PARTICULAR MORPHOLOGICAL ASPECTS OF VIRAL C CHRONIC INFECTION IN ALCOHOLIC LIVER DISEASES

Liana MOŞ[1]*, Corina ZORILĂ[1], Coralia COTORACI[2], George-Ciprian PRIBAC[3], Veronica GREC[1], Adrian WIENER[1]

[1]Department of Internal Medicine, Faculty of Medicine, Pharmacy and Dental Medicine „Vasile Goldis" Western University of Arad

[2]Department of Haematology, Faculty of Medicine, Pharmacy and Dental Medicine „Vasile Goldis" Western University of Arad

[3]Department of Cell and Molecular Biology, Faculty of Medicine, Pharmacy and Dental Medicine „Vasile Goldis" Western University of Arad

*** Correspondence:** Liana Moș, Department of Internal Medicine, Faculty of Medicine, Pharmacy and Dental Medicine „Vasile Goldis" Western University of Arad, tel/fax: +40-257-212204, email: limmos2001@yahoo.com
Received: march 2008; Published: may 2008

ABSTRACT. The aim of the study was to evaluate the histological aspects of chronic infection with hepatitis C virus in heavy alcohol drinkers. We selected 84 heavy alcohol drinkers (intake-over 80g ethanol/day for more than 10 years) with alcoholic liver disease (ALD) from wich 34 patients had chronic hepatits C virus (HCV) infection. Liver biopsy was performed in all patients. In alcoholic liver disease the presence of hepatitis C anti-virus antibodies was significantly correlated with the presence of the histological signs of non-alcoholic moderate active chronic hepatitis. The pattern of hepatic histological lesions is dominated by the histological aspects of viral hepatitis chronic infection rather than the typical aspect of alcoholic liver disease. The steatosis was present in both groups. The score of fibrosis was more severe in patients with markers of hepatitis C virus chronic infection and chronic alcohol intake. The histological signs of the alcoholic liver disease were less exhibited in hepatitis C infected group of patients, even though all patients presented the same severe consumption of alcohol. Hepatic histological pattern of the lesions is dominated rather by the histological aspects of viral hepatitis than by the typical aspect of the alcoholic liver disease. Fibrosis was more severe in hepatitis C positive groups, associated with alcohol intake. Alcohol consuming in hepatitis C chronic infection increase the liver dammage and accelerates the rate of fibrosis with mixed dispossition.

Keywords: viral C infection, alcoholic liver disease

INTRODUCTION

Alcoholics exhibit an increased incidence of the chronic infection markers with the C virus. The increased prevalence of the hepatitis C anti-virus antibodies (Ac VHC) in alcoholics has led to a series of speculations concerning the part played by alcohol intake in the evolution of chronic infection with hepatitis C virus.

The interdependence between the C viral chronic infection and the chronic consumption of alcohol is not completely revealed and explained but the mutual influence in the aggravation of liver lesions is certain. The aim of this study is to identify particular aspects regarding the chronic consumption of alcohol and the C viral infection among the middle-age population characterized by a severe chronic consumption of alcohol without lethal implications that could influence the condition of the immune system. In the case of these patients we monitored the way in which constant chronic consumption of alcohol and the C viral infection become interconnected and the extent to which this double association influences the gravity of the hepatitis C disease.

MATERIAL AND METHODS

SRAC Test was used to evaluate the quantity and Duration of Alcohol Consumption Questionnaire (adapted after Skinner and Sheu; 1997).

Alcohol consumption questionnaire

1. Have you been drinking for more than ten years? YES/NO

2. What age did you start consuming alcohol?

3. How many days a month do you consume alcohol?

4. How many doses of alcohol a week have you been drinking? A dose represents the equivalent of 10g pure alcohol contained in 30ml of aqua vitae (cognac, whiskey, and tzuica), 100ml of wine or 250ml beer.

5. What was the maximum quantity consumed during a day?

6. How would you rate your own manner of alcohol consumption? 1 = occasionally; 2 = at the weekend; 3 = frequently; 4 = every day.

7. Is there any difference in your consumption as compared to previous periods of time? YES/NO.

8. What age were you when you started consuming alcohol somewhat differently than before? Back to question no. 3

The total consumption of alcohol was calculated according to the formula: Total quantity of alcohol = no. of days/month × quantity of alcohol/day (g of pure alcohol) × time period; 10g alcohol are contained in 30ml of aqua vitae (cognac, whiskey), 100ml of wine or 250ml of beer.

C Viral Infection Diagnosis – in order to identify the Hepatitis C anti-virus antibodies (Ac anti HCV) we used the MEIA technique, equivalent to the 2nd generation ELISA, with over 95% success rate in identifying the C viral infection (National Institutes of Health (NIH) consensus 1997).

Laboratory investigations - the biochemical samples are determined with the KONELAB-30 analyser and Clini-lab reagents.

Histological aspects – the hepatic biopsy puncture was performed on all patients without general cautions who accepted exploration. The histological parts were placed in B-5 fixative, processed and wrapped in Aradit plastic, sectioned in 3 microns sections and coloured with haematoxylin-eosin, MGG or Masson trachoma and interpreted in optical microscopy. In order to discriminate between necro-inflammatory lesions in Hepatitis C associated to alcoholic liver disease we used the study grid of the METAVIR group by envisaging a separate score for fibrosis. Fibrosis and the final score have been separately quantified.

