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Evaluation and quantification of morphological characteristics associated to hepatitis C virus infection: comparative study with hepatitis B

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Abstract

Hepatic histological features described in hepatitis C virus (HCV) infection include bile duct damage, lymphoid follicles and/or aggregates in portal tracts, large- and small-droplet fat, Mallory body-like material in hepatocytes, liver cell dysplasia and multinucleation, and activation of sinusoidal inflammatory cells. We have examined the frequency of these lesions in 189 liver biopsy specimens, to distinguish HCV from hepatitis B infection. We have analyzed a set of three features of tures more likely to be seen in HCV than in HBV infection: lymphoid follicles and/or aggregates (68%/20%), bile duct damage (44%/22%) and large-droplet fat (77%/64%). A fourth lesion, Mallory body-like material, was seen more frequently in HCV than HBV (25%/5%). These four histological lesions are useful pathological parameters in the diagnosis of liver disease caused by HCV.

***Keywords*:** liver biopsy, hepatitis C virus, histologic features.

Introduction

Recent progress in molecular biology led to the cloning and sequencing of hepatitis C virus (HCV), the agent responsible for most cases of non-A, non-B hepatitis, as well as many cases of "cryptogenic" cirrhosis and the subsequent development of a clinically useful assay [1–3].

HCV is an RNA virus related to *Flaviviridae* and some RNA plant viruses. It is a 50–60 nm, enveloped, single-stranded, positive-polarity RNA virus. The genome is approximately 10 Kb with a single open reading frame [4, 5].

Chronic HCV infection occurs all over the world [6]. It is estimated that in 40% to 50% of HCV patients the disease progresses to chronic hepatitis, and as many as 20% of such patients become cirrhotic [7–9]; moreover, HCV may augment liver lesions in chronic alcoholism [10, 11] and it is connected to the development of hepatocellular carcinoma [12, 13], epidemiologic studies suggesting a strong association between chronic HCV infection and the development of liver carcinoma in about 15–30 years after infection [14].

Although the various clinical and laboratory evaluations can often define the specific nature of chronic hepatitis, the liver biopsy remains important in the evaluation of the patient. The liver biopsy can confirm the clinical diagnosis and also allows the exclusion of many diseases that can manifest as "chronic hepatitis" [15–18].

Many studies have tried to correlate viral infection to microscopic liver lesions [19].

Immunoperoxidase methods utilizing antibodies to both HBsAg and hepatitis B core antigen (HBcAg) are readily available. HBsAg may be present diffusely or focally in the cytoplasm, and sometimes may also have a membranous expression. HBcAg is expressed in the liver cell nuclei in the majority of cases, but both intranuclear and granular intracytoplasmic expression may be simultaneously present, especially with high levels of viral replication [20].

There is no reliable immunohistochemical method to demonstrate hepatitis C virus in formalin- fixed, paraffin-embedded tissue. A commercially available antibody that gives reliable results is not available. Hepatitis C virus antigen can be demonstrated in cryostat-prepared sections, but the antibody for this antigen is not widely available [21–23]. The gold standard for diagnosis has been the identification of viral RNA using the polymerase chain reaction.

Although, there are still no cellular markers analogous to HCV infection, a series of papers has helped define the key histologic features of chronic hepatitis C including:

- marked and patchy expansion of portal tracts by predominantly lymphocytic infiltrate with minimal interface hepatitis into adjacent lobule, often with
- a well defined lymphoid aggregate, including true follicle formation with a well-defined germinal center;

▪ varying degrees of bile duct damage and even focal bile duct loss [24];

▪ varying degrees of steatosis, including micro-and macrovesicular fat;

▪ sinusoidal cell hyperplasia.

Less commonly seen features include: slight lobular necrosis, liver cell dysplasia, multinucleation and accumulation of Mallory-like material in hepatocytes.

These features are not pathognomonic and have been recognized in other forms of chronic hepatitis, including hepatitis B, but they are most often seen in hepatitis C [25–35].

In order to quantify histologic features that differentiate HCV infection, we examined the frequency of these seven lesions in liver biopsies obtained from two groups of patients.

Material and methods

The studied material consisted of liver parenchyma fragments collected by liver biopsy specimens, totalizing a number of 189 cases, divided into two groups following clinical, serological and histological investigation. The first group included 125 chronic hepatitis C patients, most of them with serum ALT levels situated at the upper limit or a persistently increased level of at least 1.5 times the normal level (and an average level of 91.42 U/l). Patients tested positive for HCV, with personal histories most frequently including blood transfusions and occupational exposures. The 64 patients with chronic B hepatitis who were included into the second group (for the comparative study of histologic features) tested positive for HBsAg, showing an increased level of serum ALT, with an average of 184.5 U/l.

