

1 Original article

2 **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

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**78 Introduction**

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

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

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

91 Infliximab is a chimeric monoclonal antibody that binds avidly to tumor necrosis factor  $\alpha$ , resulting  
92 in its inactivation. It is currently indicated for CD, UC and several rheumatologic disorders[5].  
93 Infliximab was considered to be a safe medication in terms of liver impairment even though it is  
94 known to result in asymptomatic elevation of liver enzymes[6-8]. Post-marketing reports however  
95 have shown that severe liver injury can be induced by this agent resulting even in liver transplantation  
96 [9].

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

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## 103 **Materials and Methods**

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### 105 **Study population**

106 All consecutive IBD patients treated with infliximab from 2008-2013 in the Department of  
107 Gastroenterology, Royal Free Hospital, London, were retrospectively evaluated. The diagnoses of  
108 CD and UC were established by standard clinical, radiological, histological, and endoscopic criteria  
109 [10, 11]. Patients diagnosed with known liver related disease, such as primary sclerosing cholangitis  
110 (PSC) or chronic viral hepatitis, were excluded from the study. Laboratory parameters were obtained  
111 the day before infliximab induction as baseline and all subsequent follow up blood tests (full blood  
112 count and liver enzymes) were recorded. Elevation of transaminases was categorized as mild,  
113 moderate or severe ( $ALT \leq 2ULN$ ,  $ALT = 2-3ULN$ ,  $ALT \geq 3ULN$  respectively). In those patients with  
114 raised liver enzymes post infliximab, we retrospectively checked their liver biochemistry up to one  
115 year before baseline and we recorded any other events of liver impairment during that time. Data  
116 were retrieved from medical records regarding concomitant medications, especially those potentially  
117 related to hepatotoxicity (immunomodulators, aminosalicylates, antibiotics). Liver enzymes were  
118 recorded until each patient's last follow-up.

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

122

### 123 **Definitions**

124 Abnormality or increase of liver enzymes was defined as any elevation in alanine transaminase (ALT  
125 upper limit of normal (ULN) 33 U/l), aspartate transaminase (AST ULN 31 U/l), alkaline phosphatase  
126 (ALP ULN 129 U/l),  $\gamma$ -glutamyl transferase (gGT ULN 36 U/l) and/or bilirubin (Bil ULN 21  $\mu\text{mol/l}$ ).  
127 Elevation of transaminases was defined as mild in  $ALT \leq 2ULN$ , moderate in  $ALT \times 2-3ULN$  and  
128 significant in  $ALT \geq 3ULN$ . The R value was calculated as the ratio  $[ALT/ALT ULN : ALP/ALP$

129 ULN].  $R \geq 5$  corresponds to a hepatocellular pattern of drug-induced liver injury (DILI),  $R \leq 2$  to a  
130 cholestatic pattern and R between 2 and 5 is defined as a mixed type of DILI[12].

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### 132 **Treatment**

133 All patients treated with Infliximab were taking the standard regimen dose of 5mg/kg at weeks 0, 2,  
134 6 and every 8 weeks thereafter [13]. Total duration of treatment in months was recorded. The use of  
135 azathioprine, 6-mercaptopurine, methotrexate, aminosalicylates and steroids was recorded when they  
136 were prescribed during the period of the study. Full blood count and liver biochemistry were  
137 monitored every 8 weeks for patients on infliximab and every 3-6 months for the rest of the  
138 population.

139

### 140 **Statistical analysis**

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

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## 155 **Results**

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### 157 **Patients' characteristics**

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

164 Mean age was  $40 \pm 14.7$  years and 164 were males (53.8%). 223/305 (73%) had Crohn's disease and  
165 82 had ulcerative colitis. Mean duration of infliximab treatment was  $23.1 \pm 21.7$  months.

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

169

### 170 **Abnormal liver enzymes in the infliximab population**

171 176/305 patients (57.7%) were treated with infliximab and ALT increase was observed in 69 (39.2%)  
172 of them, with spontaneous resolution in 76% of cases. Mean age was  $37.8 \pm 14.1$  years, 105/176  
173 (59.7%) were males and 138/176 (80%) had Crohn's disease. **All patients had been checked for**

174 **hepatotropic viruses prior to initiation of infliximab (HAV, HBV, HCV, CMV, EBV, VZV).**

175 Nearly half of them were taking immunomodulators, i.e azathioprine, 6-MP or methotrexate. Of the  
176 69 patients who received infliximab, mean duration of treatment was  $23.1 \pm 21.7$  months, 31 (44.9%)  
177 had an elevation of ALT within the first 3 months of treatment and 41 (59%) had at least one episode  
178 of elevated liver enzymes one year prior [Table 2]. **37 (53.6%) patients had autoimmune testing**  
179 **on appearance of abnormal liver tests and 12 (32%) had positive antinuclear antibodies but**  
180 **with low titres (less than 1:80) and normal immunoglobulins.**

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

191 In multivariate analysis, abnormal ALT in the subgroup of patients treated with infliximab was  
192 significantly associated with elevated ALT prior to infliximab induction (OR 3.854, 95% CI 1.800-  
193 8.251, P=0.001) and longer duration of infliximab treatment (OR 1.030, 95% CI 1.013-1.047,  
194 P=0.001).

195

#### 196 **Abnormal liver enzymes in the whole cohort**

197 111/305 patients (36.4%) were found to have at least one episode of elevated liver enzymes, which  
198 resolved spontaneously in 73% of cases. **Time to resolution was less than three months in 43% of**  
199 **these patients. Thirty eight patients with prolonged time to resolution (48 %) had only mildly**  
200 **abnormal transaminases (<2 ULN), possibly reflecting a non-acute aetiology such as fatty liver**  
201 **disease.** The elevation was moderate in 10.2% and severe in 7.9% of cases. Transaminases were  
202 predominantly elevated compared to cholestatic enzymes in the majority of patients (89.2%). Twelve  
203 patients (10.8%) had a preponderance of elevated cholestatic enzymes (ALP, GGT).

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



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

213

#### 214 **Patients with significant liver injury**

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

220 4/24 patients (16.6%) had positive antinuclear antibodies (3/4 on anti-TNF). Thirteen patients  
221 (54.1%) of those with significant liver injury were under some immunomodulatory agent. Ultrasound  
222 and/or computed tomography of the liver was performed in the majority of patients, showing fatty  
223 liver in 10 (41.6%), other lesions such as gallstones or hemangiomas in 4 (16.6%) and normal  
224 findings in 6 (25%) [Table 5].

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

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

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

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

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

244

## 245 **Discussion**

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

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

254 Several liver conditions have been frequently associated with IBD, the most common being PSC.

255 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

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

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

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

293 In the ATTRACT study group, patients receiving infliximab for rheumatoid arthritis developed ANA  
294 and anti-ds DNA antibodies in 23% and 16% respectively versus 6% and 0% of placebo recipients  
295 [32]. Autoimmune hepatitis may accompany the positivity of autoantibodies with markedly elevated  
296 transaminases and a typical liver biopsy showing interface hepatitis with lymphoplasmacytic infiltrate  
297 [33].

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

300 Overall, infliximab may result in liver abnormalities via different mechanisms, raising issues  
301 regarding its safety. An Austrian consensus about infliximab use in IBD after ten years on the market  
302 proposes discontinuation of treatment in cases of significant liver enzymes' elevations (more than  
303 3ULN) [7].

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

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

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

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

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

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

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

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

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

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355 I P collected the data and wrote the paper

356 J O'B designed the study

357 R E R collected the data

358 E Theocharidou analyzed the data

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

360 All authors read and approved the final manuscript

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