Journal of Viral Hepatitis, 2008, **15,** 523–530 doi:10.1111/j.1365-2893.2008.00976.x

Liver eosinophilic infiltrate is a significant finding in patients with chronic hepatitis C

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Received August 2007; accepted for publication November 2007

SUMMARY. Eosinophilic infiltrate of liver tissue is described in primary cholestatic diseases, hepatic allograft rejection and drug-induced liver injury, but its significance and its implications in chronic hepatitis C are unknown. The aim of this study was to investigate the clinical significance of eosinophilic liver infiltrate in patients with chronic hepatitis C. We retrospectively evaluated 147 patients with chronic hepatitis C. The presence of eosinophilic infiltrate was investigated in liver biopsies, and a numeric count of eosinophilic leucocytes in every portal tract was assessed. An eosinophilic infiltrate of liver tissue (≥3 cells evaluated in the portal/periportal spaces) was observed in 46 patients (31%), and patients who consumed drugs had an odds ratio (OR) of 4.02 (95% CI: 1.62–9.96) to have an eosinophilic infiltrate in liver biopsy. By logistic regression analysis, the presence of steatosis was independently associated with eosinophilic infiltrate (OR 5.86; 95% CI: 2.46–13.96) and homeostasis model assessment-score (OR 1.18; 95% CI: 1.00–1.39). Logistic regression analysis also showed that fibrosis staging ≥ 2 by Scheuer score was associated with grading >1 by Scheuer score (OR 6.82; 95% CI 2.46–18.80) and eosinophilic infiltrate (OR 4.00; 95% CI 1.23–12.91). In conclusion, we observed that the eosinophilic infiltrate of liver tissue was significantly more frequent in patients who assumed drugs, and found a significant association between eosinophilic infiltrate, liver steatosis and liver fibrosis. These preliminary data could lead to a constant assumption of drugs as a co-factor of eosinophils-mediated liver injury in chronic hepatitis C.

Keywords: chronic hepatitis C, drugs, eosinophilic infiltrate, liver biopsy, liver fibrosis, liver steatosis.

INTRODUCTION

Chronic hepatitis C in developed countries is a common cause of chronic hepatic injury, liver transplantation and liver related death [1]. In HCV hepatic disease, the co-factors of liver damage are viral co-infections, liver steatosis, alcohol abuse and liver iron overload [2]. The presence of concomitant diseases and, consequently, of chronic drug assumption has not been investigated as a possible risk factor for severe liver damage in chronic hepatitis C. In this context a tissue infiltrate of eosinophilic leucocytes has very rarely been described, and its significance is unknown. Conversely an infiltration of eosinophilic leucocytes has been described in various liver diseases, including primary biliary cirrhosis (PBC) [3–9], primary sclerosing cholangitis (PSC) [10–12], idiopathic hypereosinophilic syndrome [13–16], hepatic allograft rejection [17–26], graft-*vs*-host disease [27] and drug-induced liver injuries [28–34].

Experimental models have reported that activated Kupffer cells play a key role in producing the cytotoxicity of eosinophils by releasing TNF-α [35,36] a process that in evidenced, specifically, by liver biopsy of patients with drug-induced liver injuries [37,38].

Our study was designed to examine the prevalence of eosinophilic infiltrate (EI) in liver biopsies of patients with chronic hepatitis C and to investigate the relations between eosinophilic infiltration of liver tissue and clinical features, current and/or recent assumption of drugs, and histological features.

Abbreviations: HCV, hepatitis C virus; EI, eosinophilic infiltrate; PBC, primary biliary cirrhosi; PSC, primary sclerosing cholangitis.

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METHODS

Patients

We retrospectively analysed the clinical records of 335 consecutive patients with chronic hepatitis C admitted to

our Liver Unit from January 2005 to December 2006 for liver biopsy. Inclusion criteria of the patients were: (i) HCV-RNA positive with histological diagnosis of chronic hepatitis with any degree of fibrosis; (ii) a detailed pharmacological anamnesis to define current and/or recent assumption of drugs; (iii) availability of adequate liver biopsy and serum stored upon admission to hospital for histological and biochemical evaluations. Patients were excluded if they had: (i) post-transplant recurrent hepatitis C; (ii) chronic co-infection with HBV and/or HIV; (iii) acute hepatitis; (iv) values of serum alanine aminotransferase (ALT) of more than 15 times the upper normal limit (UNL); (v) an incomplete or absent anamnesis for concomitant diseases and/or drug assumption.