For routine histologic assessment of liver biopsies, tissue fragments fixed in 10% formalin, paraffin-embedded and sectioned at 3–4 μm, were stained by Hematoxylin–Eosin, van Gieson, Gömöri's Trichrome, PAS and silver method techniques.

On liver biopsy samples we analyzed and graded portal and periportal necrosis, inflammation (portal and lobular) and fibrosis, obtaining a significant score, which allowed the classification of biopsies according to the type of chronic hepatitis.

Moreover, on biopsies performed in patients from the HBV group we noted the presence of ground-glass hepatocytes, quantifying them as: 0 – absent, 1 – low number of ground-glass hepatocytes, 2 – moderate number, and 3 – high number.

The seven histologic features considered as useful for the diagnosis of HCV-induced liver disease were defined and graded according to the Lefkowitch criteria (1993) as follows [29]:

Portal *lymphoid follicles or aggregates* were assessed as absent or present. We noted the presence of lymphoid follicles with germinal centers surrounded by small lymphocytes or the aggregation of portal tract, closely packed small lymphocytes, often adjacent to interlobular bile ducts.

We classified as *bile duct damage* the presence of interlobular bile ducts surrounded by a lymphocyte-plasma cell infiltrate (the lesions consisting of migration of inflammatory cells through the basal membrane into/or between epithelial cells), layer stratification, formation and polarity loss in epithelial cells, variations of nuclear chromatine, vacuolization of epithelial cells, degeneration or mitotic activity or combinations of these.

Small-droplet (microvesicular) fatty change. Small vacuoles of lipid within hepatocytes arranged such that the nucleus is maintained in position at or near the cell's center, were graded: 0 – absent, 0–1 – few, 1 – mild, 2 – moderate, 3 – numerous.

Large-droplet fatty change. Vacuoles of lipid occupying the majority of the hepatocyte cytoplasm, pushing the nucleus to the cell periphery, were graded: 0 – absent, 0–1 – few, 1 – mild, 2 – moderate, 3 – numerous.

The eosinophilic Mallory-like material (*absent or present*). Clumped, ropelike or serpigenous eosinophilic material within liver cell cytoplasm was found typically in periportal hepatocytes or dispersed intralobulary.

Activation of sinusoidal inflammatory cells: the presence of lymphocytes and Kuppffer cells inside sinusoidal capillaries, of mononuclear cells in intralobular spots and in intertrabecular lines were graded as: 0 – absent, 1 – low number, 2 – moderate number, 3 – high number.

Multinucleate liver cells such as giant hepatocytes, containing three or more nuclei (indicating multinucleation), were labeled as present or absent.

Liver cell dysplasia (absent or present). Enlarged hepatocytes with atypical nuclei, including hyperchromia, multiple nuclei, and multiple, prominent nucleoli, indicated the presence of liver cell dysplasia.

The data were statistically analyzed using the χ^2 test.

Results

Inside the portal spaces we observed a dense, diffuse or nodular mononuclear *inflammatory infiltrate*, organized as lymphoid aggregates or forming true lymphoid follicles centered on activated B lymphocytes (CD_{20}-positive) and follicular dendritic cells surrounded by non-activated B-lymphocytes, and peripherically enveloped by T-lymphocytes.

Table 1 reveals the frequency of the mentioned histologic features, on liver biopsy samples obtained from patients with HCV as compared to HBV patients.

Table 1 – ***Liver biopsy features in HCV and HBV infections***

Histologic features	HCV [%]	HBV [%]	P	
LF/LA	68% (85/125)	20% (13/64)	P < 0.001	ES
BDL	44% (55/125)	22% (14/64)	P = 0.002791	VS
mMHS	77% (96/125)	64% (41/64)	P = 0.063502	NS
MM	25% (31/125)	5% (3/64)	P = 0.000657	ES
ASIC	68% (85/125)	70% (45/64)	P = 0.745421	NS
MLC	6.2% (5/125)	9% (6/64)	P = 0.655879	NS
DLC	8.7% (7/125)	15.6% (10/64)	P = 0.157682	NS

LF/LA – lymphoid follicles and/or lymphoid aggregates; BDL – bile duct lesions; mMHS – micro-macrovacuolar hepatocyte steatosis; MM – Mallory material; ASIC – activation of sinusoidal inflammatory cells; MLC – multinucleate liver cells; DLC – dysplastic liver cells; ES – extremely significant; VS – very significant; NS – not significant.

On the studied material, we identified the presence of portal *lymphoid follicles (LF)* and *lymphoid aggregates (LA)* in 68% of the HCV as compared to 20% in HBV infection cases, either isolated or as part of the portal lymphoid infiltrate (with active germinal centers in some of the follicles), being located in the immediate vicinity of interlobular bile ducts (Figure 1), around them (Figure 2).

