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Histopathology of chronic hepatitis C in relation to virus genotype

Histopatološke manifestacije hroničnog hepatitisa C u odnosu na genotip virusa

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Abstract

Background/Aim. The natural history of hepatitis C virus (HCV) infection is variable and the factors determining the course of the illness are unclear. There are geographical variations in the distribution of different HCV genotypes, and some of them are related to the specific infection routes. Regarding our country, the dominant genotype is genotype 1b. It is unclear and still remains a question whether the distinct histopathological manifestations are related to the particular genotypes of HCV. Thus, the aim of this study was to determine whether the distinct histopathological manifestations of HCV infection might be in relation to the individual virus genotype. **Methods**. In this study we examined 126 patients with chronic HCV infection regarding the histopathological features, demographic data, and virus genotype. The observed groups of patients were predominantly infected with HCV genotypes 1b and 3a. **Results.** In this study we found that the patients infected with HCV genotype 1b had more frequently moderate or severe necroinflammatory activity of the disease, significantly higher grading score as compared with other genotypes ($p < 0.0001$). A higher degree of fibrosis was, also, more common in the patients infected with genotype 1b of HCV as compared with other genotypes ($p < 0.05$). There were no significant correlations between the necroinflammatory activity of the disease and the stage of fibrosis in 1b, 4 and mixed genotypes. **Conclusion.** The present data support the hypothesis that distinct genotypes of HCV are associated with the particular histopathological manifestation of the disease.

Key words:
hepatitis c, chronic; hepacivirus; histology; genotype.

Apstrakt

Uvod/Cilj. Prirodni tok hronične hepatitis C virusne infekcije (HCV) je različit i nije potpuno objašnjeno koji sve faktori utiču na tok bolesti. Poznato je da postoje geografske varijacije u distribuciji različitih genotipova virusa i ova različitost najčešće je povezana sa specifičnim načinom prenosa bolesti u pojedinim regionima. U našoj zemlji dominanatan je genotip 1b. Uprkos novijim saznanjima o samoj HCV infekciji i njenom lečenju, još uvek je nejasno i postavlja se pitanje da li je i u kojoj meri histopatološki nalaz povezan sa genotipom virusa izazivača bolesti. Cilj ove studije bio je da se utvrdi da li bi jasne histopatološke manifestacije HCV infekcije mogle biti u vezi sa individualnim genotipom virusa. **Metode**. Našim istraživanjem bilo je obuhvaćeno 126 bolesnika sa verifikovanom hroničnom HCV infekcijom i praćena je povezanost histopatološkog nalaza, demografskih podataka i genotipa virusa. U našoj grupi ispitanika dominirali su bolesnici sa genotipom 1b i 3a. **Rezultati.** Histopatološka evaluacija pokazala je da bolesnici inficirani genotipom 1b imaju češće srednje tešku ili tešku nekroinflamatornu aktivnost bolesti, kao i značajno viši *grading* skor u poređenju sa bolesnicima zaraženim drugim genotipovima virusa ($p < 0,0001$). Fibroza je, takođe, češća kod bolesnika sa genotipom 1b u poređenju sa drugim genotipovima ($p < 0,05$). U našoj grupi ispitanika nismo pronašli značajnu korelaciju između nekroinflamatorne aktivnosti bolesti i stadijuma fibroze kod inficiranih genotipom 1b, 4 i mešanim genotipom. **Zaključak**. Naše istraživanje potvrđuje hipotezu da genotip virusa može da bude značajan za histopatološke karakteristike bolesti.

Ključne reči:
hepatitis c, hronični; hepacivirus; histologija; genotip.

