

Histological factors that predict the liver fibrosis in patients with chronic hepatitis C

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Abstract

In chronic hepatitis, pathologies reveal a prominent inflammatory infiltrate portal consisting mostly of lymphocytes and plasma cells invading the portal spaces, although one can also identify macrophages, neutrophils or eosinophils. In all the forms of chronic hepatitis, fibrosis starts in the portal area, namely periportal, subsequently extends towards the lobules to the central veins, causing septa, followed by fibrosis. We studied 52 patients with chronic hepatitis C, who underwent a hematological, biochemical, virological and histopathological investigation. We found that the severity degree of the portal inflammation was in direct relation to the hepatitis activity index (HAI) and to the degree of fibrosis. The portal inflammation is dependent to the degree of fibrosis. The degree of inflammation significantly changes the distribution of cases with different degrees of fibrosis (*chi-square* $p=0.00011 < 0.001$). Periportal inflammation, periportal necrosis and focal necrosis are the morphological aspects of the necroinflammatory process best correlated to the occurrence and development of fibrosis.

Keywords: chronic hepatitis C, portal inflammation, focal necrosis, fibrosis, predicting factors.

Introduction

Chronic hepatitis is characterized by the occurrence of several types of lesions, of which the most important is piecemeal necrosis. This necroinflammatory transformation originally destroys the basal membrane of the liver cells and, in untreated patients, there is a continuous progressive erosion involving the liver parenchyma and portal territory [1]. Piecemeal necrosis cannot equally affect all regions of the portal level, but can affect a segment or the entire perimeter of the portal area. Degenerative changes affecting the liver cells in areas of necrosis are the bloating of piecemeal liver cells, cytoplasmic rarefaction, aggregation of cytoplasmic organelles, nuclear and membrane lysis and ultimately apoptosis [2]. This necroinflammatory process extends from a lobe to another, from the portal to the central area, actually causing a bridge. The evolution is progressive and irreversible, nodular regeneration is constant, widespread fibrosis and cirrhosis being the ultimate result [3].

Portal inflammation often varies in intensity and included infiltration with lymphocytes and plasma cells. There may be aggregated lymphoid follicles with reactive centers, these being considered typical changes but not necessarily pathognomonic for chronic hepatitis C [4]. These lymphoid follicles were found to contain activated B-cells in germinal centers surrounded by a network of

follicular dendritic cells. An area-surrounding mantle of B-cells activated the B-cells. B-cell follicles in turn are surrounded by an area of T-cells.

A characteristic feature of infection with hepatitis C virus (HCV) is the tendency to develop into a chronic infection that induces both injury and inflammation of liver fibrosis responsible for its emergence. During the chronic infection persistent intrahepatically, the HCV replication cycle causes an inflammatory chemokine secretion [5]. They recruit inflammatory cells, which secrete cytokines and generate free radicals that cause cell damage, thus triggering fibrogenesis [6]. It was found that plasma cells in areas of piecemeal necrosis in chronic hepatitis produce interleukin-1, which together with other mediators may stimulate the collagen production by hepatocytes in these areas [7]. Other studies showed that beta1 growth factor was found to be activated in chronic hepatitis C, and was considered to contribute to the process of hepatic fibrogenesis [8]. Other authors found that the histological fibrosis and chronic hepatitis C activity is correlated with the level of soluble receptors for the tumor necrosis factor, especially sTNF-R75 [9]. The TNF- α receptor is also active in the liver, particularly in chronic viral B hepatitis (HBV) and it is considered to play an important role in the pathogenesis of liver injury and viremia. It was also observed a decreased antioxidant

capacity of the liver, while the increase of free radicals concentration is in fact due to the presence and activity of HCV [10].

Fibrogenesis can be defined as a dynamic process characterized by cellular synthesis of the matrix constituents, a complex mixture of glycoproteins and proteoglycans organized in a three-dimensional network [11]. It is a non-specific protection mechanism, which limits the extension of inflammation and lasts as long as the pathogenic agent persists in the liver. In all forms of chronic hepatitis, fibrosis starts in the portal area, specifically periportally and subsequently extends towards the lobules to the central veins causing septa, followed by fibrosis [12]. The final stage of fibrosis is cirrhosis, with portal fibrosis extending to the central area, together with the emergence of regenerative nodules in the liver parenchyma.

