liver enzymes in the infliximab population

176/305 patients (57.7%) were treated with infliximab and ALT increase was observed in 69 (39.2%) of them, with spontaneous resolution in 76% of cases. Mean age was 37.8±14.1 years, 105/176 (59.7%) were males and 138/176 (80%) had Crohn's disease. **All patients had been checked for hepatotropic viruses prior to initiation of infliximab (HAV, HBV, HCV, CMV, EBV, VZV).** Nearly half of them were taking immunomodulators, i.e azathioprine, 6-MP or methotrexate. Of the 69 patients who received infliximab, mean duration of treatment was 23.1±21.7 months, 31 (44.9%) had an elevation of ALT within the first 3 months of treatment and 41 (59%) had at least one episode of elevated liver enzymes one year prior [Table 2]. **37 (53.6%) patients had autoimmune testing on appearance of abnormal liver tests and 12 (32%) had positive antinuclear antibodies but with low titres (less than 1:80) and normal immunoglobulins.**

These 69 patients were compared to the rest of the infliximab subpopulation that did not have any ALT abnormality during the period of the study (n=107). No statistically significant difference was found among age (P=0.654), gender (P=0.152), **diagnosis (UC or CD) (P=0.219)** and use of immunomodulators, aminosalicylates or steroids between the two groups (P=0.235, P=0.713 and P=0.699 respectively). 25/69 (36.2%) of patients with ALT abnormalities after infliximab induction had also at least one episode of elevated ALT up to one year before, compared to 16/107 (15%) of those with normal liver enzymes post infliximab treatment (P=0.002). Mean duration of anti-TNF treatment was 29.5 months in patients with abnormal liver enzymes, and this was significantly longer compared to patients with normal liver biochemistry; mean duration of infliximab treatment 11.5 months, P<0.0005 [Table 3].

In multivariate analysis, abnormal ALT in the subgroup of patients treated with infliximab was significantly associated with elevated ALT prior to infliximab induction (OR 3.854, 95% CI 1.800-8.251, P=0.001) and longer duration of infliximab treatment (OR 1.030, 95% CI 1.013-1.047, P=0.001).

Abnormal liver enzymes in the whole cohort

111/305 patients (36.4%) were found to have at least one episode of elevated liver enzymes, which resolved spontaneously in 73% of cases. **Time to resolution was less than three months in 43% of these patients. Thirty eight patients with prolonged time to resolution (48 %) had only mildly abnormal transaminases (<2 ULN), possibly reflecting a non-acute aetiology such as fatty liver disease.** The elevation was moderate in 10.2% and severe in 7.9% of cases. Transaminases were predominantly elevated compared to cholestatic enzymes in the majority of patients (89.2%). Twelve patients (10.8%) had a preponderance of elevated cholestatic enzymes (ALP, GGT).

We compared patients with normal ALT (194/305) to those with at least one ALT abnormality since entry into study (111/305). There was no significant difference in age (P=0.134), gender (P=0.165), **IBD diagnosis (UC or CD) (P=0.262),** anti-TNF treatment (P=0.284) and use of aminosalicylates or

steroids between the two groups (P=0.487 and P=0.240 respectively). 85/111 (76.6%) patients with abnormal ALT were taking immunomodulators compared to 105/194 (43.9%) with normal ALT and this was statistically significant (P=0.001). The correlation of immunomodulators to ALT abnormalities was also confirmed in multivariate analysis (OR 2.666, 95% CI 1.576-4.511, p<0.001), while use of infliximab, steroids or aminosalicylates, age, gender and diagnosis were not statistically significant (P=NS) [Table 4].

Patients with significant liver injury

Twenty-four patients out of the whole cohort (24/305, 7.9%) had an ALT more than three times the upper limit of normal and twelve (50%) of them were treated with infliximab. **All but one (95%) had Crohn's disease**. All were screened for HAV, HBV, HCV and CMV serology and found negative. The rest of liver screening tests, such as immunoglobulin G, ferritin, alpha1- antitrypsin, copper and ceruloplasmin levels were tested and found normal.

4/24 patients (16.6%) had positive antinuclear antibodies (3/4 on anti-TNF). Thirteen patients (54.1%) of those with significant liver injury were under some immunomodulatory agent. Ultrasound and/or computed tomography of the liver was performed in the majority of patients, showing fatty liver in 10 (41.6%), other lesions such as gallstones or hemangiomata in 4 (16.6%) and normal findings in 6 (25%) [Table 5].

All but one patient under infliximab had a hepatocellular type of damage with an R value>5. A cholestatic pattern of liver injury was found in four patients not on anti-TNF treatment. MCRP was performed in those with elevated cholestatic enzymes and there was no bile duct dilation or evidence of PSC.