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Introduction

Chronic hepatitis C virus (HCV) infection is a disease with a wide clinical spectrum ranging from ″asymptomatic carriers″ to patients with fatal decompensated liver cirrhosis and primary liver cancer [1–6]. According to the World Health Organization (WHO) Report of 2002 [7], in 2001, chronic liver diseases were responsible for 1.4 million deaths, including 796 000 due to cirrhosis and 616 000 due to primary liver neoplasms. At least 20% of these deaths are probably attributable to HCV infection – more than 280 000 deaths. Chronic hepatitis C (CHC) is not the consequence of the direct destruction of hepatic cells by the virus. Rather, it results from an intermediate immune response that is large enough to induce hepatic cell destruction and fibrosis but not enough to eradicate the virus from its reservoirs [8]. Quantitatively, HCV specific CD4 and CD8 T-cell responses are weaker in the chronic phase than in the acute phase of the infection [9]. Histopathologically, inflammatory activity is less pronounced in chronic HCV infection when compared to liver lesions caused by hepatitis B virus infection. Also, the typical ″ground glass″ inclusions within hepatocytes, which are characteristic for cytoplasmic hepatitis B surface antigen expression, are not apparent in liver sections from chronically HCV-infected patients [10]. In contrast, typical hepatic lesions in chronic HCV infection might include bile duct damage, steatosis, or intraportal lymphoid clusters or follicles [11, 12]. HCV consists of six genotypes which do not change during the course of infection. Genotype-specific differences both within the translated and nontranslated regions of the genome have been suggested to be responsible for the different clinical manifestations of the disease. For instance, group-specific sequences both at the 3' and 5' terminus of the HCV genome have been suggested to be related to the differences in viral replication, translation, and pathogenicity among the different groups of HCV isolates [13, 14]. The knowledge of genotype is helpful for the prediction of sustained virological response and the choice of treatment duration [15]. Also, several European studies have suggested that genotype 1b might be linked to the severity of liver disease [16, 17], while some others might not [18, 19]. However, it has not been sufficiently clarified whether the prognosis differs among hepatitis C patients infected with different genotypes.

The present study was aimed to evaluate whether distinct histopathological manifestations of HCV infection might be associated with the individual viral genotypes.

Methods

Patients. A total of 126 patients infected with HCV as diagnosed by the presence of anti–HCV antibodies and HCV RNA in serum were studied prospectively (57 male, and 57 female patients, mean age 38 years, range 18–65). The patients were considered the candidates for the peginterferon-α2a therapy. Liver biopsies were performed as a part of a routine clinical evaluation. Chronicity was proven by histopathology, virologically and biochemically according to the established criteria: active virus replication (HCV RNA positivity), presence of anti–HCV antibody, elevated serum alanine aminotransferase (ALT) activities observed for a period longer than 6 months. The patients with active hepatitis B virus or human immunodeficiency virus infection and alcohol or drug abusers were excluded from the study. The following exclusion criteria were applied: clinical and biochemical evidence of the advanced disease, such as decompensate cirrhosis, serum albumin < 35 g/l, platelet count < 100 000/μl, white cell count < 3 500/μl, anemia (hemoglobin concentration of < 120 g/l in women and 130 g/l in men), psychiatric conditions, uncontrolled diabetes, autoimmune diseases, pregnancy or concomitant significant medical illnesses.

Histopathological evaluation

Since 2002, 167 transcutaneous liver biopsies have been performed. Hepatic biopsy is the gold standard exam to estimate the severity of tissue damage in chronic hepatitis and to determine histological activity. The estimation of fibrosis progression and the knowledge concerning associated factors in chronic hepatitis C is extremely important for understanding its natural history. The patients were submitted to a percutaneous hepatic biopsy, when clinically indicated. In this study, only liver biopsies of at least 20 mm length with a minimum of 11 portal tracts were included. The biopsy fragments were submitted to the conventional histological procedures. All the samples were fixed in formalin, embedded in paraffin wax and stained with haemotoxylin and eosin, chromotrope aniline blue, Perl's iron stain, PAS after diastase digestion, and Shikata's orcein. Grading for activity and staging of fibrosis were performed by the Ishak's scoring system [20]. Ishak's histological activity index (HAI) score consists of the following separate categories:

Grading HAI score

Necroinflammatory score consist of:

– periportal or periseptal interface hepatitis (piecemeal necrosis, range 0–4);

– confluent necrosis (range 0–6);

– focal (spotty) lytic necrosis, apoptosis and focal inflammation (range 0–4);

– portal inflammation (range 0–4);

Staging HAI score

– staging of fibrosis: architectural changes, fibrosis and cirrhosis (range 0–6)

Thus, necroinflammatory changes or the activity of hepatitis, respectively, were graded: absent (score 0), mild (score 1), moderate (score 3), or severe/marked (4 for all categories, except for category B (score 4–6). Architectural alterations were graded: absent (score 0, no fibrosis), mild (score 1–2, portal fibrosis expansion), moderate (score 3–4, portal-portal septa and portal-central), marked (score 5, bridging fibrosis, P-P and/or P-C septa and occasional nodules) and cirrhosis (score 6).

Histopathological evaluation was performed by trained pathologist without the knowledge of the patients' biochemical or clinical data, respectively.