In infection with HCV, the factors associated with a rapidly progressive fibrosis are represented by alcohol abuse, male gender, obesity, age over 40 years old, diabetes, alanine aminotransferase (ALT) serum increase, degree of necroinflammation and fibrosis at liver biopsy and co-infection with HBV and human immunodeficiency virus (HIV) [13]. The fibrosis progression rate varies significantly from person to person, therefore assessing the degree of fibrosis and necroinflammation progression speed can help the development of treatment strategies capable of improving prognosis [14].

In chronic hepatitis C, liver biopsy evaluation is considered the gold standard in the assessment of the degree of necroinflammation and fibrosis stage, helping in guiding treatment and determining prognosis. The assessment of liver biopsy in chronic hepatitis C is important for determining the degree (severity) of necroinflammation and evidence of disease progression, which is measured by the disease stage (fibrosis). This has therapeutic implications, as it is helpful in determining the response to antiviral treatment. It also has prognostic implications, as it predicts the progression of the disease.

In this study, we tried to identify the morphological parameters, which correlate with the occurrence and progression of the liver fibrosis process, in a group of 52 patients with chronic hepatitis C.

☐ Patients, Materials and Methods

We studied 52 patients with chronic hepatitis C, who underwent a hematological, biochemical, virological and histopathological investigation. The patients were monitored and treated with antiviral medication over a period of two years within Medical Clinic II of the Emergency County Hospital of Craiova, Romania. The chronic hepatitis C diagnosis was suggested by the clinical examination, supported by serum tests (anti-HCV antibody screening), and confirmed by liver biopsy and virological tests (quantitative HCV RNA test).

The study was performed with the approval of the Ethics Committee of the University of Medicine and Pharmacy of Craiova and a written informed consent was obtained from every patient in the study.

The hematological and biochemical investigations were performed in the Hospital Laboratory using Celltac

Nihon Kohden hematology and Vitros 250 dry chemistry analyzers. For the immunological and virological determinations we used Chiron Riba 3.0 HCV SIA and Cobas Amplicor HCV 2.0 methods.

The severity of liver fibrosis and necroinflammatory lesions obtained from liver biopsy was evaluated by Ishak score. The liver biopsy was performed on the patients from the study group using the percutaneous method, using the special kit Hepafix (B. Braun Melsungen AG). The removed liver tissue samples (sized between 10–25 mm and 1–1.4 mm) were placed in formalin solution and then analyzed according to the standard protocol performed in the Laboratory of Pathological Anatomy, Emergency County Hospital of Craiova.

The selection criteria for patient inclusion were the following: age group from 18 to 70 years old; presence of anti-HCV antibodies; detected viral load HCV RNA; normal values of hematological (platelet count $>150\,000/\text{mm}^3$) and biochemical parameters (prothrombin index $>70\%$). The criteria for patient exclusion were: clinical and paraclinical signs of cirrhosis (hemorrhagic syndrome, edema, bleeding esophageal varices, ascites); autoimmune diseases (autoimmune hepatitis, autoimmune thyroiditis, collagenosis); mental disorders (chronic ethylism, high risk drug addiction and hepatotoxic drugs) and non-cooperative patients.

Statistical analysis was performed by Department of Medical Informatics and Biostatistics of the University of Medicine and Pharmacy of Craiova, using Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA) for data processing. To test the normality of the data, we used the Anderson–Darling test. Because the numerical variables investigated had a normal data distribution, globally or in every studied group, we were allowed to use the parametric statistical tests (*e.g.*, Student's *t*-test, ANOVA) and the results were summarized as the mean value \pm standard deviation. For all statistical tests, *p*-values less than 0.05 were considered significant. We used the *chi*-square (χ^2) test to highlight the relationship between the two factors; the test was used to interpret the incidence tables generated by cross tabulation of the two factors monitored in the study. To assess correlation, we decided to use the Spearman ρ (rho) rank correlation coefficient, because of the repeating values recorded for some of the investigated parameters.

