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Diagnostic and prognostic significance of hepatic steatosis in patients with chronic hepatitis C

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Abstract

Knowing that hepatic steatosis (HS) is a common occurrence in patients with chronic hepatitis C (CHC), it is essential to establish what are the factors that predispose to its occurrence and what is the role of HS in the evolution and prognosis of patients with CHC who develop this feature. To achieve these aims, we performed a retrospective clinical study in 33 patients with CHC hospitalized, diagnosed, and monitored in the 2nd Medical Department of the Emergency County Hospital, Craiova, Romania, in a period of two years (2011–2012). Following clinical, hematological, biochemical, immunological, and pathological investigations of the 33 patients with CHC selected, only 14 patients showed pathological changes of the HS. The appearance of steatosis in patients with CHC results from a complex interaction between the particularities of the host and viral factors. The main risk factors of the host, which contributed to the appearance of HS were sex, age, body mass index (BMI), body weight, and personal history of pathology (obesity, metabolic syndrome). Virus-related factors involved in HS were viremia and viral genotype. In conclusion, HS is a common finding (42.42%) in patients with CHC, particularly genotypes 1 and 2. Early detection of HS by invasive or non-invasive methods is an important objective of monitoring patients with CHC, because HS is correlated with a high degree of fibrosis. Therefore, early correction of metabolic factors and early introduction of antiviral therapy are important targets for treating of patients with CHC.

Keywords: hepatic steatosis, hepatitis C virus genotypes 1 and 2, prognosis chronic hepatitis.

Introduction

Worldwide, hepatitis C virus (HCV) infection is a major public health problem. According to the *European Association for the Study of the Liver* (EASL), in 2015 there were approximately 71 million people chronically infected with HCV [1]. Liver biopsy puncture performed in patients with CHC highlights two factors involved in liver disease progression, namely, periportal necrosis and hepatic steatosis (HS) [2]. HS is defined by the accumulation of lipids in hepatocytes. Physiologically, hepatocytes contain small amounts of lipids necessary for metabolism, and when lipids exceed 5–10% of the weight of hepatocytes,

HS occurs [3]. HS is a common histopathological feature of patients with CHC [4–6]. The incidence of HS in patients with CHC varies between 40% and 86%, with an average of 55% [7]. In patients with CHC, many factors that predispose to HS: viral factor (genotype 3), host factors (overweight, dyslipidemia, hypertension, type 2 diabetes, and insulin resistance) and certain drugs (glucocorticoids, Amiodarone, Methotrexate) [8]. Two forms of HS can be found in these patients. “Metabolic” steatosis can coexist with the CHC, regardless of genotype, and occurs in people at risk, such as obesity, dyslipidemia, high blood pressure, type 2 diabetes, and insulin resistance. The second form of steatosis, “viral steatosis”, specific to genotype 3, results

from the direct cytopathic effect of the viral infection on the infected hepatocytes [9]. Liver biopsy is currently the “gold standard” for diagnosing and assessing of the severity of HS [10]. On pathological examination, HS has a macrovesicular appearance and is predominantly distributed in the periportal area and less in the centrilobular region [11]. The progression of liver disease is supported by portal inflammation, periportal necrosis and liver fibrosis [12], so the high incidence of steatosis in patients with CHC is evidence that it plays a significant role in disease progression [13, 14]. Risk factors, such as old age, male sex, chronic alcohol consumption, obesity and type 2 diabetes are progressive factors for both HCV infection and HS [4, 12, 15, 16].

Aim

The main objective of the study was to emphasize the importance of establishing the diagnosis of steatosis in the evaluation of the prognosis of patients with CHC, particularly genotypes 1 and 2.

☐ Patients, Materials and Methods

We conducted an open clinical retrospective study in which we analyzed HS in liver biopsy fragments performed in 33 patients with CHC, diagnosed and monitored in the 2nd Medical Department of the Emergency County Hospital, Craiova, Romania, within two years. The clinical examination of the patients included in the study was followed by paraclinical investigations, such as antibodies against HCV, quantitative tests [HCV–ribonucleic acid (RNA)], hematological, enzymatic, biochemical, immunological tests and liver biopsy. The criteria for inclusion in the study were the presence of antibodies against HCV, serum HCV–RNA titer detected, liver biopsy puncture, the presence of metabolic syndrome components (type 2 diabetes, dyslipidemia, obesity and hypertension), lipid and carbohydrate profile [total cholesterol, triglycerides (TG), blood sugar] and liver function tests [total bilirubin (TB), γ -glutamyltransferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albuminemia, prothrombin time (PT), total protein, γ -globulins, immunoglobulins]. The positive diagnosis of HS was suggested in base of the abdominal ultrasound and confirmed by liver biopsy puncture. Exclusion criteria were clinical and paraclinical criteria for decompensated liver disease (coagulation disorders, rupture of esophageal varices, ascites and hypoproteinemic edema), the presence of autoimmune diseases (collagenosis, autoimmune hepatitis), the presence of genetic diseases (hemochromatosis, α_1 -antitrypsin deficiency, Wilson’s disease) and chronic alcohol and drug use (Amiodarone, Methotrexate, and glucocorticoids). The COBAS[®] HCV ver. 2.0 Amplicor method and the CHIRON RIBA HCV 3.0 Strip Immunoblot Assay system were used to perform virological and serological tests. The biochemical and hematological tests were processed in the Clinical Laboratory of the Emergency County Hospital, Craiova, using the Celltac Nihon Khoden system and Vitros 250 dry chemistry analyzers. Liver biopsy was performed using the percutaneous method using the special Hepafix kit (Braun Melsungen AG). The fragments harvested from liver tissue (with a diameter between 1–1.4 mm/10–25 mm) were fixed in 10% neutral buffered