Qualitative HCV analysis

Serological blood examination evidenced anti–HCV positivity in two samples, which were obtained in an one-month interval. Serum HCV RNA positivity was determined

using PCR method (*Amplicor, Roche Diagnostics*). The results of viral RNA titers in clinical samples are expressed as the number of viral copies per ml (copies/ml).

Determination of the genotype

Viral genotype was determined using the Line probe assay (*LiPa, Innogenetics, Ghent, Belgium*). The HCV genotypes were designed according to the nomenclature proposed by the Simmond's classification, which distincts five HCV RNK genotypes: 1b, 2a, 3, 4 and mixed genotype.

Statistical analysis

Descriptive statistical analyses were performed, and the results were presented as the mean ± standard error (SEM). Baseline data were descriptively summarized and the assessment of the differences was completed using a Microsoft Excel program and analysis was carried out using a GrapPad Prism/Instat 1.1 (GraphPad Software, California, USA). Differences between parameters were tested by one of the following tests: the Students *t* test, Fisher's exact test and one-way analysis of variance (ANOVA) followed by Dunnett's or Bonferroni's multiple comparison post-hoc significance testing. Absolute frequencies were statistically compared using the χ^2 test. Correlations were tested by Spearman or Pearson tests. A *p* value of less than 0.05 was considered to indicate significance ($p > 0.05$ considered not statistically significant).

Results

Analysis of demographic parameters in patients with chronic HCV infection in relation to virus genotype

Age and sex distribution of 126 patients with chronic HCV infection was compared with respect to virus genotype (Table 1). The patients infected with HCV type 1b were found to be older on average than the patients infected with type 3a and 4 ($p < 0.001$, $p < 0.05$ respectively, ANOVA, Bonferroni's multiple comparison *post-hoc* test). Also, the patients infected with HCV type 2 were found to be older on average than the patients infected with type 3a, 4 and mixed genotype ($p < 0.001$, $p < 0.001$, $p < 0.01$ respectively, ANOVA, Bonferroni's multiple comparison *post-hoc* test). The whole group had the similar duration of the disease before the study: 2 years on average, (ranging from 6 months to 8 years), and we did not find any significant difference in the duration of the disease in respect to virus genotype.

Histopathological manifestations of chronic HCV infection in relation to virus genotype

A total of 126 liver biopsy specimens from the patients being chronically infected with HCV genotype 1b, 2, 3a, 4 and mixed were evaluated histopathologically.

The data were analyzed by the statistical means and significant differences in absolute frequencies were indicated by bold characters (Table 2).

Also, we compared the degree of necroinflammatory activity between chronically infected patients with HCV genotype 1b versus other genotypes (2, 3a, 4 and mixed). In this study we found that the patients infected with HCV genotype 1b had more frequently moderate or severe necroinflammatory activity of the disease, a significantly higher grading score in comparison with other genotypes ($\chi^2 = 23.67$; $p < 0.0001$) (Figure 1a).

Table 1

Analysis of demographic characteristics of patients with chronic hepatitis C virus infection in relation to virus genotyp

Genotype	n (%)	Gender (male/female)	Mean age (year)	Duration of disease (year)
Genotype 1b	67 (53%)	37/30 (55%/45%)	41.66±1.516	2.33±0.167
Genotype 2	8 (6%)	4/4 (50%/50%)	53.0±3.30	2.13±0.479
Genotype 3a	32 (26%)	19/13 (59%/41%)	32.06±1.467	2.44±0.265
Genotype 4	8 (6%)	4/4 (50%/50%)	28.88±3.492	2.13±0.479
Mixed genotype	11 (9%)	5/6 (45%/55%)	33.27±2.316	2.36±0.411
Total	126 (100%)	69/57 (55%/45%)	37.94±1.108	2.37±0.134

Table 2

Histopathological manifestation of chronic hepatitis C infection in relation to various viral genotypes [20]

HCV genotypes*						Statistical analysis (*p* value, Fisher's exact test)									
HAI	1b	2	3a	4	Mixed	1b vs. 2	1b vs. 3a	1b vs. 4	1b vs. mixed	2 vs. 3a	2 vs. 4	2 vs. mixed	3a vs. 4	3a vs. mixed	4 vs. mixed
Grading score†	4±0.08	3.13±0.54	4.03±0.39	2.75±0.41	3.27±0.384	**0.027**	0.9201	**0.0128**	**0.0014**	**0.0271**	0.4287	0.3457	**0.0398**	**0.0321**	0.0419
Staging score‡	3.1±0.24	2.63±0.99	2.56±0.31	2.13±0.85	2.18±0.644	0.0614	0.2639	0.1830	0.3349	**0.0351**	0.3447	0.1986	0.1129	0.2134	0.3432
Total score (HAI)§	7.1±0.31	5.75±1.54	6.59±0.59	4.88±1.09	5.45±0.846	**0.0096**	**0.0316**	**0.0255**	**0.05**	0.1455	0.1911	0.0991	0.4461	0.2847	0.3772