☐ Results

The patient group consisted of 12 men and 40 women, aged between 18 and 70 years old, with a body weight varying from 55 to 120 kg (Table 1).

Depending on the HCV RNA viral levels, we observed moderately high levels (less than 2 000 000 copies/mL) in 40 patients, and very high levels (over 2 000 000 copies/mL) in 12 patients.

The most frequent histopathological aspects described in patients with chronic viral C hepatitis were portal inflammation (52 patients; 100%), periportal or piecemeal necrosis (52 patients; 100%), focal necrosis (52 patients; 100%) and liver fibrosis (51 patients; 98%). The histopathological changes rarely found in these patients were:

confluent (bridging) necrosis (32 patients; 61.5%), hepatic steatosis (17 patients; 32.6%), lymphoid infiltrates (24 patients; 46.1%) and biliary duct lesions (10 patients; 19.2%).

Table 1 – Hematological, biochemical and virological parameters

Parameter	Mean value	Limit values
Hemoglobin [g%]	13.2±2.98	9.1–16.6
Leukocyte count [/ mm^3]	6535.5±2184.88	3130–9530
Platelet count [/ mm^3]	215 143.25±93 857.08	90 000–366 000
Total bilirubin [mg%]	1.16±0.4	0.8–2.2
ALT [U/L]	107.8±67.5	21–258
AST [U/L]	96.64±47.82	24–300
Albumin [mg%]	3.73±0.54	3.1–4.5
Prothrombin index [%]	92.9±7.4	79–100
HCV RNA titer [copies/mL]	1 040 477.05±497 842.96	807–3 510 000

The assessment of the histological activity index through Ishak score determined the presence of: mild chronic hepatitis (hepatitis activity index – HAI 1–6) in 12 (23.1%) patients, moderate chronic hepatitis (HAI 7–10) in 21 (40.4%) patients, and severe chronic hepatitis (HAI 11–16) in 19 (36.5%) patients (Table 2, Figure 1).

Table 2 – HAI distribution in our group of patients

HAI value	<7	7–10	>10	Total
Total	12 (23.08%)	21 (40.38%)	19 (36.54%)	52 (100%)

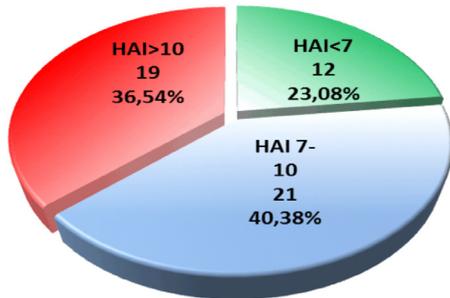


Figure 1 – HAI distribution in our group of patients.

Portal inflammation, found in all patients with chronic viral C hepatitis (52 pts; 100%), was the result of the presence of an inflammatory infiltrate inside the portal tracts consisting of lymphocytes, plasmocytes and polymorphonuclears. The severity degree of the portal inflammation was in direct relation to the chronic hepatitis activity, thus the average severity degree of inflammation was: 1 in mild chronic hepatitis; 1.72 in moderate chronic hepatitis, and 2.52 in severe chronic hepatitis. The degree of portal inflammation varied depending on the degree of fibrosis: 1 in first degree of fibrosis; 1.65 in second degree of fibrosis; 2.08 in third degree of fibrosis; 2.57 in fourth degree of fibrosis; 3 in fifth degree of fibrosis (Table 3).

In chronic hepatitis, the pathology reveals a prominent inflammatory infiltrate portal, consisting mostly of lymphocytes and plasma cells invading the portal spaces, although one can also identify macrophages, neutrophils or eosinophils. Occasionally, there could also be identified primary lymphoid follicles with germinal centers. Besides the various degrees of fibrosis, bile ducts reactivity may also be identified in these portal spaces (Figure 2).