Statistic analysis: All comparisons were made between the two groups – with and without positive Ac anti HCV through: x^2 test in order to compare percentages; Studdent t test in order to compare medium values; Mann–Whitney test for ± medium values' comparison. For statistic significance Pearson correlation. The necroinflammatory activity score has a normal distribution and is considered as a quantity variable and the statistic analysis was made through multiple quantity regression of quality variables.

RESULTS

We selected 84 the patients who were included in our study, 38 with hepatitis C infection, all alcohol consumers.

Table 1

THE STRUCTURE OF THE SAMPLE AND THE RELATIONSHIP BETWEEN THE PRESENCE AND THE ABSENCE OF AC ANTI HCV, THE CLINICAL BACKGROUND AND LABORATORY DATA

	ALD with negative Ac anti HCV (n = 50) average values or %	ALD with positive Ac anti HCV (n=34) average values or %	Statistical P
Age	45 ± 6 years	43 ± 5 years	**NS *****
Daily average intake of alcohol	55,5 ± 1,7g/day	54,9 ± 2,7g/day	**NS *****
Duration of alcohol intake	12 ± 5 years	11 ± 4 years	**NS****
Total amount of alcool intake	134,55 ± 27,34g	109,94 ± 14,65g	**NS****
ASAT (U/l)	147 ± 65	165 ± 108	**NS *****
ALAT (U/l)	71,5 ± 12	154,5 ± 34	**0,0030*****
ASAT/ALAT	> 2	< 1	**< 0,0010****
FA (U/l)	152,65 ± 32	174,85 ± 65	**NS ****
GGT (mmol/l)	206 ± 12	245 ± 23	**NS *****
GGT/FAL	2,4	1,9	**NS ****

*x^2 test in order to compare percentages; **Studdent t test in order to compare medium values; ***Mann–Whitney test for ± medium values' comparison. *S = statistically significant for p<0.05. IS = statistically insignificant: ALD- alcoholic liver disease*

Table 2

THE RELATIONSHIP BETWEEN ALCOHOLIC LIVER DISEASE, HISTOLOGICAL ASPECTS AND PRESENCE OF AC ANTI HCV IN THE STUDIED PATIENT'S SAMPLE

Histological aspect	ALD negative Av anti HCV n = 50 (%)	ALD positive Ac anti HCV n=34 (%)	Statistical P
Steatosis	76 % (38)	88,23 % (30)	**NS***
Focal necrosis	68 % (34)	55,88 % (19)	**NS***
Piecemeal necrosis	0	94,11 % (32)	**< 0,010***
Bridging necrosis	0	11,7 % (4)	**0,0010***
Polimorphonuclear inflammatory infiltrate focal, portal or lobular	90 % (45)	29,41 % (10)	**0,05***
Lymphocytic inflammatory infiltrate in portal spaces	0	100% (34)	**0.0010***
Lymphoid aggregates formation in portal spaces	0	14,11% (5)	**< 0,001***
Lymphocytic inflammatory infiltrate in hepatic lobuli	1,9% (1)	70,58 % (24)	**< 0,010***
PMN mixt infiltrate	16% (8)	5,88% (2)	**0,001***
Apoptotic hepatocytes with Councilmann bodies	18%(9)	29,41% (10)	**0,05***
Periportal fibrosis	0	85,29% (29)	**0,0010***
Perivenular/pericellular fibrosis	46% (23)	52,94% (18)	**NS***
Portocentrolobular fibrosis	8%(4)	14%(5)	**0,0010***
Intrahepatic colestasis	4% (2)	5,9% (2)	**NS***

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Alcoholic acute hepatitis	16% (8)	5,8 % (2)	**0,05***

S = statistically significant for p<0.05. IS = statistically insignificant
** x^2 test in order to compare percentages*

Table 3

STATISTIC COMPARISON OF THE HISTOLOGICAL MODIFICATIONS NOTICED IN THE PATIENTS SAMPLE ACCORDING TO THE PRESENCE OR ABSENCE OF AC ANTI HCV

	Negative Ac anti HCV n = 50	Positive Ac anti HCV n = 34	Statistical P
Steatosis	45 (89%)	27 (78%)	**NS****
Alcoholic hepatitis	8 (16%)	2 (5,88 %)	**0,05****
Active chronic hepatitis	0	29 (85,29 %)	**0,0010****
Pericentrolobular fibrosis	18 (36%)	16 (47,05%)	**NS****
Periportal fibrosis	0	24 (70,58%)	**0,05*****

*S = statistically significant for p<0.05. IS = statistically insignificant. ***Mann–Whitney test for ± medium values' comparison. **** Multiple quantity regression of quality variables*

Table 4

COMPARATIVE HISTOLOGICAL ACTIVITY IN ALD WITH POSITIVE AC ANTI HCV AND ALD

Histologic activity	ALD	ALD with Ac anti HVC	Statistical P
HAI score	1,9±0,5	9,6 ± 3,8	**<0,0001*****
"Piecemeal necrosis"	0	3,1±2,9	**<0,0001*****
Intralobular deneresccence	1,6±1,0	1,3±0,8	**NS*****
Portal inflammatory infiltrate	1,4±1,0	4,7±1,0	**0,0020*****
Fibrosis	1,7±1,1	3,5±1,3	**0,030*****

*S = statistically significant for p<0.05. IS = statistically insignificant. ***Mann–Whitney test for ± medium values' comparison.*

DISCUSSIONS

Generally speaking, the alcoholic liver disease and chronic hepatitis C infection are examples of inducing hepatic lesions with an individual evolution, with a necro-inflammatory activity of a rather slow progression in the case of hepatitis C.