*Mean ± SEM; Statistically significant differences ($p < 0.05$ or $p < 0.01$, Fisher's exact test) in mean values or ratios, respectively, are indicated by bold characters.

†Score of HAI grading - Necroinflammatory scores: A. Periportal or periseptal interface hepatitis (piecemeal necrosis - range 0–4); B. Confluent necrosis (range 0–6); C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation (range 0–4) ; D. Portal inflammation (range 0–4).

‡Staging of fibrosis: Modified staging: architectural changes, fibrosis and cirrhosis (range 0–6).

§ Score of histological activity index (HAI) components: grading plus staging.

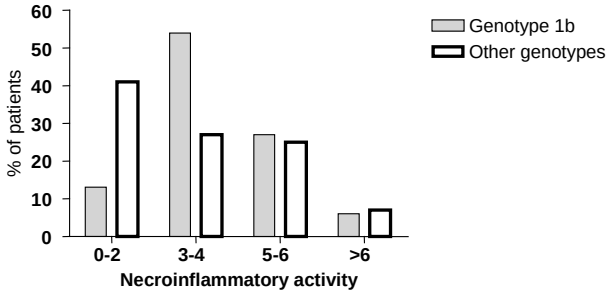

Fig. 1a – Comparison of a degree of necroinflammatory activity between chronically infected patients with HCV genotype 1b versus other genotypes (2, 3a, 4 and mixed)

A higher degree of fibrosis was, also, more common in the patients infected with genotype 1b infection in comparison with other genotypes ($\chi^2 = 12.95$, $p < 0.05$) (Figure 1b).

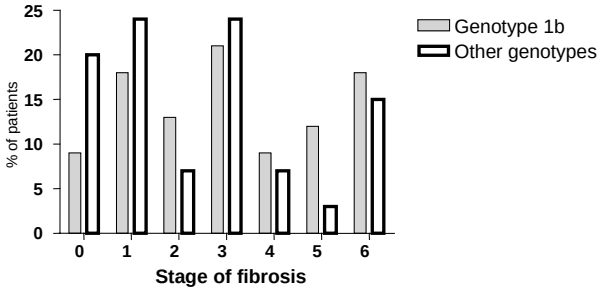

Fig. 1b – Comparison of a stage of fibrosis between chronically infected patients with HCV genotype 1b versus other genotypes (2, 3a, 4 and mixed)

According to the previous results, the patients infected with genotype 1b had significantly higher total HAI score in comparison with other genotypes ($\chi^2 = 21.73$, $p < 0.001$) (Figure 1c).

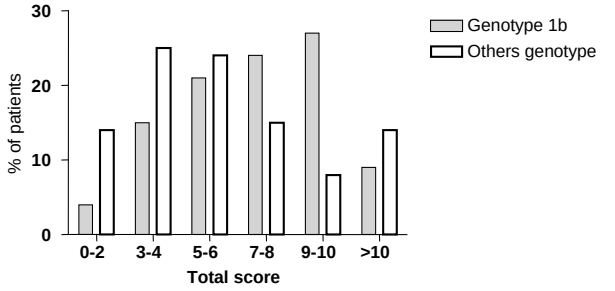

Fig. 1c – Comparison of total HAI score between chronically infected patients with HCV genotype 1b versus other genotypes (2, 3a, 4 and mixed)

There were no significant correlations between necroinflammatory activity of the disease and the stage of the fibrosis in 1b, 4 and mixed genotype (genotype 1b: r = 0.1151, p = N.S; genotype 4: r = 0.4187, p = N.S; mixed genotype: r = 0.3111, p = N.S.) (Figure 2a, 2d and 2e). Also, these two parameters of HAI were significantly correlated in genotype 2 and 3a (genotype 2: r = 0.8944, $p < 0.01$; genotype 3a: r = 0.3852, $p < 0.05$) (Figure 2a and 2b).