Table 3 – Correlation between degree of fibrosis and portal inflammation

Fibrosis stage	Portal inflammation				Total
	1	2	3	4	
1	8 (15.38%)	0 (0%)	0 (0%)	0 (0%)	8 (15.38%)
2	6 (11.54%)	15 (28.85%)	2 (3.85%)	0 (0%)	23 (44.23%)
3	3 (5.77%)	5 (9.62%)	4 (7.69%)	0 (0%)	12 (23.08%)
4	0 (0%)	4 (7.69%)	2 (3.85%)	1 (1.92%)	7 (13.46%)
5	0 (0%)	0 (0%)	2 (3.85%)	0 (0%)	2 (3.85%)

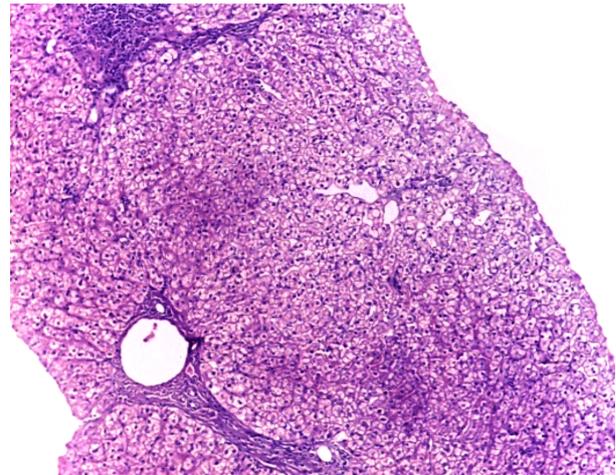


Figure 2 – Portal inflammation (HE staining, ×100).

Portal inflammation is related to the degree of fibrosis, the degree of inflammation, significantly changing the distribution of cases with varying degrees of fibrosis (*chi-square* $p=0.00011 < 0.001$). Calculating the Spearman correlation coefficient, we obtained $\rho=0.609$, thus supporting the existence of a highly significant correlation ($p < 0.001$) between the degree of fibrosis and portal inflammation (Figure 3).



Figure 3 – Correlation between degree of fibrosis and portal inflammation.

Outside the portal spaces, hepatocyte necrosis in chronic hepatitis can be focal or show a confluent appearance. Focal necrosis of the intralobular hepatocytes, occurred in all selected patients (52 patients; 100%), presented different degrees of severity in accordance with the hepatitis activity index (HAI): average severity degree of 1.1 in mild chronic hepatitis, average degree of 1.8 in moderate chronic hepatitis, and average severity degree 2.8 in severe chronic hepatitis. At the same time, the degree of focal necrosis recorded different values depending on

the degree of fibrosis: 1.125 in first degree of fibrosis; 1.65 in second degree of fibrosis; 1.83 in third degree of fibrosis; 2.57 in fourth degree of fibrosis; 2.5 in fifth degree of fibrosis (Table 4).

Table 4 – Correlation between degree of fibrosis and focal necrosis

Fibrosis stage	Focal necrosis			Total
	1	2	3	
1	7 (13.46%)	1 (1.92%)	0 (0%)	8 (15.38%)
2	8 (15.38%)	15 (28.85%)	0 (0%)	23 (44.23%)
3	0 (0%)	10 (19.23%)	2 (3.85%)	12 (23.08%)
4	0 (0%)	3 (5.77%)	4 (7.69%)	7 (13.46%)
5	0 (0%)	1 (1.92%)	1 (1.92%)	2 (3.85%)

Chronic inflammatory cells spread in the parenchyma together with the presence of apoptotic bodies, Kupffer cell necrosis and degeneration of surrounding hepatocytes (Figure 4).

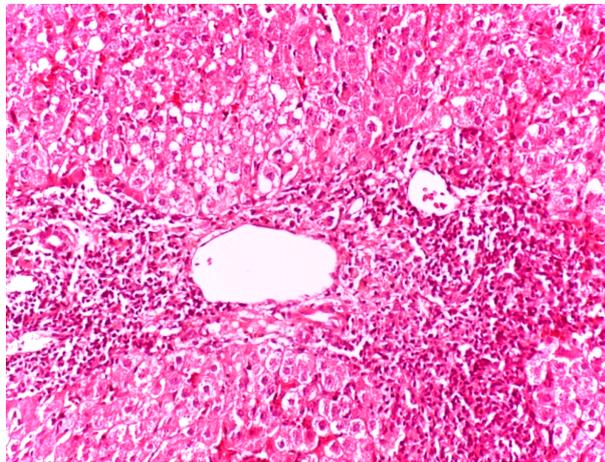


Figure 4 – Focal necrosis (HE staining, ×100).

