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FACTORS ASSOCIATED WITH SEVERE FIBROSIS IN CHRONIC HEPATITIS C

A. GEORGESCU[1] D. GEORGESCU[1]
I. ZAHARIA-KEZDI[1] C. CHIRIAC[1] B. ŢILEA[1]

Abstract: *The paper presents an epidemiological, biological, virological and histological study of patients suffering from chronic viral hepatitis C with severe fibrosis whose aim was to identify the main etiological factors of the disease. The main characteristics for the group were advanced age, alcohol consumption, severe cytolytic syndrome, high viral load values, severe necroinflammatory lesions, alcohol induced histological lesions, biliary pathways pathology – leading to a pathological entity that is very severe, with a strong potential towards cirrhosis and hepatocellular carcinoma.*

Key words: *hepatitis, C, fibrosis.*

Introduction

The chronic viral hepatitis C is together with the alcoholic hepatopathy the leading cause of hepatic morbidity in our geographical area. Despite the progress in early diagnosis and therapy it remains a challenge for the clinician and a major public health concern [12].

The most important histological aspect in the chronic viral hepatitis C is the severity of the fibrosis, representing the main prognostic factor for the evolution towards hepatic cirrhosis and hepatocellular carcinoma [5], [9], [13-14]. The fibrosis and its progression speed in chronic viral hepatitis C closely reflect the combined action of the hepatitis C virus and the host reaction to the viral aggression [8-9]. Studying patients with chronic viral hepatitis C and severe fibrosis is very important, allowing us to identify the factors that promote fibrosis and control them efficiently.

Objectives

The study aims to identify and assess the demographical, biological, histological and virological elements associated with severe fibrosis. By sorting the chronic viral hepatitis C patients according to the severity of fibrosis and comparing data derived from sex and age distribution, alcohol consumption, hepatic enzyme profile, histological lesions other than fibrosis and viral load we can eventually determine which of them correlates better with severe fibrosis. Identifying these factors is extremely important, allowing us to select patients at high risk and to create a true profile of the patient with severe fibrosis. Last but not least some of the factors are amenable to treatment, so we could turn them into therapeutic targets in order to improve the prognosis for these patients.

[1] University of Medicine and Pharmacy Târgu-Mureş.

Material and Methods

Our cases consist of 129 patients selected between 2009 and 2010 suffering from chronic viral hepatitis C.

The chronic viral hepatitis C diagnosis was based on the presence of VHC antibodies, detectable HCV RNA and chronic hepatitis lesions on hepatic biopsy.

The inclusion criteria were age between 25 and 65, fibrosis score between 1 and 3 (Metavir score), VHC antibodies present and detectable HCV RNA.

The exclusion criteria for this group were age below 25 or above 65, hepatic cirrhosis, hepatic pathology other than viral hepatitis C: viral B, B+D, autoimmune, primary or secondary biliary, diabetes, obesity (BMI over 25), pathology that goes with stasis liver, liver thesaurismosis, congenital jaundice.

Our choice for age limits was based on the following: below the age of 25 many patients have a fibrosis score of 0 and there isn't enough time for other factors such as alcohol to make an impact. Above 65 years of age the antiviral therapy is seldom prescribed and we regard performing hepatic biopsy on pure scientific grounds to be ethically unjustified; moreover at this age many patients suffer from comorbidity, especially diabetes, obesity and heart condition.

VHC antibodies were determined by 2nd or 3rd generation ELISA in 2 independent laboratories; HCV RNA was determined by real time PCR considering 15 copies/ml as the lower detection limit.

We considered assessing accurately the degree of alcohol consumption to be essential. Alcohol intake evaluation was based on medical history (own and family), gamma-glutamyl transpeptidase values, tests such as MAST (Michigan Alcoholism Screening Test) and ESPAD. All patients went through the former test to document alcohol abuse; the ESPAD test was used to quantify the alcohol consumption in patients with more than 3 MAST positive answers, corroborating it with medical history. We defined alcohol abuse as an intake of more than 20 grams/day in both men and women.

Main demographical data, body mass index, serum transaminase (SGOT, SGPT) values and colestase enzyme (ALP, GGT) values were recorded for all patients.

All patients underwent hepatic biopsy, the necroinflammatory and fibrotic lesions being quantified according to the Metavir score. Besides the fibrosis and necroinflammatory scores we also recorded particular histological aspects specific to the viral hepatitis C, such as hepatic steatosis, sanded nuclei, portal lymphatic nodes, epithelial lesions along the bile ducts, proliferating bile duct epithelial cells and cholestatic lesions [5], [7].