We selected 50 patients with alcoholic liver disease and 34 patients with chronic hepatitis C and concomitant alcohol intake, daily, for more than ten years.

In our selected patients, the values described by the biochemical parameters in the alcoholic liver disease with hepatitis C concomitant infection are generally comparable to those of patients sample with alcoholic liver disease, except AST/ALT ratio.

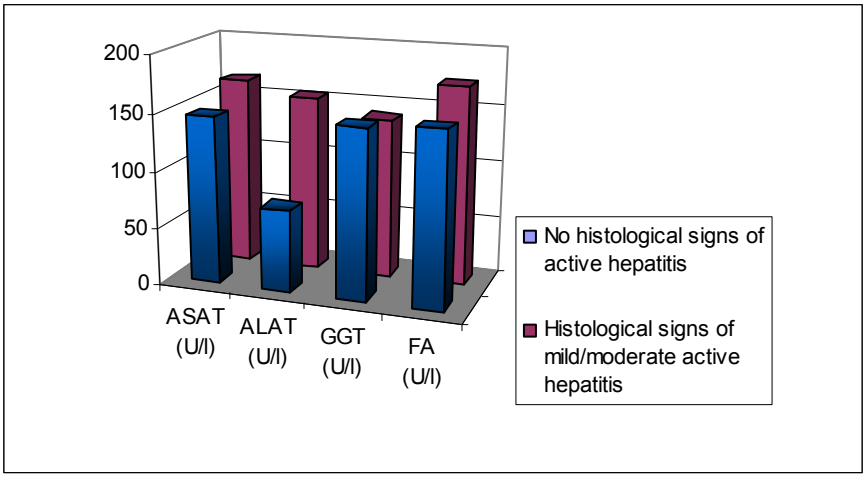

Fig. 1 Correlation of the biological samples with the necro-inflammation in our studied patients

More elevated transaminases are present in the alcoholic liver disease with chronic hepatitis C infection than in the group of patients without chronic hepatitis C infection (p=0,002), which certifies that there is a factor independent of the alcohol consumption which influences and amplifies the necrosis of hepatocytes.

In alcoholic liver disease without chronic hepatitis C associated infection aspartataminotransferase (AST) is more prone to an increase than alanine aminotransferase (ALT), the ratio of the average values of AST/ALT being over 2, while in the case of a mixed hepatic disease, determined by the consumption of alcohol associated with chronic hepatitis C infection, the AST/ALT ratio was less than 1. These patients exhibit convergent hepatocytic necrosis and a more severe hepatic dysfunction, have cholestatic forms of disease and histological signs of active hepatitis.

Sub unitary AST/ALT ratio is significantly statistically correlated with the presence of histological

active hepatitis and exclusively with the presence of the C viral infection (Figure 1).

For stabilising the histological lesions in the alcoholic liver disease associated to hepatitis C chronic infection the METAVIR grid was used. After each histological diagnosis for each biopsy a total score was calculated as the sum between the necrosis score (periportal, intralobular and focal), the portal inflammation, respectively, the histological activity index (HAI) score (table 6). Generally, chronic viral hepatitis C has certain histological aspects considered characteristic: the inflammatory portal diffuse infiltrate consists of lymphocytes cells, organised in aggregates and lymphocyte follicles. The tendance of inflamatory limphocityc cells to organize in follicles is mediated by the inflammatory cytokines secreted by the T helper CD4 lymphocytes present in the intra hepatic inflammatory infiltrate, respectively on the Th1/Th2 ratio.

Based on our histological criteria, active chronic hepatitis associated to the alcoholic liver disease was defined by the presence of the portal and periportal lymphocyte inflammatory infiltrate with or without polymorphonuclears, with the loss of the integrity of the hepatic lobules, respectively 'piecemeal necrosis' and with lobular (focal or diffuse) inflammatory infiltrate (Table 2).

The histological stigmata of alcoholic liver disease were present in all cases regardless of the presence or absence of the C viral infection with generally macro vesicular steatosis, pericellular and pericentrolobular fibrosis, focal necroses, polymorphonuclear or/and mixed lymphocytes inflammatory cells infiltrate and polymorphonuclear with predominance of polymorphonuclears (figure 2, 3).

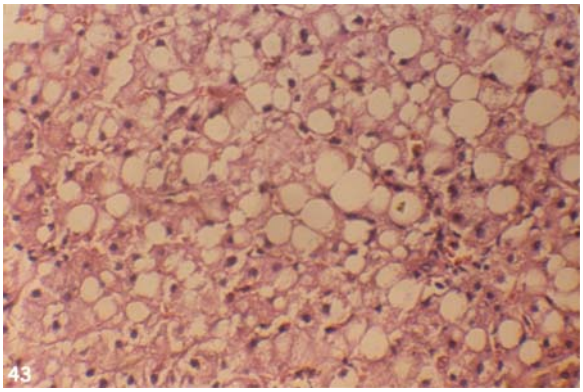

Fig. 2 AST/ALT ratio >2, negative for hepatitis C infection; severe steatosis, with pericentrolobular focal inflammatory infiltrate with focal heptocytes necrosis