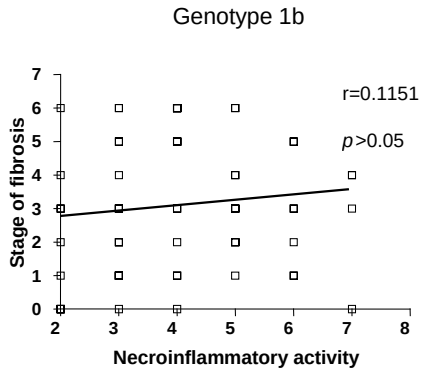

Fig. 2a – Correlation between necroinflammatory activity of disease and stage of the fibrosis in patients with genotype 1b

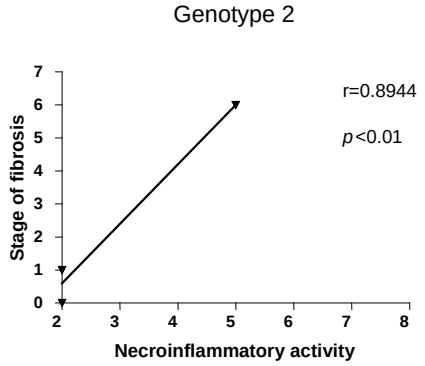

Fig. 2b – Correlation between necroinflammatory activity of disease and stage of the fibrosis in patients with genotype 2

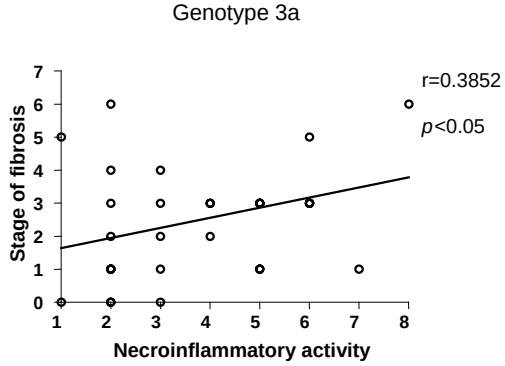

Fig. 2c – Correlation between necroinflammatory activity of disease and stage of the fibrosis in patients with genotype 3a

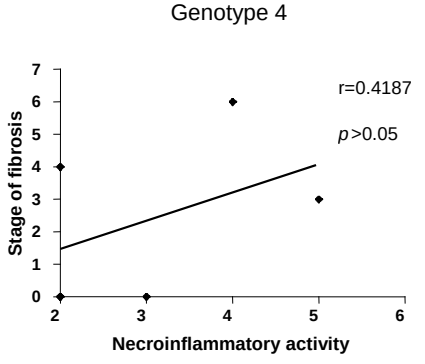

Fig. 2d – Correlation between necroinflammatory activity of disease and stage of the fibrosis in patients with genotype 4

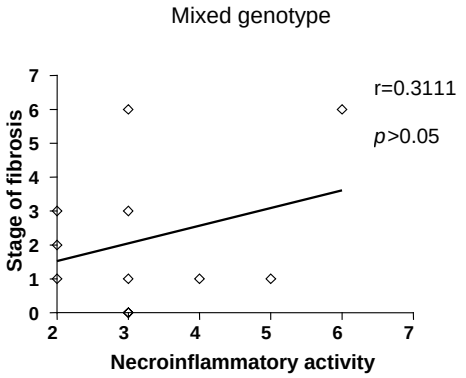

Fig. 2e – Correlation between necroinflammatory activity of disease and stage of the fibrosis in patients with mixed genotype

None of the histopathological parameters analyzed were found to be related to age, sex, or the history of drug abuse (p = N.S.) for each combination (data not shown).

Discussion

Progression to cirrhosis will occur in up to 30% of all cases [21] and the risk of developing hepatocellular carcinoma after longstanding infection is clearly increased [22]. Chronic HCV infection is often an insidious illness with a slow rate of progression in the majority of patients during the first two decades after the acute onset of the disease [23]. The reason why some people, not others, develop a severe disease may be determined by the effect of a large number of both host and viral variables that can influence disease progression. The recent paper by Datz et al. [24] suggests that rates of progression are not always linear and accelerated progression can occur in same cases without clear triggers, which emphasizes the necessity for continuing follow up of patients even when the disease progression appears to be slow. Host factors that may play a role include the route of infection, patient sex, age at time of infection, and alcohol consumption. Viral factors of relevance include viral genotype [25], viral load [26], and genetic heterogenecity [27], together with coinfection with hepatitis B virus [28] or HIV [29]. Several reports have suggested a difference in pathogenicity according to HCV genotype. Silva et al. [6] in their recent published data did not find an association between the genotypes of HCV and the evolution of fibrosis. Pozzato et al. [30] suggested that patients with genotype 1b had more severe liver disease compared to those with genotype 1a.