formalin, then processed by paraffin inclusion and stained with Hematoxylin–Eosin (HE) and van Gieson, according to the standard protocol practiced in the Laboratory of Pathological Anatomy of the Emergency County Hospital, Craiova. The pathological samples obtained were analyzed with a microscope with a built-in 5 Mpixel digital camera (MOTIC manufacturer), equipped with software integrated in the microscope. The pathological aspects were evaluated independently by two investigators, using the parameters provided in the Ishak score [17]. The presence or absence of steatosis in patients with CHC was analyzed in relation to the mean values of the main anthropometric, clinical, hematological, biological, immunological, and pathological parameters. Statistical data were processed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and IBM Statistical Package for the Social Sciences (SPSS) Statistics 20.0 (IMB Corporation, Armonk, NY, USA). Numerical variables showed a normal distribution of data in each group studied or globally, so we were allowed to use parametric statistical tests. The results thus obtained were summarized as mean \pm standard deviation (SD). The relevant values for the statistical tests were *p*-values lower than 0.05, being considered significant. For the use of patient data, the study was performed using informed consent.

☐ Results

The patients’ group structure

The study was performed on a group of 33 patients with CHC, from which 16 (48.48%) were men and 17 (51.51%) were women. The mean age of the studied patients was 47.87 ± 11.11 years. The distribution by age was: nine (27.27%) patients were in the group age under 40 years, 18 (54.54%) patients were in the group age 41–60 years and five (15.15%) patients in the group age over 61 years. In the studied patients with CHC, the mean value of body weight was 71.78 ± 8.51 kg, body mass index (BMI) was 25.12 ± 1.65 kg/m² and abdominal circumference (AC) was 90.82 ± 10.02 cm (Table 1).

The positive diagnosis of CHC was established based on HCV–RNA viremia in the 33 (100%) of the studied patients (Table 1). The mean viremia value in the studied patients was $1\,499\,548.49 \pm 283\,970.53$ copies/mL. In this group of patients, the liver biopsy puncture demonstrated the presence of HS in 14 (42.42%) patients.

The epidemiological study analyzed the incidence of the main endogenous risk factors in the occurrence of HS (Tables 1 and 2). In this group of patients, HS was present mainly in men, the observed male/female ratio being 10:4. In the patients with CHC studied, the mean age of those with HS was 49.36 ± 12.11 years, while the mean age of patients without HS was 45.85 ± 9.84 years. In patients with CHC analyzed, the mean body weight was 71.78 ± 8.51 kg, with patients with HS having a significantly higher mean weight ($p=0.005$) (75.46 ± 11.85 kg) compared to those without steatosis (68.21 ± 5.17 kg). A similar behavior was reported in the case of BMI, which showed significantly higher mean values ($p=0.004$) in patients with HS (26.21 ± 1.72 kg/m²), compared to those without HS (24.32 ± 1.07 kg/m²). Also, patients with CHC and HS showed a significantly higher mean value ($p=0.034$) of AC (95.57 ± 9.56 cm), compared to patients who did not develop HS (87.32 ± 9.04 cm) (Table 2).

Table 1 – Mean values and SDs of anthropometric and virological parameters in the group of patients with CHC, as well as mean values and standard errors of these parameters, depending on the presence or absence of HS, together with the value of the statistical test for comparative analysis of studied parameters in the two subgroups of patients

Parameter	Mean value and SD in patients with CHC	Mean value and SD in patients with CHC with steatosis	Mean value and SD in patients with CHC without steatosis	*Statistical significance p-value
Age [years]	47.87±11.11	49.36±12.11	45.85±9.84	0.31
Men [n]	16	10	6	0.02
Women [n]	17	4	13	0.02
Body weight [kg]	71.78±8.51	75.46±11.85	68.21±5.17	0.005
BMI [kg/m ²]	25.12±1.65	26.21±1.72	24.32±1.07	0.004
AC [cm]	90.82±10.02	95.57±9.56	87.32±9.04	0.034
Viremia [copies/mL]	1 499 548.49±283 970.53	1 743 423.36±332 478.43	1 255 673.5±238 577.96	0.001
Genotype 1 [n]	31	13	18	0.46
Genotype 2 [n]	2	1	1	0.82

AC: Abdominal circumference; BMI: Body mass index; CHC: Chronic hepatitis C; n: No. of cases; HS: Hepatic steatosis; SD: Standard deviation. *The statistical significance of the comparison of the mean values recorded in the patients with CHC and steatosis versus the patients with CHC without steatosis.