The current study was performed in accordance with the principles of Good Clinical Practice, the principles of the Declaration of Helsinki and its appendices, and local and national laws. To maintain patient privacy, patients' names were replaced in the database with codes, dates of birth, and/or ages.

Clinical and laboratory assessment

Upon admission to hospital, the age and gender of patients, presence of other chronic diseases and a detailed pharmacologic anamnesis to define current and/or recent assumption of drugs were recorded. After an overnight fast, venous blood was drawn to evaluate the serum levels of ALT, γ-glutamyltransferase (γ-GT), alkaline phosphatase (AP), total cholesterol, triglycerides, plasma glucose concentration, platelet count and blood eosinophil count (normal values < 550 cells/mm^3). Serum insulin was measured on stored serum by a two-site enzyme ELISA (Mercodia Insulin ELISA, Arnika). The detection limit was less than 1 μU/mL. Insulin resistance (IR) was determined with the homeostasis model assessment (HOMA) method by using the following equation: Insulin resistance (HOMA-IR) = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 [39]. HOMA-IR has been validated in comparison with euglycaemic/hyperinsulinaemic clamp technique in both diabetic and non-diabetic subjects [40]. The same day, serum was collected to perform HCV RNA qualitative PCR assay (Cobas Amplicor HCV Test version 2.0; limit of detection: 50 IU/mL) and to determine HCV genotype by INNO-LiPA (HCV II, Bayer).

Assessment of liver biopsy

Percutaneous liver biopsies, performed with a 16-gauge needle, were formalin fixed and paraffin embedded. We used 4 μm sections that were rewashed, rehydrated and stained with Hematoxylin-Eosin, Shikata's orcein, PAS diastase and Gomori stain for reticular fibres. Slides of liver specimens were coded and read by a single pathologist (D.C.), who was unaware of patients' identities and clinical features. Only biopsies containing more than eight portal tracts were read. Portal, peri-portal and lobular necro-inflammatory activity (grading) and fibrosis (staging) were investigated by applying Scheuer's 1991 histological score [41]. Liver steatosis was assessed as the percentage of hepatocytes containing macrovescicular fat droplets. It was coded as absent if 0 to 4%, or present if ≥ 5% of hepatocytes were affected. Portal and peri-portal eosinophilic leucocyte infiltrate was assessed in every portal tract of haematoxylin/eosin stained sections. We counted eosinophils in all portal tracts (at least eight) and reported in the data-base the three highest values of eosinophils count. The presence of 3 or more eosinophils in portal and peri-portal space was considered relevant. We also investigated, histological features more frequently reported to be related to drug induced hepatitis, i.e. canalicular cholestasis, peri-venular lipofuscinosis and small intra-lobular granulomas.

Statistical methods

Continuous variables were summarized as mean ± SD and categorical variables as frequency and percentage. Significant differences between patients with or without drug assumption were calculated using a chi-square test for categorial variables and *t* Student test for continuous variables. Multiple logistic regression models were used to assess the relationship of steatosis, fibrosis and eosinophilic infiltrate with demographics, history of drug assumption, and metabolic and histological features of the patients.

In the first model the dependent variable was steatosis coded as 0 or absent if <5% of the hepatocytes were affected, and 1 or present if ≥5% of the hepatocytes were affected. As candidate risk factors for presence of histological steatosis we selected age, gender, history of drug assumption, ALT, γ-GT and AP levels, cholesterol, triglycerides, HOMA-score, EI (<3 *vs* at least three eosinophils observed), grading score (≤ 1 *vs* >1 according to Scheuer score) and fibrosis score (1 *vs* 2–4 of Scheuer score).

In the second model the dependent variable was fibrosis coded as 0 (stage 1 of fibrosis according to Scheuer score) or 1 (stage 2–4 according to Scheuer score). We considered as explanatory variables age, gender, history of drug assumption, platelet count, ALT, γ-GT, AP, cholesterol, triglycerides, HOMA-score, EI (<3 *vs* at least 3 eosinophils observed), grading score (≤ 1 *vs* >1 according to Scheuer) and steatosis (<5% *vs* ≥5% of hepatocytes affected).