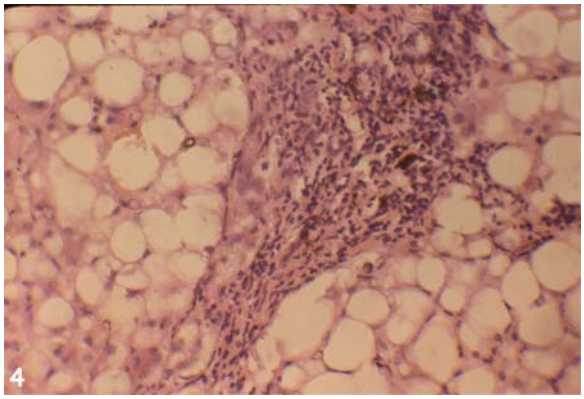

Fig. 3 AST/ALT>2, negative for hepatitis C infection; massive steatosis, mixed pericentrolobular inflammatory infiltrate, with massive steatosis

In the majority of the histological samples from the patients with alcoholic liver disease without hepatitis C concomitant infection (n=50), steatosis was present in all samples, with hepatocellular focal necrosis and vacuolated degenerescence of hepatocytes. Inflammatory infiltrates with lobular or portal focal disposition was present in only 24 patients (48%), without criteria of histological activity.

Patients with hepatitis C infection (n=34) have a series of particular aspects: the majority of patients, 29 (85.2%) exhibit predominantly lymphocytes inflammatory infiltrate which infiltrates the portal spaces, but only in 5 patients (14.11%) there is a tendency towards the formation of lymphocytes aggregates typical for hepatitis C infection.

24 patients with alcoholic liver disease and concomitant hepatitis C infection (70.58%) presented lymphocytes inflammatory infiltrate in the hepatic lobule, statistically significant as compared to the group without hepatitis C infection present. In 10 patients (29.41%) intra-cytoplasmatic acidophil inclusions (Councilman) have been noticed, considered as the histological markers of apoptosis. Apoptosis is present both in the alcoholic liver disease but also in hepatitis C as the main form of cell death. Apoptotic hepatocytes are significantly more numerous in patients with a concomitant C viral infection than in patients with alcoholic liver disease without hepatitis C infection.

The majority of patients with hepatitis C concomitant infection exhibit moderately active forms of chronic hepatitis, with a histological activity score between 5.6 and 11.2. (figure 4)

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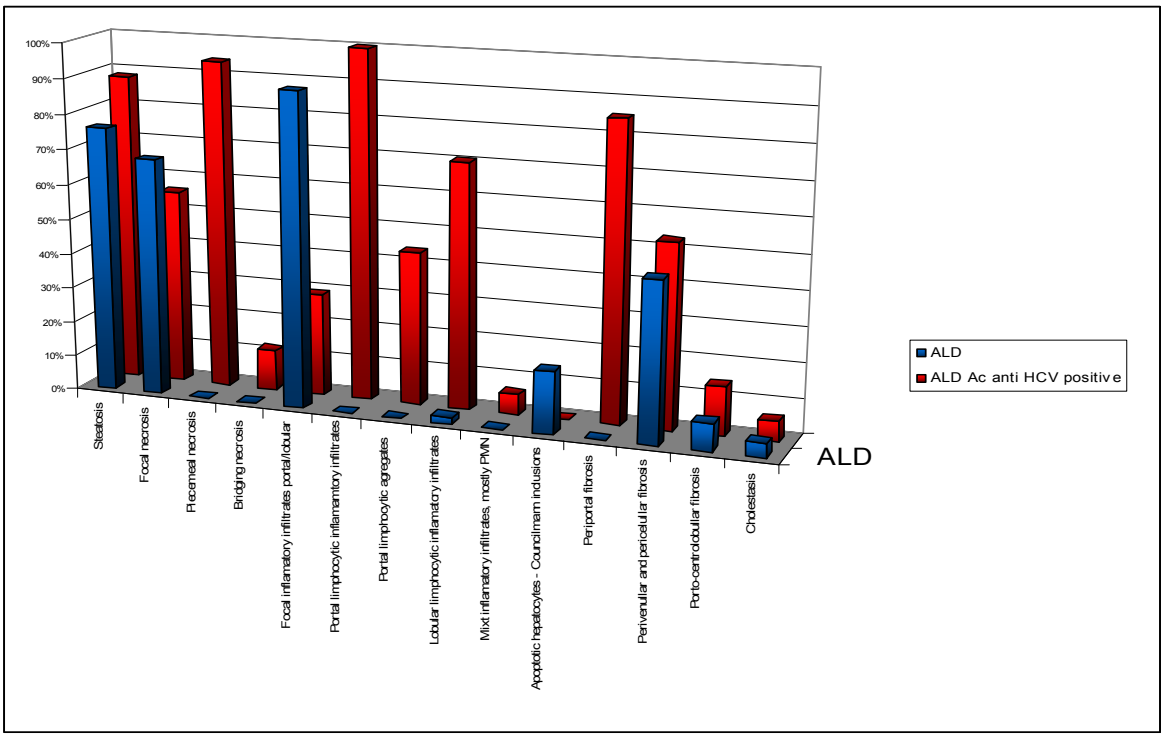

Fig. 4 Comparative main histological modifications our two groups of patients

Only 4 patients have had the histological character of active aggressive chronic hepatitis, with bridging necrosis and multi lobular necrosis unusual for hepatitis C chronic infection. The presence of C viral infection introduces in the patients' hepatic sample the progressive character of hepatic suffering through the presence of "piecemeal necrosis". (figure 5, 6)

The classic histological signs of the alcoholic liver disease were present on all preparative of the patients in a variable proportion. In patients with alcoholic liver disease we may notice the relative incidence of the pericentrolobular polymorphonuclear inflammatory infiltrate, with persistent features of the inflammation, while in the alcoholic disease with associated hepatitis C infection this aspect was described only in four patients. (figure 7, 8, 9).