The patients were classified according to the fibrosis score. The cases comprised patients (n=48) with a Metavir score of 3 and controls consisted of patients with a Metavir score of 1 or 2 (n=81).

For database analysis we used Microsoft Access and Microsoft Excel, and statistical work was done using Graph Pad Instat and Medcalc software, considering $p<0.05$ as the significance threshold.

Results and discussions

The distribution across sexes shows no statistically significant difference ($p=1.00$) between cases (39.50% men, 60.50% women) and controls (39.58% men, 60.41% women). The data is a little surprising at least at first glance given the fact that alcohol abuse is much more prevalent among men, so we would expect a more severe fibrosis among them [12], [15]. A good explanation for the roughly equal sex distribution would be women's increased susceptibility to alcohol aggression, accounting for the mismatch in prevalence of alcohol consumption among

sexes [9]. Another explanation is the lack of homogeneity for the cases comprising more women, the viral hepatitis C being quite often an incidental finding in women who generally undergo more often than men routine check-ups. Although the female sex is predictive for a better response to antiviral therapy, it doesn't prevent severe hepatic lesions. Severe hepatic fibrosis associated with viral hepatitis C is as common in women as in men [15].

The mean age for the cases is 50.72±2.33 compared to controls 44.58±2.36, the difference being statistically highly significant ($p<0.0001$). Such results are to be expected given the fact that fibrosis is an irreversible lesion progressing in time [13-14], [16]. The same pattern holds true for alcohol induced lesions [13]. The time related difference between the two groups confirms perfectly well the natural course of viral hepatitis C fibrosis as described by medical literature. Passing to the superior degree of fibrosis takes around 5 years or less, especially if the viral infection occurs in late age [8,15]. All available data points to the significance of age as a predictive factor for severe fibrosis, making it very important to diagnose the condition at an early age, when fibrotic lesions are less severe and the chances of getting a decent response to therapy are much better [2], [9], [15]. Finally, the mean age of 50.72 for patients with severe fibrosis means we are dealing with an active segment of the population both on social and professional level. That makes it even more important to aim for therapies that prevent or delay the natural progression towards cirrhosis and hepatocellular carcinoma [2]. By looking at the fibrosis progression curve we expect patients with severe fibrosis to suffer from hepatic cirrhosis at a mean age of 54.32±3.55, meaning that the time span available for diagnosis and treatment is extremely small.

The relation between chronic viral hepatitis C and alcohol consumption is well known, the alcoholics having an increased risk for infection with the hepatitis C virus on one hand, and on the other hand the alcohol is known to promote the lesions generated by the virus and its replication process [8-9], [14]. We had pathological alcohol intake being present in 50% of the cases compared to 18.51% in controls, $p=0.0003$. The difference is extremely significant statistically and underlines the prevalence of alcohol consumption in patients with chronic hepatitis C patients and its effect upon the evolution of the disease, leading to an aggravated fibrosis and accelerated progression towards cirrhosis given the low response rate to therapy. [2]. Data suggests that alcohol consumption should be accurately diagnosed, even doses considered socially acceptable, the threshold that we used being a moderate one (20 grams pure alcohol per day). Alcohol intake is one of the few liver aggression factors that can be managed and it's relevant since 50% of the patients with severe fibrosis have a pathological level of alcohol intake.

Serum transaminase values are significantly higher in cases compared to controls. Thus for SGOT we found a value (U/l) of 83.87±12.15 versus 67.46±7.62, $p=0.0197$ and for GPT 121.44±16.34 versus 96.41±12.74, $p=0.0198$. The more severe cytolytic syndrome in patients with severe fibrosis comes as no surprise and, as explained later in the paper, is due to the more advanced necroinflammatory lesions and higher viral load [10], [15]. The GPT/GOT quotient is above 1, a typical viral pattern. Despite frequent alcohol consumption SGOT is not higher than SGPT. Although trying to extrapolate histological data from biological data is not always successful we consider that a true positive correlation between the severity of

the cytolytic syndrome and the severity of the fibrosis in patients with chronic viral hepatitis C exists [15].