In the third model the dependent variable was EI coded as absent (<3 eosinophils) or present (≥3 eosinophils). We selected as possible related variables age, gender, drug assumption, platelet count, eosinophil count, ALT, γ-GT levels, AP, cholesterol, triglycerides and HOMA-score. Variables found to be associated with the dependent variables on univariate logistic regression at a probability

threshold of <0.10 were included in multivariate logistic regression models. Regression analysis was performed using PRO LOGISTIC subroutine in SAS (SAS Institute, Inc., Cary, NC, USA) [42].

RESULTS

Patients' characteristics

Among the 335 patients who underwent liver biopsy, 147 patients satisfied inclusion/exclusion criteria and were evaluated in our study. The clinical and histological features of those 147 patients were similar to the remaining 188 patients.

The characteristics of the 147 patients are shown in Table 1. The mean age was 51 ± 13 years. HCV genotype 1 was predominant (91%), and all other genotypes of HCV [2,3,4] were present in the measure of 3% each. Thirty-eight percent of patients had concomitant diseases and constantly took medications: 14% of patients assumed antihypertensive drugs for blood hypertension; 6% of patients assumed L-tiroxina for hypothyroidism, 4% of patients assumed benzodiazepines for psychiatric disturbances, 4% of patients assumed inhibitor protonic pump for ulcer-like dyspepsia; 3% and another 3% of patients assumed oral hypoglycaemic drug and alpha blocker, respectively, for diabetes and prostatic hypertrophy, and another 4% assumed various drugs for various conditions.

Concerning the prevalence of allergic diseases in our series, two patients were affected by allergic rhinitis occasionally treated with anti-histaminic drugs, and another patient had a history of asthmatic bronchitis periodically treated with corticosteroid drugs.

Only two patients had a mild increase of blood eosinophil count with values of 620 and 660 cells/mm^3 respectively. These two patients assumed no drugs and their liver biopsies show no increase of eosinophils in the liver parenchyma.

Histological findings

Regarding the histological features, 40 patients (27%) had mild inflammation, 84 (57%) had moderate inflammation and 23 (16%) severe inflammation. Overall, 107 patients (73%) had a Scheuer's grading score of greater than one. A moderate/severe fibrosis (Scheuer's staging score ≥2) was present in 99 patients (67%). Histological steatosis was observed in 52 patients (35%). Hepatic eosinophilic infiltrate (≥3 cells evaluated in the portal/periportal spaces) was observed in 46 patients (31%). A canalicular cholestasis was present in two patients though they neither took drugs nor showed eosinophilic infiltrate in portal tracts. Peri-venular lipofuscinosis was present in three patients and two of them were taking drug with EI in the liver parenchyma. Small intra-lobular epithelioid granulomas and epithelioid granuloma-like aggregates were present in 31 patients (21% of cases), with no differences between drug taking and not in drug taking patients.

Table 1 Demographic, laboratory and histological features of 147 patients with chronic hepatitis C

Mean age (years), mean ± sd	51 ± 13
Gender, *n* (%)	
Male	74 (50.3)
Female	73 (49.6)
Genotypes HCV, *n* (%)	
1	134 (91)
2	4 (3)
3	5 (3)
4	4 (3)
Patients with drug assumption, *n* (%)	56 (38)
Antihypertensives	21 (14)
L-tiroxin	8 (6)
Benzodiazepin	6 (4)
Inhibitor protonic pump	6 (4)
Oral hypoglicaemic drug	5 (3)
Alfa-blocker	4 (3)
Other	6 (4)
ALT (UNL)	2.4 ± 2.0
Platelet count ($\times 10^3/mm^3$)	206 ± 58
Eosinophils count (cells/mm^3)	184.9 ± 143.7
γ-GT (UNL)	1.08 ± 0.8
Alkaline Phosphatase (UNL)	0.7 ± 0.2
Cholesterol mg/dL (n.v. ≤220)	177 ± 35 (43–294)
Triglycerides mg/dL (n.v. ≤175)	97 ± 47 (40–404)
Blood glucose (mM/L)	5.2 ± 1.33 (3.7–13.7)
Insulin (μU/mL)	12.6 ± 7.2 (2.0–42.0)
HOMA-score	3.1 ± 2.3 (0.4–17.0)
Histology at biopsy (Scheuer score)	
Grade of inflammation (code)	
1 (0)	40 (27%)
2 (1)	84 (57%)
3 (1)	23 (16%)
Stage of fibrosis (code)	
1 (0)	48 (33%)
2 (1)	71 (48%)
3 (1)	25 (17%)
4 (1)	3 (2%)
Steatosis	
Absent (<5%)	95 (65%)
Present (≥5%)	52 (35%)
Eosinophilic infiltrate	
Absent (<3 cells)	101 (69%)
Present (≥3 cells)	46 (31%)

Continuous variables: mean ± SD (minimum–maximum). Categorial variables: absolute value (%). Abbreviations: ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; UNL, upper normal limit; n.v., normal values.