In the group of patients with alcoholic disease and hepatitis C virus infection the histological aspect was dominated by the portal and lobular diffuse mononuclear inflammatory infiltrate as compared to the group without concomitant hepatitis c infection($p=0.0010$) statistically significant. In the majority of cases histological activity was moderate with „piecemeal necrosis". (figure 10)

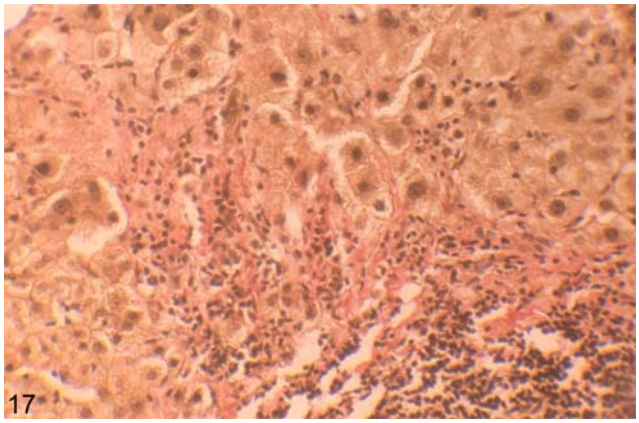

Fig. 5 AST/ALT ratio<1, hepatitis C infection present; portal and lobular inflammatory infiltrate with piecemeal necrosis, ballooned hepatocytic cells with dystrophy along the necro-inflammatory septum generated in the portal space

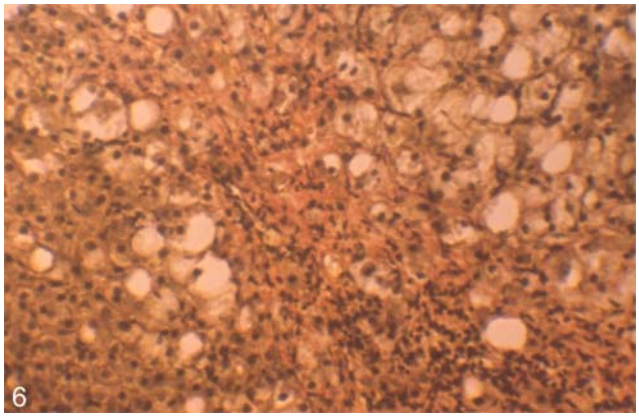

Fig. 6 AST/ALT ratio<1, hepatitis C infection present; macro vesicular steatosis, portal and lobular diffuse inflammatory infiltrate with "piecemeal necrosis" and „bridging necrosis"

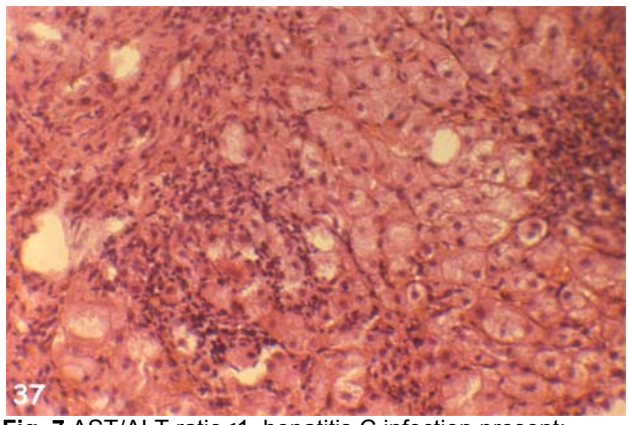

Fig. 7 AST/ALT ratio<1, hepatitis C infection present; portal and lobular diffuse inflammatory infiltrate with piecemeal necrosis, slight macro vesicular steatosis and fibrosis 3

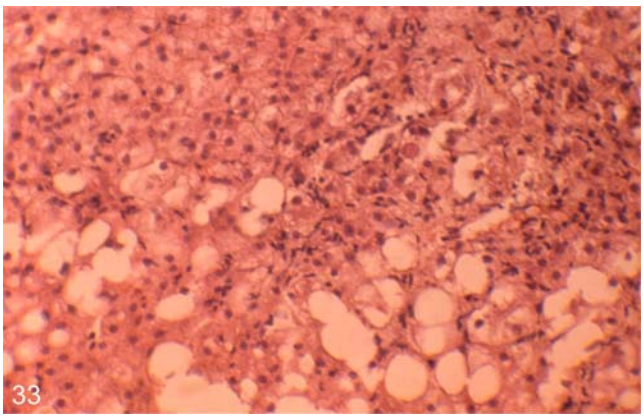

Fig. 8 AST/ALT ratio<1, hepatitis C infection present; aspect of aggressive hepatitis with bridging necrosis, macro- and micro vesicular mixed steatosis with cellular dystrophies and alcoholic Mallory hialin