The existence of highly significant differences between the distribution of various degrees of fibrosis according to the degree of necrosis focus is proven by the *chi*-square test, the result being $p=0.0000127 < 0.001$. Due to this fact, we checked the correlation between the degree of fibrosis and the necrosis focus. By calculating the Spearman correlation coefficient, we obtained a value $\rho=0.716$, which supports the existence of a highly significant correlation ($p < 0.001$) between the degree of fibrosis and the necrosis focus (Figure 5).

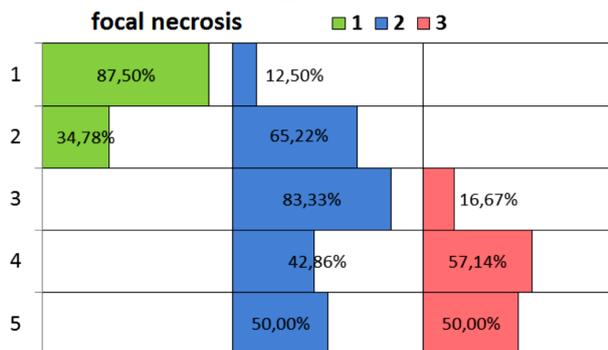


Figure 5 – Correlation between degree of fibrosis and focal necrosis.

Confluent necrosis (CN), found in 32 (61.5%) patients, had the severity degree in accordance with the hepatitis activity level, the determined values being: 0.4 in mild chronic hepatitis, 0.88 in moderate chronic hepatitis and 1.42 in severe chronic hepatitis. Confluent necrosis severity varied depending on the degree of fibrosis: 0.8 in first degree of fibrosis; 0.78 in second degree of fibrosis; 1.41 in third degree of fibrosis; 1.28 in fourth degree of fibrosis; 0.5 in fifth degree of fibrosis (Table 5).

Table 5 – Correlation between degree of fibrosis and confluent necrosis

Fibrosis stage	Confluent necrosis				Total
	0	1	2	3	
1	3 (5.77%)	5 (9.62%)	0 (0%)	0 (0%)	8 (15.38%)
2	11 (21.15%)	6 (11.54%)	6 (11.54%)	0 (0%)	23 (44.23%)
3	3 (5.77%)	2 (3.85%)	6 (11.54%)	1 (1.92%)	12 (23.08%)
4	2 (3.85%)	2 (3.85%)	2 (3.85%)	1 (1.92%)	7 (13.46%)
5	1 (1.92%)	1 (1.92%)	0 (0%)	0 (0%)	2 (3.85%)

Performing *chi*-square test to evaluate the influence of confluent necrosis degree on cases with varying degrees of fibrosis, we obtained that a value $p=0.293 > 0.05$, not statistically significant (Figure 6). As expected, after verifying the correlation between the confluent degree of necrosis and the extent of fibrosis, we obtained non-significantly statistical results, Spearman $\rho=0.229$ (equivalent to $p=0.103 > 0.05$). Performing the *chi*-square test for the influence of CN degree on case distribution with various degrees of fibrosis, we obtained a value $p=0.293 > 0.05$, not statistically significant. As expected, verifying the correlation between the CN degree and the extent of fibrosis, we obtained non-significantly statistical results, Spearman $\rho=0.229$ (equivalent to $p=0.103 > 0.05$).

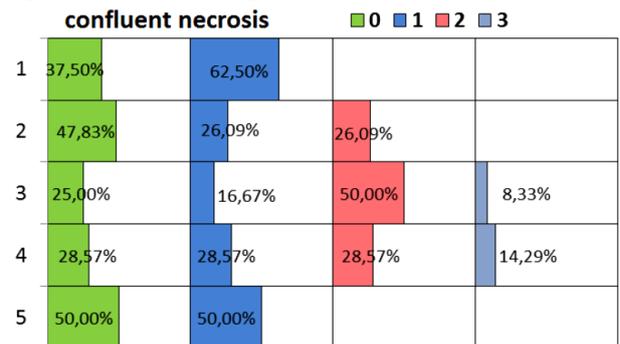


Figure 6 – Correlation between degree of fibrosis and confluent necrosis.