Comparison between patients with and without chronic drug assumption

Table 2 shows the significant differences between patients with and without history of drug assumption. The drug-taking patients were 10 years older ($P < 0.0001$), had higher ALT ($P < 0.009$) and alkaline phosphatase ($P < 0.003$) levels, presented a more severe grading score ($P < 0.02$), and a more frequent presence of EI ($P < 0.0001$), but not of an elevated blood eosinophil count, than non drug-taking subjects.

Factors associated with histological hepatic steatosis

We performed univariate and multivariate analyses to identify risk factors associated with the presence of histological steatosis. The results are reported in Table 3. At univariate analysis, drug assumption, ALT serum levels,

Table 2 Demographic, laboratory and histological features of the 147 patients in according to drug assumption

Variables	Patients without drug assumption (91)	Patients with drug assumption (56)	*P*
Age (years)	48 ± 14 (18–69)	57 ± 9 (29–70)	<0.0001
Gender (M/F)	46/45	28/28	
ALT (UNL)	2.05 ± 1.4	2.9 ± 2.5	0.009
γ-GT (UNL)	1.0 ± 0.7	1.22 ± 0.89	0.09
Alkaline phosphatase (UNL)	0.63 ± 0.21	0.74 ± 0.23	0.003
Platelet count ($\times 10^3/mm^3$)	210.7 ± 59.5	199.4 ± 54.7	0.25
Eosinophil count ($cells/mm^3$)	188 ± 152,2	180 ± 129.4	0.7
HOMA-IR	2.9 ± 2.6	3.3 ± 1.9	0.31
Histological features			
Grading >1	60 (66%)	47 (84%)	0.02
Staging ≥2	56 (61.5%)	42 (75%)	0.1
Steatosis	28 (31%)	24 (43%)	0.18
Eosinophilic infiltrate	16 (17.6%)	30 (53.6%)	<0.0001

Abbreviations: ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; UNL, upper normal limit; n.v., normal values.

Table 3 Univariate and multivariate analysis of risk factors for liver steatosis in 147 patient with chronic hepatitis C

Variable	Univariate analysis			Multivariate analysis		
Independent	*P*	OR	95% C.I.	*P*	OR	95% C.I.
Age (years)	0.15	1.018	0.994–1.053			
Sex (M/F)	0.52	0.724	0.361–1.451			
Drug assumption	0.04	2.133	1.00–4.552	0.63	0.811	0.346–1.903
ALT–UNL	0.03	1.005	1.000–1.050	0.26	1.003	0.998–1.009
γ-GT–UNL	0.16	0.99	0.98–1.002			
AP–UNL	0.50	1.005	0.99–1.018			
Cholesterol (mg/dL)	0.42	0.99	0.98–1.006			
Triglycerides (mg/dL)	0.23	0.99	0.98–1.003			
HOMA score	0.04	1.173	1.001–1.374	0.046	1.181	1.003–1.391
Eosinophilic Infiltrate	<0.001	4.83	2.242–11.418	<0.0001	5.86	2.464–13.962
Grading	0.11	0.59	0.35–1.015			
Staging	0.17	0.72	0.46–1.14			

Abbreviations: ALT, alanine aminotransferase, γ-GT, γ-glutamyltransferase; AP, alkaline phosphatase; UNL, upper normal limit.

HOMA score and histological EI were significantly associated with liver steatosis ($P < 0.10$). Multivariate analysis showed that HOMA score (OR 1.18; 95% CI 1.00–1.39) and EI (OR 5.86; 95% CI 2.46–13.96) were independent and significant risk factors for histological steatosis.