A liver biopsy was done in four patients (16.6%), three of whom were treated with infliximab. Liver histopathology revealed a probable drug-induced liver injury (DILI) in 3/4 patients and non-alcoholic

steatohepatitis in the other one. Of the three patients with established DILI one was on azathioprine, one on infliximab (which was discontinued) and one on combination of infliximab and 6-MP (6-MP was subsequently discontinued).

ALT values normalised in 20/24 patients (83.3%). **Out of the four patients with no spontaneous resolution of their abnormal ALT, one was on the infliximab group**. **Fatty infiltration of the liver was the cause of abnormal ALT (as confirmed by CT imaging) in two patients with persistent liver enzyme elevations and these were offered lifestyle changes and close monitoring**. **No obvious reason for an intermittently elevated ALT despite extensive investigation was found in the other two patients.**

Resolution occurred in 21/24 (87.5%) patients without discontinuation of IBD treatment. Infliximab was stopped in two patients: one with a biopsy showing DILI and one due to absence of response who was switched to adalimumab. Azathioprine was discontinued in one patient with a biopsy-proven DILI.

Discussion

Hepatobiliary manifestations in patients with IBD are relatively frequent [14]. In our study, we confirmed the increased prevalence of elevated liver enzymes in an IBD population and we found that this is not associated with infliximab treatment. Furthermore, we showed that these elevations are usually not severe and they spontaneously resolve in the majority of cases, thus rarely requiring specific management.

Liver disease in IBD can develop due to an immune-mediated pathogenetic mechanism, as a complication of structural and pathophysiological changes due to IBD or from drug induced hepatotoxicity [15].

Several liver conditions have been frequently associated with IBD, the most common being PSC. Nearly 80% of patients diagnosed with PSC have IBD and a percentage of up to 7% of IBD patients

256 will finally develop PSC [5]. Autoimmune hepatitis (AIH) more often occurs in UC patients than in
257 general population [16]. Cholelithiasis is more common in CD patients than in matched controls and
258 this is associated with bile salt absorption affected by the number of surgeries and extent of ileal
259 resections [17]. Patients with IBD are also prone to thrombosis [18]. Portal or hepatic vein thrombosis
260 can lead to portal hypertension and liver cirrhosis. Non-alcoholic fatty liver disease (NAFLD) appears
261 to be more common in IBD patients, with prevalence as high as 35% [17].
262 Medications used for IBD treatment are greatly associated with hepatotoxicity. Thiopurines can result
263 not only in asymptomatic liver enzyme elevation but also in DILI or even liver injury due to
264 generation of reactive oxygen species, via oxidation of azathioprine or 6-mercaptopurine (6-MP) by
265 xanthine oxidase [19]. Thiopurine methyltransferase (TPMT) is the enzyme that catalyzes 6-MP
266 methylation and shows great variation in its activity due to multiple genetic polymorphisms. As a
267 result, toxic metabolites such as 6-MMP can accumulate in some patients leading to a greater extent
268 to liver impairment[20]. Moreover, azathioprine has been linked to nodular regenerative hyperplasia
269 (NRH) of the liver and non-cirrhotic portal hypertension [12]. The prevalence of 6-MP and
270 azathioprine-induced liver impairment varies in several studies. In a recent study of 3931 IBD
271 patients, 95% of who were on azathioprine, the incidence of hepatotoxicity was 4% [13]. Earlier
272 studies have reported higher incidence of abnormal liver enzymes in IBD patients treated with
273 thiopurines (10-20%) [21, 22].
274 5-ASA is probably the responsible hepatotoxic moiety of aminosalicylates. Abnormal hepatic
275 biochemistry attributed to these agents has been described since 1989 [14]. The hepatotoxic potential
276 of methotrexate was firstly recorded in patients treated for psoriasis several decades ago. However,
277 few trials have been conducted in IBD patients so far. In a meta-analysis of 13 trials, Khan et al
278 showed that liver injury induced by methotrexate occurs in a comparable incidence to that of
279 thiopurines [15].
280 Infliximab (IFX) was initially approved for the treatment of moderate to severe Crohn's disease and
281 rheumatoid arthritis in 1998 [23]. Most serious side effects include an increase in frequency of

infections, allergic reactions and generation of autoantibodies (mostly antinuclear and double-stranded DNA). Elevation of liver enzymes is relatively common due to infliximab but significant liver damage was considered to be extremely rare [24].

Several case reports have been published reporting hepatitis, frequently immune-mediated, that occurred after infliximab induction and resolved after discontinuation of the drug [25-28]. In a recent study Ghabril et al. described 34 cases of DILI induced by anti-TNFs [9]. Most of them follow a hepatocellular pattern of injury, have an autoimmune background and resolve after discontinuation of treatment. Switching infliximab to adalimumab seems to be well tolerated, suggesting absence of cross-toxicity between the two agents [29].