Ichimura et al. [31] suggested that genotype 1b was found more often in patients with chronic liver disease than in HCV carriers who were identified among apparently healthy blood donors, whereas the opposite relation was seen for genotype 2a. Yamada, Tanaka et al. [32] analyzed data for 4 176 patients with type C chronic liver diseases and suggested that the prevalence of genotype 1 increased and that of genotype 2 decreased in accordance with the progression of the severity of chronic liver disease. Kobayashi et al. [33] concluded that patients infected with genotype 2 HCV show more favorable histological outcome compared with those with genotype 1b. Deterioration of the grade of liver histology during the follow-up period was seen in 68% of the patients with genotype 1 as compared with 41.7% of those with genotype 2 ($p < 0.01$). Similarly, the deterioration of the stage of liver histology was more common in the former group than in the later one (63% and 38.9%, respectively; $p < 0.05$). The mean serum HCV RNA titer was significantly higher in the patients with genotype 1 than in those with genotype 2 ($p < 0.001$), and multivariate analysis showed the titer was one of the independent factors of the deterioration of the stage ($p = 0.0044$). This phenomenon may be related in part to the difference in pathogenicity between the two HCV genotypes [33].

In this study, liver histology was analyzed by assessing the grade of necroinflammatory process and the stage of fibrosis according to the Ishak's scoring system. We found that patients infected with HCV genotype 1b had more frequently moderate or severe necroinfalammatory activity of the disease, significantly higher grading score in comparison with other genotypes ($p < 0.0001$).

Also, higher degree of the fibrosis was more common in patients infected with genotype 1b infection in comparison with other genotypes ($p < 0.05$). This study confirmed the results of other authors reporting that genotype 1b is predominant in Europe, as well as significantly higher incidence of viremia in patients with genotype 1b infection in relation to other HCV genotypes ($p < 0.05$). Over 70% of patients infected by genotype 1b had more than 2×10^6 virus copies in 1 ml of blood, while in the event of genotypes 2, 3a and 4, the percentage was 40%, 38.5% and 30%, respectively [34]. This phenomenon may relate in part to the difference of pathogenicity between the genotypes.

The majority of studies have failed to show a relationship between hepatic necroiflammation and the progression of fibrosis, but there are studies with conflicting results. Using the Ishak and modified METAVIR scoring system, Rosenberg et al [35] found that moderate/ severe fibrosis was independently associated with the severity of necroinflammation in 275 patients with chronic hepatitis C. Their study included 221 patients with predominantly mild hepatitis C and dual biopsies without intervening treatment and it found that the intensity of necroinflammation was significant univariate predictor of fibrosis progression. Whereas patients with fibrosis progression (> 2 points on the Ishak's scale) had a mean necfoinflammatory score of 4.35, the mean score in patients without progression was 2.55 ($p = 0.007$). Serra et al. [36] in his study described that older age at the time of infection, increases the duration of the disease and alcohol consumption had a strong association with progression of fibrosis. On the other hand, HCV genotype did not seem to be an important determination for progression of liver disease.

In our study there were no significant correlations between necroinflammatory activity of disease and stage of the fibrosis in 1b, 4 and mixed genotype. Also, none of the histopathological parameters analyzed was found to be related to age, sex or the history of drug abuse for each combination. In addition, we should not neglect the fact that the histopathological result which we got through biopsy doesn't necessarily reflect the complete state of the liver disease and

severity of liver damage. Namely, sampling variability accounts for the high discordance rate (greater than 20%) between the stages of fibrosis or grades of activity when biopsy samples are taken from different parts of the liver at the same time [37]. Sampling variability of liver fibrosis in chronic hepatitis C has been recently thoroughly described by Bedossa et al. [38]. The coefficient of variation of staging of 15 mm biopsy sample, the usual mean length of biopsy samples in routine, is 55%.

Conclusion

In conclusion, our results suggest that distinct genotypes of HCV appear to be associated with a particular manifestation of the disease and that HCV genotypes seem to be an important determination for the progression of liver disease. An improved understanding of factors which determine differences in the natural course of HCV infection is needed.

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