Periportal (piecemeal) necrosis, determined in all selected patients (52 patients; 100%), had different degrees of severity in accordance with the hepatitis activity index: average degree 1 in mild chronic hepatitis; average degree 2.57 in moderate chronic hepatitis; and average degree 3.1 in severe chronic hepatitis. This change showed a series of alterations, such as: ballooning degeneration, acidophilic cytoplasm, apoptosis, as well as pseudo-glandular pattern of hepatocytes (rosette formation). Periportal necrosis (PN) severity varied depending on the degree of fibrosis: 1 in first degree of fibrosis; 1.91 in

second degree of fibrosis; 2.5 in third degree of fibrosis; 3.14 in fourth degree of fibrosis; four in fifth degree of fibrosis (Table 6).

Table 6 – Correlation between degree of fibrosis and periportal necrosis

Fibrosis stage	Periportal necrosis				Total
	1	2	3	4	
1	8 (15.38%)	0 (0%)	0 (0%)	0 (0%)	8 (15.38%)
2	7 (13.46%)	11 (21.15%)	5 (9.62%)	0 (0%)	23 (44.23%)
3	0 (0%)	6 (11.54%)	6 (11.54%)	0 (0%)	12 (23.08%)
4	0 (0%)	1 (1.92%)	4 (7.69%)	2 (3.85%)	7 (13.46%)
5	0 (0%)	0 (0%)	0 (0%)	2 (3.85%)	2 (3.85%)

Interface hepatitis or piecemeal necrosis is a key feature of chronic hepatitis, and it is usually distributed focally, but it can even be absent in samples from patients with minimal necroinflammatory activity. The lymphocytes and plasma cells from the portal spaces can be associated with degenerating hepatocytes at the interface between the fibrocollagenous tissue of the portal space and the Remak's hepatocyte cords (or the limiting plate). Hepatocytes affected by piecemeal necrosis appear swollen, and with clumped cytoplasm, sometimes with the presence of apoptotic bodies (Figure 7).

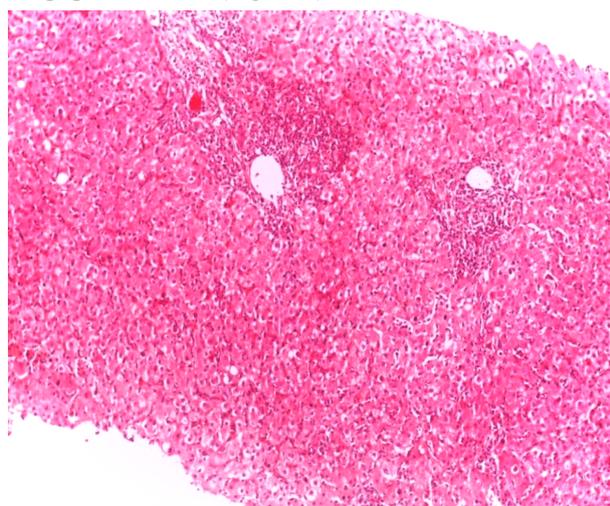


Figure 7 – Periportal necrosis (HE staining, ×100).

Chi-square test result, $p=6.6 \times 10^{-9} < 0.001$, indicating a highly significant difference between the distribution of various degrees depending on the degree of fibrosis and periportal necrosis, we checked whether there is a correlation between the degree of fibrosis and periportal necrosis. Calculating the Spearman correlation coefficient $\rho=0.749$, we proved the existence of a highly significant correlation ($p < 0.001$) between the degree of fibrosis and periportal necrosis (Figure 8).

Hepatic fibrosis was diagnosed in the majority of patients with chronic hepatitis C (98%; 51/52), and the severity degree of fibrosis was correlated with the hepatitis activity index, the average degree of fibrosis being 1.25 in patients with mild chronic hepatitis; 1.76 in patients with moderate chronic hepatitis and 3.57 in patients with severe chronic hepatitis.