In 42% of cases, we observed the presence of LF and/or LA in more than 1/3 of the portal spaces, portal areas with such structures alternating with others diffusely infiltrated with lymphocytes, histiocytes and rare plasmocytes.

Analyzing the presence and absence of the intraportal LF/LA, depending on the severity of necrosis, the histologic activity index (necro-inflammation) and the Knodell score in both types of viral infection (with HCV and HBV) we observed a direct relation between their high incidence and moderate/severe and bridging periportal necrosis (Figure 3); moreover, we obtained a higher average value of the histologic activity index and a higher Knodell score in the intraportal LF/LA group of patients (9.43 *vs.* 6.45, and 14.0 *vs.* 7.35 in HCV; 9.84 *vs.* 6.48, and 11.69 *vs.* 7.34 in HBV) (Figures 4 and 5).

We observed interlobular bile duct damages in 36.5% of cases (in 44% HCV *vs.* 22% HBV cases) (Figure 6), often associated to bile duct hyperplasia. More frequently observed were multilayer bile duct epithelium with cell depolarization, cytoplasmic vacuolization and acidophilia, loss of nuclear polarity with hyperchromic, picnotic nuclei, lymphocytic exocitosis among ductal epitelial cells, (but, in most cases, with preservation of the integrity of the ductal basement membrane revealed by PAS method as a continous eosinophilic contour) (Figure 7); in two cases we observed severe lesions of the canalicular epithelium with epithelial focal necrosis but with continuity of the basement membrane, and in the third case we observed hepatocytic metaplasia of the bile duct epithelium.

Hepatocyte steatosis observed in 72.48% of cases is valuable for the etiologic diagnosis of hepatitis C, especially associated to the other histologic features. In the studied group of patients, the incidence of hepatocyte steatosis was 72.48–77% in HCV as compared to 64% in HBV cases, mild and medium micro-macrovacuolar mixed aspects being predominant in both situations (Figure 8).

The analysis of these features on liver biopsies reveals that the set of histologic lesions associated to hepatitis C (rather than hepatitis B) includes lesions of the bile ducts, lymphoid follicles and/or lymphoid aggregates in the portal spaces and micro-macrovacuolar predominantly mixed hepatocyte steatosis.

Mallory-like material (Figure 9), more frequently identified on HCV-positive liver sections (25% in HCV *vs.* 5% in HBV) represents the next lesion, which proved to be much more probably associated to HCV infection. We observed the presence of acidophilic material, with predominantly periportal (in the acinar zone 1), periseptal or intralobular location, often surrounded by lymphocytes and/or neutrophilic leukocytes (Figure 10).

We frequently encountered hepatocytes containing Mallory-like material, characterized by increased volume and rarefaction of the cytoplasm (similar to those in alcoholic hepatitis); the absence of these aspects in the acinar area (typical for the alcoholic liver disease) suggested a rather viral etiology.

In seven cases, microscopic examination of serial sections revealed the presence of apoptotic elements with perivenular disposition; their situation in the 3rd acinar area near pericellular fibrosis and panlobular hepatocyte steatosis areas, suggested a concomitant alcoholic liver disease. Most liver sections, which showed Mallory-like material, had various degrees of hepatocyte steatosis.

Activation of sinusoidal inflammatory cells, with presence of Kuppffer cells inside the sinusoidal vessels, of mononuclear cells in intralobular spots and in intertrabecular lines (Figure 11) were observed in similar proportions in both types of hepatitis.

Multinucleate liver cells such as giant hepatocytes with 2–3 nuclei, as well as the presence of large hepatocytes, with hyperchromic nuclei or multiple, prominent nucleoli indicated liver cell dysplasia.

Moreover, in HCV infection cases, we evaluated the frequency and clinical pathological significance of intrahepatic epitheloid cell granuloma. In four from 25 cases (3.2%) we identified the presence of granulomatous lesions, some of them surrounded by lymphocytes, with portal or intralobular location. In two cases we observed portal granuloma with mild/moderate lesions of the bile duct epithelium and in other two cases the intralobular presence of "sarcoid-like" giant cell granuloma.

In Table 2 we present the predictive value of isolated or combined histologic liver lesions in the diagnosis of HCV as compared to HBV infection.