Factors associated with moderate / severe stage of fibrosis

Older age, platelet count, AP levels, EI and more severe necroinflammation were significantly associated with moderate / severe fibrosis in univariate analysis ($P < 0.10$). Multivariate analysis showed that grading (OR 6.82; 95% CI 2.46–18.80) and EI (OR 4.00; 95% CI 1.23–12.90) were independent and significant risk factors for moderate / severe fibrosis (Table 4).

Variables associated with hepatic eosinophilic infiltrate

We even investigated even possible correlations between demographics, history of drug assumption, biochemical variables, blood eosinophil count, and the EI. The results are reported in Table 5. Older age, drug assumption, high ALT

Table 4 Univariate and multivariate analysis of risk factors for moderate / severe stage of fibrosis in 147 patient with chronic hepatitis C

Variable	Univariate analysis			Multivariate analysis		
Indipendent	*P*	OR	95% CI	*P*	OR	95% CI
Age (years)	0.08	1.035	0.998–1.074	0.68	1.008	0.97–1.045
Gender (M / F)	0.42	0.581	0.298–1.557			
Drug assumption	0.877	0.938	0.383–2.292			
Platelet count ($\times 10^3$ / mm^3)	0.07	0.99	0.98–1.001	0.29	0.99	0.98–0.004
ALT–UNL	0.11	0.99	0.98–1.001			
γ-GT–UNL	0.67	1.002	0.99–1.009			
AP–UNL	0.045	0.98	0.97–1.00	0.46	1.007	0.980–1.025
Cholesterol (mg / dL)	0.96	1.00	0.98–1.01			
Triglycerides (mg / dL)	0.12	1.007	0.98–1.014			
HOMA-score	0.122	1.136	0.966–1.334			
Eosinophilic infiltrate	0.06	2.31	0.98–5.478	0.02	4.00	1.23–12.9
Grading	0.0002	4.317	2.049–9.094	0.0002	6.82	**2.46–18.8**
Steatosis	0.69	1.25	0.54–2.92			

Abbreviations: ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; AP, alkaline phosphatase; UNL, upper normal limit.

Table 5 Univariate and multivariate analysis of risk factors for hepatic eosinophilic infiltrate in 147 patient with chronic hepatitis C

Variable	Univariate analysis			Multivariate analysis		
Independent	*P*	OR	95% CI	*P*	OR	95% CI
Age (years)	0.001	1.057	1.022–1.094	0.08	1.040	0.994–1.089
Gender (M / F)	0.76	0.899	0.447–1.806			
Drug assumption	<0.0001	5.409	2.547–11.485	0.002	4.022	1.622–9.969
Platelet count ($\times 10^3$ / mm^3)	0.70	0.999	0.992–1.005			
Eosinophil count (c / mm^3)	0.12	1.009	0.991–1.025			
ALT–UNL	0.007	1.008	1.002–1.013	0.18	1.005	0.998–1.012
γ-GT–UNL	0.06	1.007	1.000–1.015	0.595	1.003	0.993–1.013
AP–UNL	0.25	1.008	0.994–1.021			
Cholesterol (mg / dL)	0.819	1.001	0.991–1.011			
Triglycerides (mg / dL)	0.52	1.002	0.995–1.010			
HOMA-score	0.825	1.017	0.874–1.184			

Abbreviations: ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; AP, alkaline phosphatase; UNL, upper normal limit.

and γ-GT serum levels were significantly associated with the presence of eosinophils in the liver tissue. The multivariate analysis showed that only drug assumption (OR 4.02; 95% CI 1.62–9.97) independently and significantly correlated with the EI.

DISCUSSION

Recently there has been increasing interest in the role of liver injury cofactors in the progression of chronic hepatitis C, steatosis alcohol abuse and iron overload have been identified as cofactors of liver damage [2,43]. Conversely other variables, such as chronic drug assumption or concomitant chronic diseases have not been clearly estimated as risk factors for the progression of liver damage. In the same setting, the significance of hepatic eosinophilic infiltrate has not been investigated.

Many experimental studies have shown that activated eosinophils could play an important role in the pathogenesis of the abovementioned liver diseases (PBC, PSC, human hepatic allograft rejection, idiopathic hypereosinophilic syndrome, graft-*vs*-host-disease) through release of granules containing TNF-α, highly cytotoxic proteins such as major basic protein and eosinophilic cationic protein [3–6,17–26,44,45].