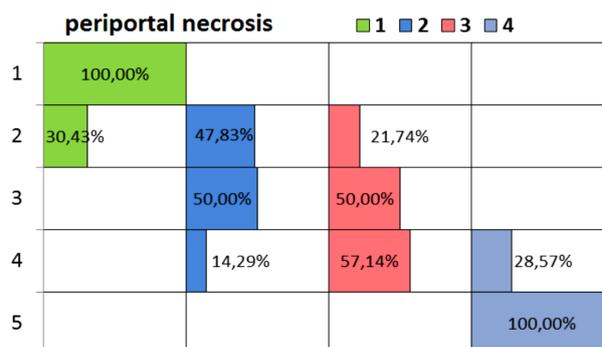


Figure 8 – Correlation between degree of fibrosis and periportal necrosis.

It occurred in stage 0 in one (1.9%) patient, in stage 1 in seven (13.7%) patients, in stage 2 in 23 (45%) patients, in stage 3 in 12 (23.5%) patients, in stage 4 in seven (13.7%) patients, in stage 5 in one (1.9%) patient and in stage 6 in one (1.9%) patient. If fibrosis occurs in stage 0 in one patient (with mild chronic hepatitis), portal fibrosis occurred in 30 patients, portal and intralobular fibrosis in 19 patients, and liver cirrhosis in two patients (with severe chronic hepatitis). Depending on the fibrosis degree (FD) score value, we found: mild fibrosis (FD between 0 and 2) in 31 patients, moderate fibrosis (FD between 3 and 4) in 19 patients, and severe fibrosis or cirrhosis in two patients (FD between 5 and 6).

Regarding the histopathological changes found in these patients, we found portal lymphoid infiltrates (24 patients; 46.1%), hepatic steatosis (17 patients; 32.6%), and destruction of bile ducts (10 patients; 19.2%).

The portal lymphoid infiltrates, discovered in 24 (46.1%) patients, had a different distribution in accordance with the hepatitis activity index. This aspect was found in 25% (3/12 patients) of the patients with mild chronic hepatitis, 57% (12/21 patients) of the patients with moderate chronic hepatitis, and 47.36% (9/19 patients) of the patients with severe chronic hepatitis.

Hepatic steatosis, found in 17 (32.6%) patients, had an incidence varying in accordance with the hepatitis activity index (HAI): 16.6% of the patients with mild chronic hepatitis, 38% of the patients with moderate chronic hepatitis and 36.8% of the patients with severe chronic hepatitis.

The destruction of bile ducts, the most rarely histopathological aspect found in the patients with chronic hepatitis C (19.2%; 10/52), was an incidence varying in accordance with the hepatitis activity index: 8.3% in patients with mild chronic hepatitis, 19.04% in patients with moderate chronic hepatitis, and 26.31% in patients with severe chronic hepatitis.

Discussion

The aim of this paper was to define the histopathological parameters that are associated to liver cirrhosis and, therefore, to a poor evolution of chronic HCV infection.

The characteristic feature of chronic hepatitis is portal inflammation with or without lobular inflammation. In chronic hepatitis, the pathologies reveal a prominent inflammatory infiltrate portal consisting mostly of lym-