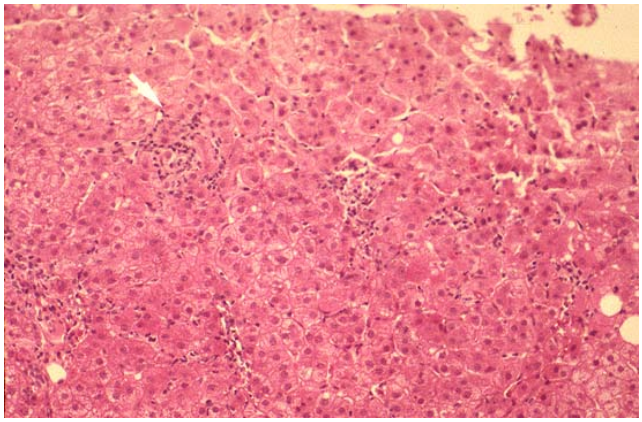

Fig. 9 AST/ALT ratio<1, hepatitis C infection present; portal and lobular diffuse inflammatory infiltrate with the tendency to form lymphoid follicles (arrow), without lesions of alcoholic hepatitis

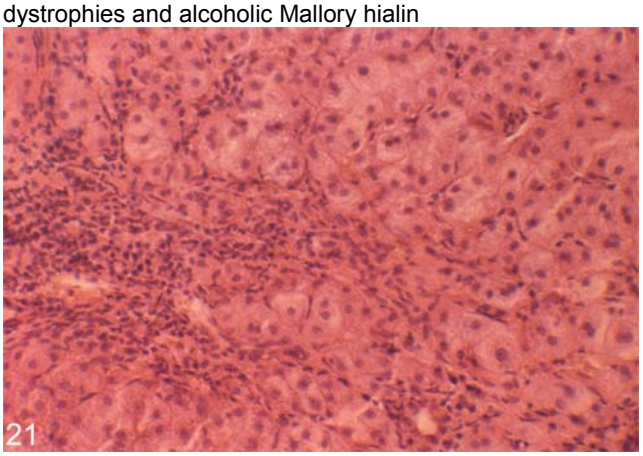

Fig. 10 AST/ALT ratio<1, hepatitis C infection present; active hepatitis with piecemeal necrosis and bridging necrosis, fibrosis 4

In our sample of patients with alcoholic liver disease the presence of the antibodies anti hepatitis C virus is relevantly associated with the presence of the histological signs of non-alcoholic active chronic hepatitis. The histological aspect of active chronic hepatitis is unusual for the alcoholic liver disease and in our sample this aspect is strictly correlated with the presence of chronic hepatitis C viral infection. "Piecemeal necrosis" was present exclusively in alcoholic liver disease associated with markers of chronic hepatitis C infection.

The histological activity has a moderately active character with the exception of four cases. For these patients the histological aspect is that of aggressive hepatitis with a histological activity index score over 13.6. These patients had cholestatic forms of disease, with severe inflammatory infiltrate in the portal and diffuse lobular spaces, with formation of periductal lymphocyte follicles (figure 11, 12).

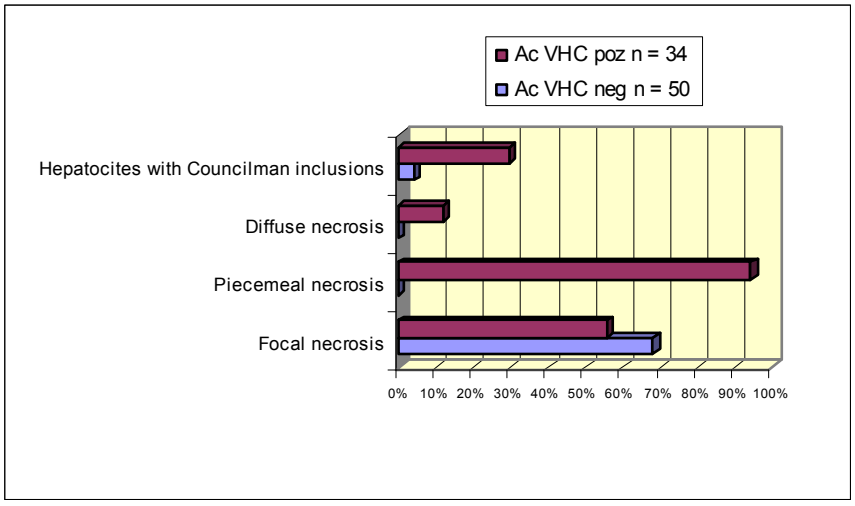

Fig. 11 The pattern of necrosis in our groups of patients

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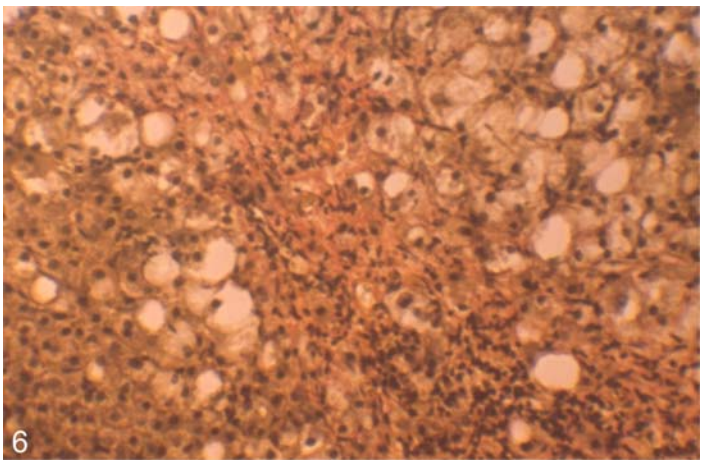

Fig. 12 AST/ALT ratio<1, hepatitis C infection present; macrovezicular steatosis, diffuse portal and lobular inflamatory infiltrtates with "piecemeal necrosis" and „bridging necrosis"

None of the patients with alcoholic liver disease exhibits aspects of histological activity of the hepatic disease. In all patients with alcoholic disease and hepatitis C infection, the histological picture is dominated by the active but moderate inflammation determined by the viral component.