Table 2 – ***Incidence and predictive value of isolated or combined histologic lesions in chronic C hepatitis***

Histologic lesions (features)	HCV [%]	HBV [%]	P	
Isolated lesions				
▪ LF/LA	68% (85/125)	20.31% (13/64)	P < 0.001	ES
▪ mMHS	77% (96/125)	64% (41/64)	P = 0.063502	NS
▪ BDL	44% (55/125)	21.85% (14/64)	P = 0.002791	VS
Combined lesions				
▪ LF/LA + BDL	39.2% (49/125)	9.37% (6/64)	P = 0.000019	ES
▪ LF/LA + mMHS	55% (69/125)	17.18% (11/64)	P = 0.000001	ES
▪ BDL + mMHS	35.2% (44/125)	17.18% (11/64)	P = 0.009881	VS
▪ LF/LA + mMHS + BDL	32% (40/125)	9.37% (6/64)	P = 0.000603	ES

LF/LA – lymphoid follicles and/or lymphoid aggregates; BDL – bile duct lesions; mMHS – micro- macrovacuolar hepatocyte steatosis; ES – extremely significant; VS – very significant; NS – not significant.

Among individual lesions, mixed micro-macrovacuolar hepatocyte steatosis represented the feature most frequently associated to hepatitis C (76.8% in HCV *vs.* 64% in HBV infection), followed by portal lymphoid follicles (68% in HCV *vs.* 20.31%

in HBV infection). Regarding combined lesions, both the association between lymphoid follicles/lymphoid aggregates with bile duct lesions or micro-macrovacuolar hepatocyte steatosis and the association between bile duct lesion and micro-macrovacuolar hepatocyte steatosis were encountered more frequently in HCV than in HBV infection.

The association between the three histologic features (LF/LA + mMHS + BDL) was encountered in 32% (40/125) of the HCV cases and in 9.37% (6/64) of the HBV cases, respectively, thus confirming the value of these lesions in the etiologic diagnosis of chronic HCV hepatitis; the more frequently these features associate, the more suggestive they are for the diagnosis of HCV infection.

After analyzing statistically the results obtained by using the chi- square test, the frequency of portal LF/LA and of the Mallory-like material was extremely significant ($p<0.001$) in patients with HCV, compared with those with HBV; we also noted a significantly high incidence ($p = 0.002791$) of BDL in HCV, compared with the HBV infection.

Although the results showed that the mixed hepatocyte steatosis represents the histologic characteristic most frequently associated with hepatitis C (76.8%), we did not observe significant differences concerning its incidence in HCV *vs.* HBV ($p>0.05$), the steatosis occurring frequently in both types of viral chronic hepatitis.

Regarding the combined lesions, the association of LF/LA with BDL (LF/LA + BDL) or with hepatocyte steatosis (LF/LA + HS), were extremely significant ($p<0.001$) in HCV *vs.* HBV infection.

The incidence of the association of BDL with HS (BDL + HS) was very significant ($p = 0.009881$), and the presence of all three histological features (LF/LA + HS + BDL) was extremely significant ($p<0.001$) in patients with HCV *vs.* those with HBV.

Discussions

Up to the present, HCV has never been identified in the hepatic tissue by electron microscopy and there are no known hepatocellular changes similar to HBsAg-specific ground-glass inclusions, allowing the microscopic identification of HCV hepatitis. As a consequence, in order to understand this form of hepatitis and in order to correlate it to positive results of serological tests for anti-HCV antibodies, as well as for the retrospective morphological study of hepatic biopsy punction and of post-mortem liver tissue examination, histological markers of the HCV infection are needed [29].

Lymphoid portal aggregates, inflammatory lesions of the bile ducts and steatosis are the most typical characteristics that allow the differentiation of HCV hepatitis from other liver diseases with progressive inflammation [14].

The presence of HCV biological markers in biopsy samples from patients with other diseases (such as alcoholic liver disease) may reveal a concomitant HCV infection [29].

In our study, performed comparatively on two groups of patients (with chronic C and B hepatitis), we analyzed the incidence of the seven HCV infection histologic markers.

Based on our data, the frequency of portal lymphoid follicles (LF), portal lymphoid aggregates (LA) and Mallory-like material (MM) was extremely significantly increased ($p<0.001$) in HCV *vs.* HBV patients. We also found that the incidence of bile duct lesions (BDL) is very significantly increased ($p = 0.002791$) in HCV, in comparison with HBV infection.

Our data showed that mixed micro-macrovacuolar hepatocyte steatosis represents the histological feature most frequently associated with C chronic hepatitis (76.8%). However, on our batch, we did not notice a significant difference regarding the incidence of steatosis in HCV *vs.* HBV patients ($p>0.05$), this lesion occurring very frequent in both types of chronic viral hepatitis.

In our study, among combined lesions, the associations between lymphoid follicles/lymphoid aggregates with bile duct lesions (LF/LA + BDL) or with mixed micro-macrovacuolar hepatocyte steatosis (LF/LA + mMHS) were extremely significantly increased ($p<0.001$) in HCV *vs.* HBV infection.

The incidence of the association between bile duct lesions and mixed micro-macrovacuolar hepatocyte steatosis (BDL + mMHS) was very significantly increased in HCV *vs.* HBV patients ($p = 0.009881$).