Of great interest is the determination of gamma-glutamyl transpeptidase values in order to properly assess the degree of alcohol abuse. For the cases we found a GGT average value of 95.41±17.12 versus 75.26±14.21 for controls, the difference being statistically significant p=0.011. Since 50% of the cases fall into the pathological alcohol consumption category we are not surprised but want to stress the fact that the enzyme retains its specificity for toxic hepatic lesions even when the viral hepatitis C is also present, serving as a very useful diagnosis tool [10]. Another explanation for the high GGT is the severe fibrosis itself, the enzyme levels increase matching the evolution towards hepatic cirrhosis [9], [12], [16].

The alkaline phosphatase is higher in cases 221.40±24.12 compared to controls 183.32±13.05, the difference being very statistically significant, p=0.0036. As an explanation we offer the hepatitis C virus tropism for the bile duct epithelium and the alterations of its architecture combined with biliary canaliculi neoformation as the fibrosis would progress towards cirrhosis, something often documented in patients with severe fibrosis [10].

The viral load values (UI/ml) are higher for the case group 171567±66535 compared to the control group 104963±27948, the difference being extremely statistically significant, p<0.0001. The explanation is on one had the causal relation between high viral loads and fibrosis progression and on the other hand by an increase in viral load secondary to alcohol abuse [2], [12]. Higher viral loads in patients with severe fibrosis lead to negative expectations regarding the response to antiviral treatment and a more rapid progression towards cirrhosis and hepatocellular carcinoma [2], [10].

Assessing the necrotic and inflammatory activity shows higher Metavir scores for the cases 12.93±0.92 compared to controls 7.81±0.52, a difference that is very statistically significant, p<0.0001. The severe fibrosis is the result of an increased necroinflammatory activity, an inverse temporal and causal relation. At the same time higher viral loads lead to more severe necroinflammatory lesions [5], [6]. Our findings also confirm the more serious cytolytic syndrome in patients with severe hepatic fibrosis [15].

Centrilobular fibrosis lesions are much more common among cases (58.33%) compared to controls (19.75%), an extremely statistically significant difference, p<0.0001. In general, perivenular fibrosis is indicative of alcohol consumption, and we found it in the same percentage as the one we found for alcohol abuse in the case group. It should be also mentioned that when the fibrosis is very severe it can affect the perivenular area even if there is no significant alcohol consumption [4], [9], [13]. However perivenular fibrosis is strongly correlated with alcohol consumption, even if it occurred many years in the past [9].

There was no significant difference regarding the fat macrovacuolar dystrophy in the two groups (cases 85.41%, controls 74.07%, p=0.184). The hepatic steatosis is common both in chronic viral hepatitis C and in alcoholic hepatopathy, leading us to expect more severe steatosis lesions in the case group. However, as the fibrosis progresses towards cirrhosis the steatosis lesions typically diminish in severity [1], [3-4], [11].

A typical histological finding for hepatitis C virus is the presence of periportal lymphocytic conglomerates [5]. Their frequency is not statistically different between the two groups, cases (70.83%) versus controls (66.66%), p=0.697. Our data shows that the higher

necroinflammatory score for the case group is the result of more severe histological lesions, such as periportal necrosis, bridge lesions and not so much the portal inflammation [6].

Another typical histological feature of the hepatitis C virus is the biliary epithelium alteration [5], [12]. Such lesions were found more often in the case group (75%) compared to the control group (41.97%), p=0.0005, an extremely statistically significant difference. The explanation resides in the parallel evolution of bile ducts lesions, necroinflammatory score and viral load [6], [13]. In a similar manner fibrosis progression leads to significant biliary canaliculi proliferation; thus we have such proliferations more often in the case group (72.91%) compared to the control group (37.02%), p=0.0001. These lesions signify progression towards cirrhosis. Both biliary epithelium lesions and biliary epithelium proliferations have a biological counterpart, the cholestatic syndrome, more often seen in patients with severe fibrosis as previously mentioned [3], [7], [10].

Conclusions

The patients with chronic viral hepatitis C with severe fibrosis, compared to those having moderate or mild fibrotic lesions, tend to be older, have the same distribution among sexes, higher alcohol consumption, a more severe cytolytic and cholestatic syndrome, higher viral load and more severe necroinflammatory lesions. The specificity of alcohol induced lesions is maintained, such as increased GGT levels and perivenular fibrosis; also the ones generated by the hepatitis C virus - portal lymphocytic conglomerates, biliary epithelium lesions and steatosis.

A particular clinical, biological, virological and histological profile of the patient suffering from chronic viral hepatitis C with severe fibrosis shapes up, having very negative expectations regarding its evolution towards cirrhosis, hepatocellular carcinoma and potential response to antiviral therapy.

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