24 of the patients with hepatitis C infection and alcohol intake (70.58%) had lymphocytes inflammatory infiltrate in the hepatic lobule. In 10 samples (29.41%) intra cytoplasmatic acidophil inclusions have been noticed, considered as the histological markers of apoptosis.

The histological signs of alcoholic liver disease were present in all cases, with macro vesicular steatosis, pericellular and pericentrolobular fibrosis, focal necroses and polymorphonuclear and/or mixed lymphocytes and polymorpho nuclear inflammatory cells infiltrate. In mixed inflammatory infiltrates in alcoholic hepatitis polymorphonuclears predominate whereas in the alcoholic liver disease with concomitant C viral disease the dominant cells in inflammatory infiltrate are lymphocytes cells (figure 13).

Even though steatosis may be correlated with the hepatic inflammation and cytolysis (FROMET et all 1996), as hepatocytes rich in fats are more vulnerable to aggressive intra- and extra cellular factors, we have not noticed relevant differences regarding the degree of steatosis according to the presence or absence of the C viral infection in patients with alcoholic liver disease which could explain the differences in the inflammation score and the presence of the active hepatic lesions in patients with alcoholic liver disease and C viral infection.

The histological activity of the inflammation in hepatitis C associated to the consumption of alcohol has a moderately active character, with the exception of four cases which exhibit the histological aspect of aggressive hepatitis, with "bridging necrosis" and a HAI score over 13.6. These patients exhibit diffuse hepatocytic necrosis, cholestatis with periductal lymphocyte follicles.

On all examined samples fibrosis was present in different degrees. Fibrosis was present in a total of over 87% of the analysed cases, more severe for patients with the C virus (p=0.001).

Our sample from patients with alcoholic liver disease, 23 (46%) have pericentrolobular, perivenular and peri cellular fibrosis characteristic to the alcoholic liver disease through area selectivity of the hepatic lesions induced by alcohol[14]. (figure 14).

In all examined samples in chronic hepatitis C associated with alcohol consumption fibrosis was present in different degrees, from moderate to severe. 27 patients (79.41%) had periportal fibrosis, in 9 samples (26.47%) proto-centrolobular or "bridging fibrosis", in 7 samples (20.58%) and severe fibrosis score 4 in 3 cases (8.82%). 14 (41.17%) samples from this group of patients exhibit also pericentrolobular fibrosis, specific to alcohol induced fibrosis, emphasises the process of fibrosis with viral and alcohol induced mechanisms.

Fibrosis was disposed periportall (p=0.01), an aspect which was not noticed in patients with alcoholic disease without hepatitis C concomitant infection, associated in some cases (p=0.001) with pericellular and perivenular disposed fibrosis, probably as a result of the effects and the area selectivity of hepatic lesions induced by chronic alcohol consumption.

The histological signs of the alcoholic liver disease, the polymorphonuclear inflammatory infiltrate and the focal necrosis, were less exhibited in hepatitis C infected group of patients, even though all patients presented the same severe consumption of alcohol. (figure 15).

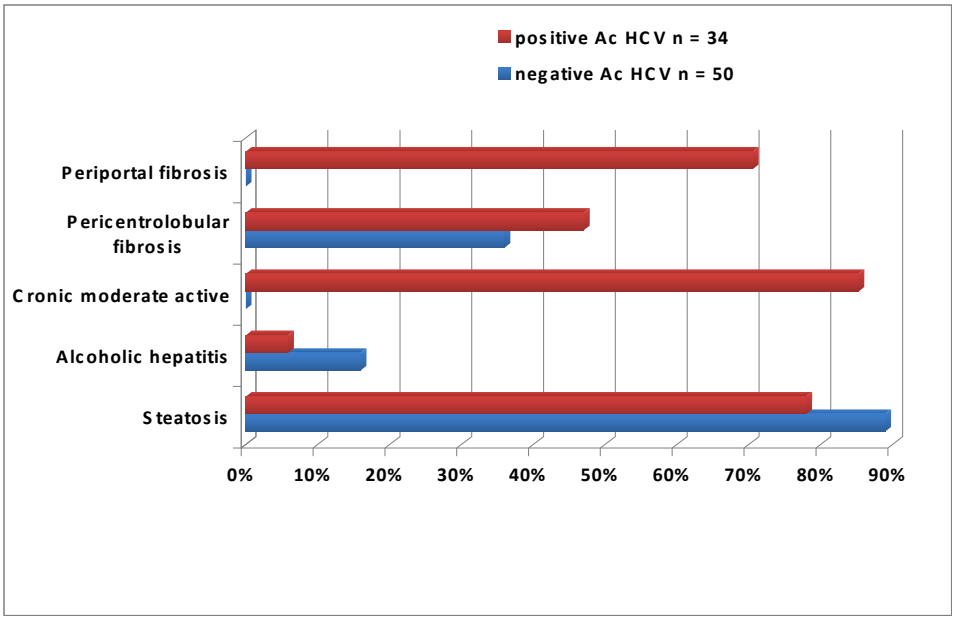

Fig. 13 Histological aspects according to the presence or absence of hepatitis C infection