We found that the presence of three histological features: portal lymphoid follicles/lymphoid aggregates, mixed micro-macrovacuolar hepatocyte steatosis and bile duct lesion (LF/LA + MMHS + BDL) was extremely significantly higher in patients with HCV infection, in comparison with HBV patients ($p<0.001$).

As for the incidence of these lesions, literature data are variable and controversial. In a study performed on 200 liver biopsies from HCV chronic hepatitis patients, the presence of lymphoid follicles represented the most frequent histologic finding, being observed in 60% of the cases; microvacuolar mixed hepatic steatosis was observed in 38% of the cases (mild and moderate intensity in 30% and severe in 8% of cases), whereas bile duct alteration was noted in 15% of cases [36].

In another study that included 124 HCV infected patients, Gerber identified bile duct alteration in 60% of cases, lymphoid follicles and aggregates in 57% of patients and steatosis in 52% of the cases; moreover, the authors noted the association between two of these histologic features in 50% of cases [37].

The incidence of these lesions varies very much and on a wide range, also including the values obtained by us. Comparing our data with reference values on histologic lesions characteristic for chronic HCV hepatitis, we opinate that their exact interpretation requires a unitary quantification and reporting system. Each of these lesions needed an evaluation in the context of other histopathological features and in an appropriate context.

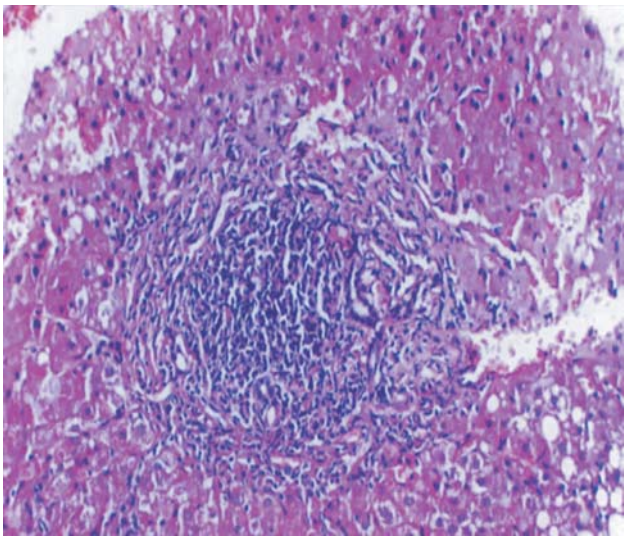

Figure 1 – *HCV with portal lymphoid follicles as part of the lymphoid infiltrate, in the immediate vicinity of the interlobular bile ducts (HE staining, ×200)*

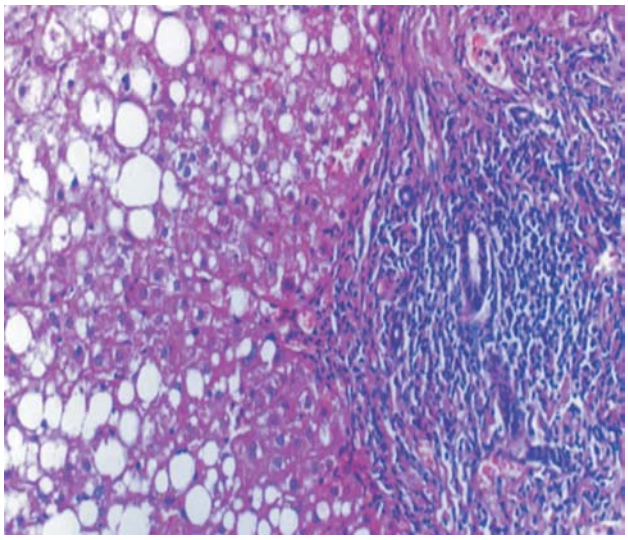

Figure 2 – *Intraportal lymphoid follicles surrounding the interlobular bile ducts (HE staining, ×200)*