phocytes and plasma cells invading the portal spaces, although one can also identify macrophages, neutrophils or eosinophils [15]. The inflammation is of variable intensity, and varies among different portal tracts in one biopsy, among various biopsies from one patient, and also from patient to patient [16]. Occasionally, there can also be identified primary lymphoid follicles with germinal centers. Portal lymphoid infiltrates were reported to be present in 33% up to 78% of cases and are composed of small lymphocytes, some with germinal centers [17, 18]. These occur more frequently in HCV genotype 1B infection [19]. The lymphoid aggregates occur in proximity to bile ducts and are sometimes associated to the damage of the bile duct epithelium [20]. In a previous study, there was shown that portal inflammation is a common injury in all patients with acute or chronic viral hepatitis. In our study, we found that the severity degree of the portal inflammation was in direct relation to the hepatitis activity index (HAI) and to the degree of fibrosis. Portal inflammation is related to the degree of fibrosis [21], the degree of inflammation significantly changing the distribution of cases with various degrees of fibrosis (*chi-square* $p=0.00011 <0.001$). More commonly, portal inflammation involves the adjacent hepatic parenchyma, thus obscuring the sharp demarcation of portal–parenchyma interface (limiting plate), this being called interface hepatitis [16]. Interface hepatitis (formerly known as piecemeal necrosis) is a key feature of chronic hepatitis, and it is usually distributed focally, but it can be absent in samples from patients with minimal necroinflammatory activity [22]. Interface hepatitis is described by the spreading of lymphoplasmacytic inflammation across the portal–parenchymal interface, into the periportal hepatocytes [23, 24]. Interface hepatitis is focal and often associated with hepatocytic damage and apoptosis. Emperipolesis, characterized by penetration of lymphocytes into hepatocytes, was also described in chronic hepatitis [25]. Considerable attention was given to the extension of portal inflammatory cells into the adjacent liver parenchyma associated to the destruction of the limiting plate and damaging of periportal hepatocytes (interface hepatitis) [26]. One study showed that the presence of interface hepatitis in initial biopsies from patients with chronic hepatitis correlates with a subsequent development of cirrhosis [27]. Other studies showed a relation between the severity of the necroinflammatory activity (including interface hepatitis) in an initial biopsy and the development of fibrosis or cirrhosis in follow-up biopsies [28]. In our work, periportal necrosis was determined in all the selected patients. It had different degrees of severity in accordance to the hepatitis activity index, and to the degree of fibrosis. The *chi-square* test result ($p=6.6 \times 10^{-9} <0.001$) indicated a highly significant difference between the distribution of various degrees depending on the degree of fibrosis periportal necrosis, therefore we checked whether there is a correlation between the degree of fibrosis and periportal necrosis.

Intralobularly, hepatocyte necrosis can be focal or show a confluent appearance [29]. Chronic inflammatory cells from portal spaces spread into the parenchyma together with the presence of apoptotic bodies, Kupffer cell necrosis and degeneration of surrounding hepatocytes

[30]. Focal necrosis is a form of lobular hepatitis, and it is composed of small clusters of lymphocytes and/or macrophages enclosing damaged/apoptotic hepatocytes. Small clusters of macrophages containing Periodic Acid–Schiff (PAS)-positive diastase-resistant material indicate prior foci of necrosis [16]. In our study, focal necrosis of the intralobular hepatocytes was found in all selected patients, with different degrees of severity in accordance with the hepatitis activity index and the degree of fibrosis. The existence of high significant differences between the distribution of various degrees of fibrosis according to the degree of necrosis focus is demonstrated by *chi-square* test and the result was $p=0.0000127 <0.001$. Due to this fact, we checked the correlation between the degree of fibrosis and the necrosis focus. A key question for physicians is whether confluent necrosis contributes to the progression of liver disease or it is a universal lesion in the evolution of chronic hepatitis C [31].

Bridging necrosis, a severe form of lobular inflammation, extending over two vascular structures, and confluent necrosis that spans over several lobules [1] are uncommon in chronic hepatitis C. In most independent clinical practice of clinical trials, the degree of activity, unless particularly severe, is not as important as the stage of fibrosis in the decision on whether or not to pursue treatment [32–36]. In our work, confluent necrosis found in only 32 (61.5%) patients had the severity degree in accordance with the hepatitis activity index, but not with the degree of fibrosis. By performing the *chi-square* test to evaluate the influence of confluent necrosis degree on cases with various degrees of fibrosis, we obtained a value $p=0.293 >0.05$, not statistically significant.

☐ Conclusions

In chronic hepatitis C infection, the severity degree of the portal inflammation was in direct relation to the hepatitis activity index (HAI) and to the degree of fibrosis. Portal inflammation is related to the degree of fibrosis, the degree of inflammation significantly changing the distribution of cases with various degrees of fibrosis. Periportal inflammation, periportal necrosis and focal necrosis are the morphological aspects of the necroinflammatory process, best correlated with the emergence and development of fibrosis. The confluent necrosis was a morphological parameter, which was not correlated with liver fibrogenesis in patients with chronic hepatitis.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Ileana Octavia Petrescu and Viorel Biciușcă equally contributed to this work.

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