Reactivation of occult hepatitis B can result in liver impairment and it was firstly described in two rheumatologic patients that were treated with infliximab in 2003 [30, 31].

In the ATTRACT study group, patients receiving infliximab for rheumatoid arthritis developed ANA and anti-ds DNA antibodies in 23% and 16% respectively versus 6% and 0% of placebo recipients [32]. Autoimmune hepatitis may accompany the positivity of autoantibodies with markedly elevated transaminases and a typical liver biopsy showing interface hepatitis with lymphoplasmacytic infiltrate [33].

Infliximab may induce elevation of liver enzymes due to idiosyncratic drug reaction. Jaundice is a rare finding and in most cases liver impairment resolves spontaneously.

Overall, infliximab may result in liver abnormalities via different mechanisms, raising issues regarding its safety. An Austrian consensus about infliximab use in IBD after ten years on the market proposes discontinuation of treatment in cases of significant liver enzymes' elevations (more than 3ULN) [7].

In our study the prevalence of abnormal liver enzymes was remarkably high, nearly 40%. However, similarly high incidence of elevated liver tests has been reported before [34, 35]. In the majority of our population the elevation was mild to moderate and only few patients developed ALT≥3ULN. Even significant elevations tended to resolve without need for treatment discontinuation.

Immunomodulatory agents were the only significant predictors of abnormal ALT, a correlation previously reported [21, 22]. Of note, this association was not statistically significant in the infliximab population.

The incidence of increased transaminases in our study was similar in both infliximab and non-infliximab treated populations. Infliximab could possibly be associated with some cases of ALT abnormalities but these were mostly mild. Even with significantly increased aminotransferases, the outcome was favorable **and treatment was discontinued in only one patient.** The presence of abnormal ALT prior to infliximab induction was significantly related to abnormal ALT in post infliximab measurements, emphasizing that several conditions unrelated to anti-TNF therapy may impair the hepatobiliary parameters in IBD. **Moreover, presence of significant ALT elevations prior to infliximab initiation should trigger a liver screen in order to identify pre-existing liver disease.**

Another interesting finding was that the duration of infliximab therapy was significantly associated with elevated liver enzymes. This group of patients requiring anti-TNF therapy for longer is usually unresponsive to conventional treatment with longer periods of relapse and hospitalizations. Continuously active IBD may be responsible for a proportion of these liver abnormalities**.** Ascertainment bias is another possible explanation of this finding as these patients are monitored more frequently than ones having a disease of indolent course.

The main limitation of this study is that, due to its retrospective design, imaging procedures and liver biopsy were not performed in the whole cohort. Of note, 41% of patients having significant elevation of hepatic enzymes turned out to have a fatty liver in ultrasound or CT scans. Liver biopsy was also performed in a minority of patients (4/111 patients, 3.6%).

In conclusion, elevation of liver enzymes in IBD is not uncommon but is usually mild and transient, resolving spontaneously in the majority of cases. Even significant abnormalities resolved spontaneously in more than two thirds of cases. These abnormalities are likely to be multifactorial. Treatment with infliximab was not associated with deranged ALT in our study, suggesting that this

agent is generally safe. **ALT elevation should not necessarily dictate discontinuation of treatment, however investigations should be performed in order to identify the exact diagnosis and underlying liver disease should be excluded in patients treated with infliximab.** Other possible etiologies should be **considered** in cases of persistent or significant ALT abnormalities and **priority** should be given to stopping immunomodulators **first** rather than infliximab. A liver biopsy can establish the diagnosis in patients with persistent unexplained transaminase elevation and guide further therapeutic decisions, particularly regarding the continuation of immunomodulators and/or infliximab.

We therefore recommend a wait-and-watch strategy rather than immediate action in IBD patients with elevated liver enzymes. A liver biopsy would be of most importance in these cases that do not spontaneously resolve in order to obtain the correct diagnosis. Infliximab could be maintained except for those cases of established and biopsy-proven DILIs, when thiopurines are excluded as the main cause of liver function test abnormalities or of significant unexplained ALT elevations.

The optimal time point to decide upon further management in significant ALT elevations has not yet been clarified. In our study, resolution occurred usually within the first three months but this is not universal. Further prospective studies and histopathologic evaluation of similar populations could enlighten our knowledge about this common phenomenon. Moreover, the presence of other liver-related etiologies, such as NAFLD, should always influence the final decision.

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I P collected the data and wrote the paper

J O'B designed the study

R E R collected the data

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E Tsochatzis, P M, M H, C M, O E and A K B reviewed for important intellectual content

All authors read and approved the final manuscript

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