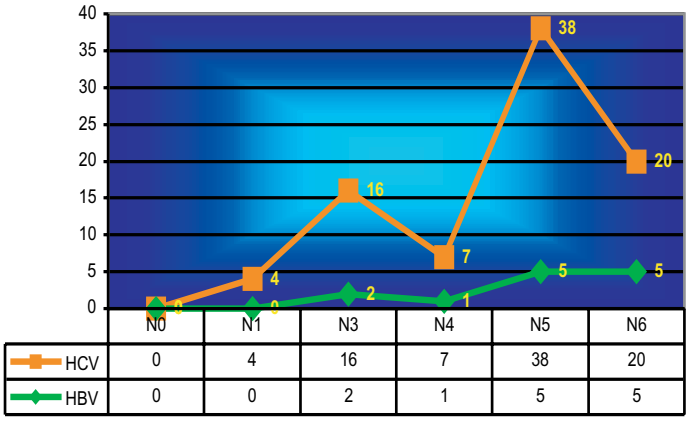

Figure 3 – *Relationship between intraportal LF/LA and severity of necrosis*

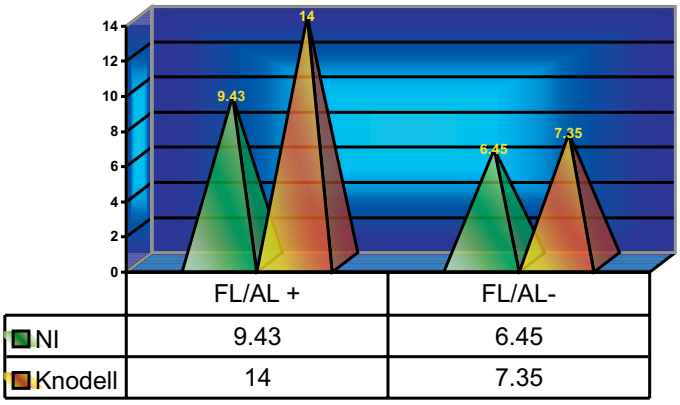

Figure 4 – *Relationship between LF/LA, necroinflammation and Knodell index in HCV*

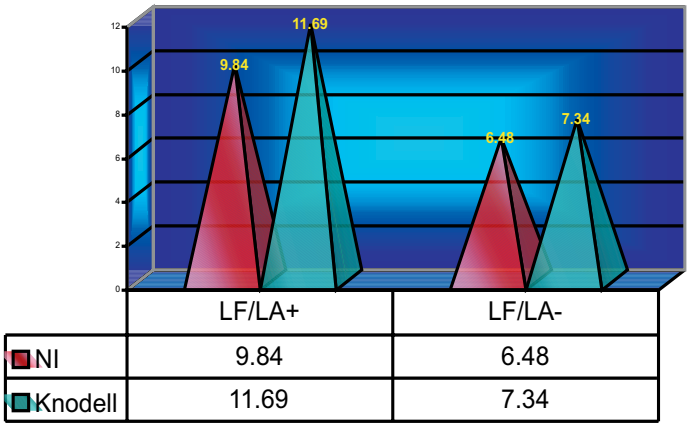

Figure 5 – *Relationship between LF/LA, necroinflammation and Knodell index in HBV*

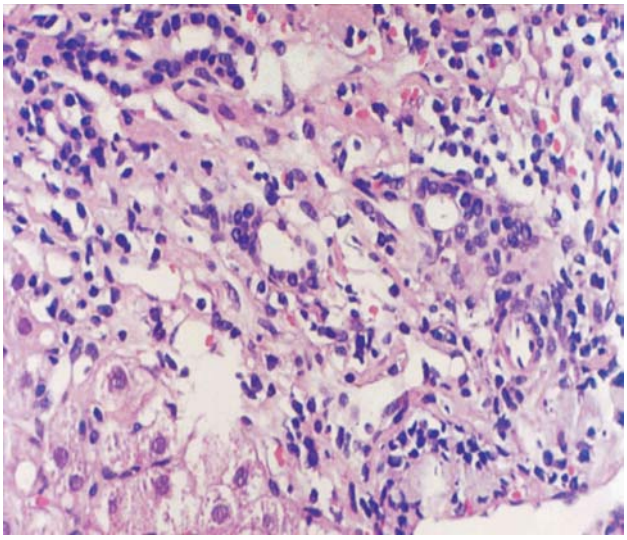

Figure 6 – ***Bile duct damage with cytoplasm vacuolization (HE staining, ×400)***

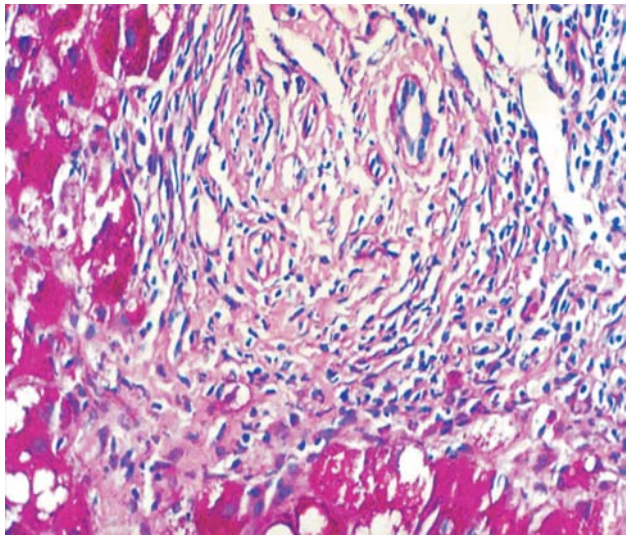

Figure 7 – ***Maintenance of basal membrane integrity of bile ducts in HCV (PAS staining, ×200)***