Several reports have described a hepatic EI in patients with drug hepatotoxicity sustained by an immunoallergic mechanism, and induced by anticonvulsivants (phenytoin, carbamazepine) [30–32] and tenoxicam [33]. Recently, some authors have studied the significance of liver EI in patients with drug-induced liver injury [34]. The first experimental model to prove *in vivo* eosinophils-induced hepatotoxicity was established by Tsuda *et al.* in 2001. They used IL-5 transgenic mice with a consequent blood hypereosinophilia. These mice, after injection of lipopolysaccharide (LPS), developed an extensive hepatic lobular necrosis associated with a transmigration of eosinophils through vascular endothelium and degranulation of cytotoxic granules in inflamed areas. These eosinophilic injuries were transient, but liver specific. Pre-administration of gadolinium chloride (GdCl3) and anti-TNF-α markedly reduced the hepatic inflammation, suggesting that LPS-activated Kupffer cells play a key role in producing the cytotoxicity of eosinophils by releasing TNF-α [35]. More recently another study by Takahashi *et al.* [36], found, with an immunohistochemical technique, an increased expression of Ecalectin / galectin-9 (ECL / GL9), an eosinophilic chemoattractant isolated from T lymphocytes, specifically in liver biopsy of patients with drug-induced liver injuries [37,38].

In consideration of this biological and clinical evidence and of eosinophils' potential capacity to induce liver injury, we investigated this histological finding in HCV chronic patients, in relation to other clinical and histological features.

In our study we showed that drug-taking patients who were significantly older than non drug-taking patients, and with higher ALT and alkaline phosphatase levels, presented more severe necro-inflammatory activity and more frequent EI in liver parenchyma than patients without drug assumption. Moreover we found that the presence of EI is strongly and independently associated with drug assumption. Therefore we could speculate that the drug assumption, more frequent in older patients, can induce hepatic EI.

In multivariate analysis, we found a clear correlation between steatosis and EI. Histological hepatic steatosis is a very frequent finding in chronic hepatitis C patients. It can be identified as viral steatosis in genotype 3, and as metabolic steatosis typically of non-3 genotypes [46]. IR represents the pathogenetic key of metabolic steatosis [43,47,48], and different viral and non-viral mechanisms have been suggested in its pathogenesis [49–51]. We could speculate that eosinophils are able to induce steatosis by interfering with insulin signaling via TNF-α [52,53].

Furthermore, in our study we found that liver fibrosis was associated with EI, as well as with necroinflammatory activity. The association between EI and liver fibrosis could be explained by the eosinophils' ability to release TNF-α and other cytokines capable of increasing an inflammatory cascade and therefore stimulating the fibrogenic activity of stellate cells [54].

We have found no significant differences in the eosinophil count in patients with or without EI and in patients with or without drug assumption. So, in our study, the number of eosinophils in liver samples was not correlated with the number of eosinophils in the blood at the time of biopsy. This is in keeping with the observation of Pham *et al.* [55], who stated that the recruitment of eosinophils in the liver tissue may depend on local mechanisms. Selective recruitment of eosinophils in the liver of patients with drug-induced liver disease may be related to the expression of specific chemoattractants.

This study presents the limits of a retrospective analysis, particularly in the recruitment of cases. In fact, patients with clearer anamnestic data were preferred, so a recall bias may have been generated. Moreover the cut-off of three eosinophils that we utilized to assign the presence of EI in liver biopsy is arbitrary, with no previous specific reports in the literature. We observed that number of eosinophils was greater in the larger portal tracts, but as we considered the feature 'eosinophilic infiltrate' a dichotomous variable (0 or 1), these differences should not affect the meaning of the study. Therefore we counted the eosinophils in all portal tracts (at least eight) and we reported the three highest values of eosinophil counts in our database.

Our evidence could be relevant for clinical management of patients with hepatitis C and chronic drug assumption. We have demonstrated the strong association between use of drugs for common chronic diseases and EI, so for this category of patients a histological assessment of liver disease may be more opportune. In the future, a collection of consecutive

cases in a prospective study should be performed to confirm these findings.

In conclusion our study provides a prevalence estimate of EI in the liver histo-morphology of patients with chronic hepatitis C and documents its significant correlation with liver injury caused by steatosis and fibrosis.

ACKNOWLEDGEMENT

The authors would like to thank Warren Blumberg for his help in editing this paper.

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