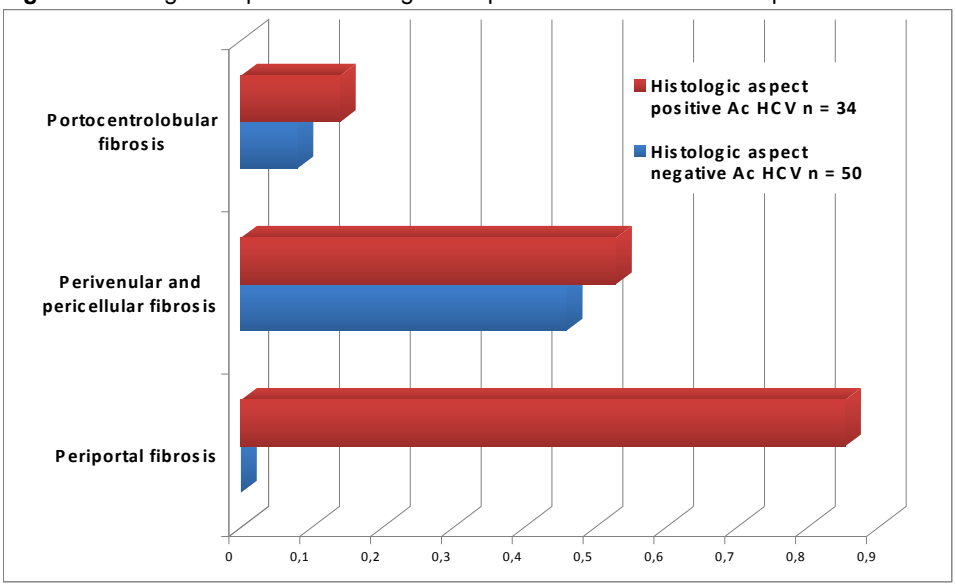

Fig. 14 Fibrosis pattern in our two groups of patients

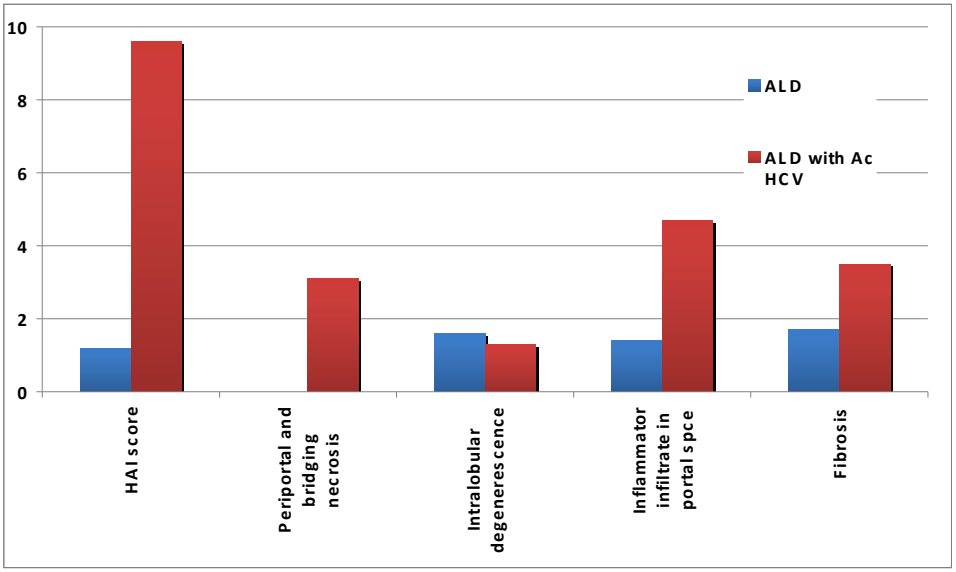

Fig. 15 Score of necro-inflammation and fibrosis in our two groups of patients

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Periportal disposed fibrosis was noticed only in the group of patients with concurrent hepatitis C viral infection, often associated with pericentrolobular fibrosis, marker of alcoholic damage (p=0.001) statistically significant.

In the group of patients with hepatitis C infection and alcohol intake fibrosis was also disposed periportal, as compared to the pericellular and perivenular fibrosis noticed on histological elements from patients with alcoholic liver disease.

In our sample of patients with hepatitis C chronic viral infection and alcoholic disease, the activity of hepatic lesions and the markers of inflammation is dominated by the viral component of the disease, with a higher degree of mixed fibrosis periportal, perivenular and pericellular disposed.

CONCLUSIONS

The histological signs of the alcoholic liver disease were less exhibited in hepatitis C infected group of patients, even though all patients presented the same severe consumption of alcohol.

Hepatic histological pattern of the lesions in mixed forms is dominated rather by the histological aspects of viral hepatitis than by the typical aspect of the alcoholic liver disease.

Alcohol consuming in hepatitis C chronic infection increase the liver dammage and accelerates the rate of fibrosis with mixed dispossition

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