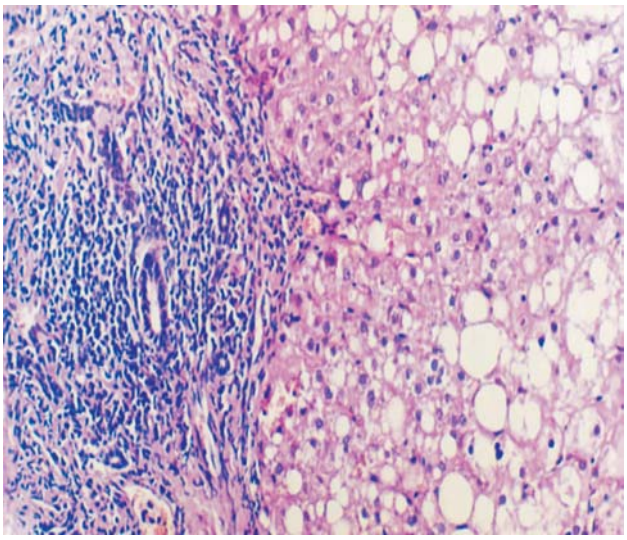

Figure 8 – ***HCV with severe micro-macrovacuolar hepatocyte steatosis (HE staining, ×200)***

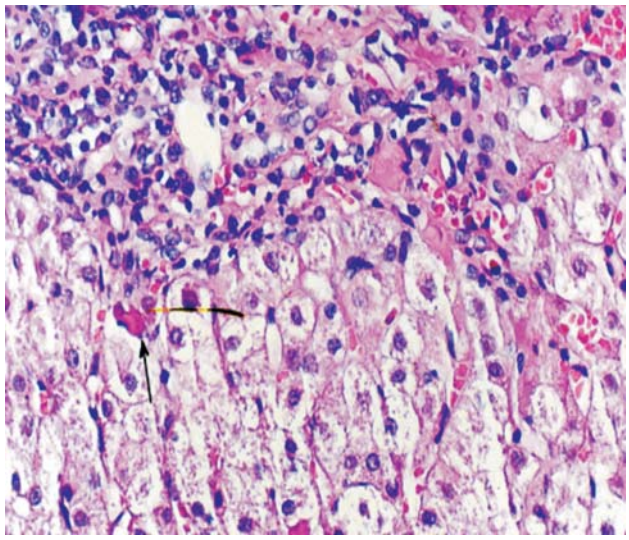

Figure 9 – ***Acidophilic bodies situated periportal – acinar zone 1 (HE staining, ×400)***

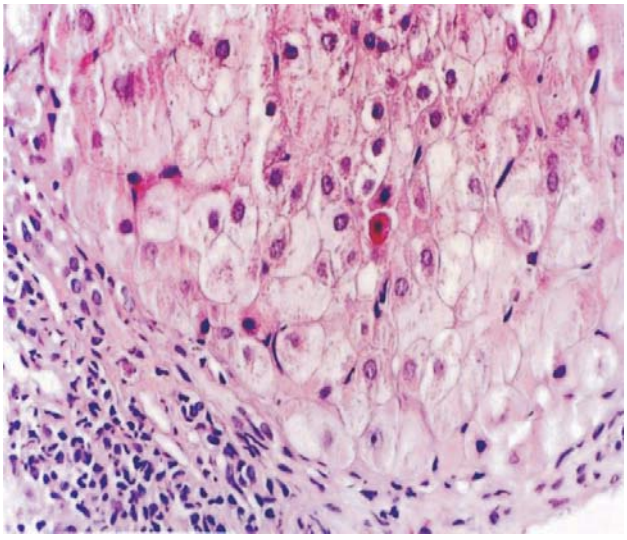

Figure 10 – ***Swelling of hepatocytes, with rarefaction of the cytoplasm and Mallory-like material (HE staining, ×400)***

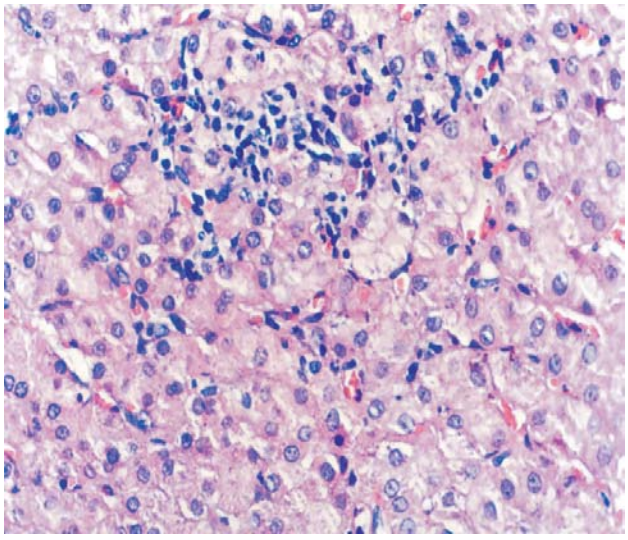

Figure 11 – ***Mononuclear inflammatory infiltrate in intralobular spots (HE staining, ×200)***

Lymphoid follicles and aggregates, bile duct lesions and steatosis, were also considered as histologic markers for hepatitis C in the study of Scheuer that included patients with serologically confirmed viral C hepatitis [38].

Bile duct lesions may be encountered in a variety of liver diseases, including primitive biliary cirrhosis (PBC), intoxications with various drugs and liver transplant rejection [39].

In PBC and in several other liver diseases, destructive lesions of the bile ducts are encountered, culminating with their disappearance and final stage biliary cirrhosis. In most cases, bile duct lesions in chronic C hepatitis do not seem to lead to ductal destruction, even though, in his study, Lefkowitch showed (in two cases) bile duct loss and proliferation of small ducts in the portal spaces. Destruction of bile ducts was also mentioned as a histologic feature of chronic C hepatitis in the study of Bach [25].

Similarly, lymphoid follicles and/or aggregates in portal spaces may also be encountered in the liver tissue of patients with other chronic liver diseases, including PBC (usually with bile duct loss) [39] and active "lupoid" autoimmune chronic hepatitis [40].

The differentiation between chronic C hepatitis and autoimmune hepatitis may be difficult but the presence of plasmocytes in high number in the portal or periportal cellular infiltrate, of periportal hepatocyte clusters organized as hepatic cell rosettes and the cirrhogenic architecture of advanced lesions sustain the diagnosis of autoimmune disease [29]. Other suggestive markers for autoimmune hepatitis include necrosis and more severe inflammation, piece-meal necrosis, multinucleate hepatocytes and large areas of liver parenchyma collapse.

The pathogenesis of these histologic lesions is controversial. Interlobular bile duct lesions may be the consequence of an immune mediated reaction towards antigens in the bile duct epithelium. These may be histocompatibility antigens (HLA) expressed in combination with the hepatitis C virus, HCV-induced antigens or antigens not yet identified (in the case of PBC).

Considering these aspects, we could observe the fact that lymphoid follicles and/or aggregates present in HBV or HCV chronic hepatitis are located in the immediate vicinity of interlobular bile ducts or around them. In the study of Lefkowitch [29], the association between bile ducts lesions and the response to α-IFN (3 mil. U) in patients with chronic C hepatitis, may be considered an indicator with prognostic value which brings new information concerning the pathogenesis of hepatitis C. These data need supplementary studies.

The presence of well-structured lymphoid structures and follicles or aggregates represents a feature encountered in several chronic inflammatory, often autoimmune, diseases (rheumatoid arthritis or Hashimoto thyroiditis); their identification in chronic C hepatitis reflects an immunological pathogenetic mechanism. Interestingly, these lymphoid structures may be early organized during the acute stage of hepatitis C [29].

The resemblance between several histologic features of chronic and acute C hepatitis may lead to a difficult differentiation between acute and chronic disease when solely histologic criteria are used.

Hepatic steatosis and Mallory-like material in hepatocytes are features included in the category of cytopathic changes reflecting a possible direct cytotoxic effect of HCV upon hepatic cells. Hepatocyte steatosis (especially lipid microvacuolae) has been described in various reports concerning viral liver infection in Venezuela natives [29]. The increased severity of steatosis in non-alcoholic patients indicates a HCV liver infection rather than B hepatitis.

The pathogenesis of periportal Mallory-like material on biopsies of HCV patients remains unknown. These patients do not have chronic cholestasis, bile duct obstructions or bile duct destructions – all these being able to generate periportal copper accumulation and the occurence of Mallory-like material [37].

Even though the study of Rugge suggested that Mallory-like material might be connected to the fixation of liver samples in Bouin solution, these are also frequently observed in formalin-fixed samples [19].

Conclusions

Similar to other studies, we observed that, even though they are not pathognomonic for hepatitis C, the presence and increased frequency of lymphoid follicles and/or portal lymphoid aggregates, bile duct lesions, increased degree of hepatocyte steatosis, predominantly macrovacuolar, and the Mallory-like material in hepatocytes, rather indicate the probability of HCV infection than a case of hepatitis B**.**

New studies are needed in order to clarify the proportion in which the immune aggression leads to a set of lesions (bile duct lesions and portal lymphoid structures), and weather the viral cytopathic effect is responsable for hepatocyte steatosis and Mallory-like material formation.

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