Table 2 – The mean values of the incidence of significant pathological history and clinical manifestations recorded in patients with CHC, depending on the presence and absence of HS

Parameter	Incidence of diseases/signs in patients with CHC	Incidence of diseases/signs in patients with CHC with steatosis	Incidence of diseases/signs in patients with CHC without steatosis	*Statistical significance p-value
Hypertension	11/33 (33.33%)	7/14 (50%)	4/19 (21.05%)	0.08
Diabetes mellitus	7/33 (21.21%)	4/14 (28.57%)	3/19 (15.78%)	0.39
Dyslipidemia	23/33 (69.69%)	10/14 (71.42%)	13/19 (68.42%)	0.85
Metabolic syndrome	12/33 (36.36%)	8/14 (57.14%)	4/19 (21.05%)	0.03
Obesity	14/33 (42.42%)	10/14 (71.42%)	4/19 (21.05%)	0.002
Pallor	9/33 (27.27%)	5/14 (35.71%)	4/19 (21.05%)	0.32
Jaundice	4/33 (12.12%)	2/14 (14.28%)	2/19 (10.52%)	0.75
Ecchymosis	2/33 (6.06%)	1/14 (7.14%)	1/19 (5.26%)	0.82
Hepatomegaly	21/33 (63.63%)	11/14 (78.57%)	10/19 (52.63%)	0.13
Splenomegaly	12/33 (36.36%)	6/14 (42.85%)	6/19 (31.57%)	0.52

CHC: Chronic hepatitis C; HS: Hepatic steatosis. *The statistical significance of the comparison of the mean values recorded in the patients with CHC and steatosis versus the patients with CHC without steatosis.

In the studied patients with CHC, the incidence of hypertension was 33.33%, which was present in half (50%) of patients with HS and in only four (21.05%) patients without HS. The incidence of diabetes mellitus in the studied patients was 21.21%, being present in four (28.57%) patients with CHC and HS and in three (15.78%) patients with CHC without HS. Dyslipidemia, recognized in the personal pathological history of 23 (69.69%) patients with CHC, was present in 10 (71.42%) patients with CHC and HS and in 13 (68.42%) patients without HS. The metabolic syndrome, a clinical entity discovered in the pathological history of 12 (36.36%) patients with CHC, was present in eight (57.14%) of the patients with HS and in only four (21.05%) patients without HS. Fourteen (42.42%) of the patients with CHC were overweight. This characteristic was present mainly in patients with HS (71.42% vs 21.05%). The clinical study of these patients allowed the calculation of the incidence of the main clinical signs, as well as the establishment of the statistical significance of these clinical parameters in the anticipation of HS in patients with CHC (Table 2). The clinical signs frequently encountered in these patients were hepatomegaly (63.63%), splenomegaly (36.36%), pallor (36.36%), jaundice (12.12%) and ecchymosis (6.06%).

The virological study

The virological study analyzed the main virus-related

factors involved in the occurrence of HS, namely viremia and viral genotype (Table 1). The mean value of viremia in patients with steatosis (1 743 423.36±332 478.43 copies/mL) was significantly higher ($p=0.001$), compared to the mean value of viremia in the patients in whom liver biopsy puncture did not show HS (1 255 673.5±238 577.96 copies/mL). Genotyping performed in these patients revealed the presence of genotype 1 in 31 (93.93%) patients and genotype 2 in two (6.06%) of the studied patients.

The hematological and metabolic studies

The hematological and metabolic study analyzed the behavior of hematological and metabolic parameters (Table 3) in selected patients, depending on the presence or absence of HS. The mean hemoglobin (Hb) value was higher in patients with HS (12.77±0.88 g/dL) compared to patients without HS (12.12±1.23 g/dL), without this difference being statistically significant (Table 3). Anemia was described in four (28.57%) patients with HS and in only three (15.78%) patients without HS. Regarding the number of white blood cell (WBC) count, patients with HS had a significantly higher mean value (5176.32±1045.67/mm³) than that reported in patients without HS (4878.57±1249.11/mm³). Leukopenia (WBC<4000/mm³) was recorded in five (35.71%) patients with HS and in only three (15.78%) patients without HS. The mean platelet (PLT) count in the group of patients with HS was lower (163 071±41 112.3/mm³) but not statistically

significant compared to the mean value in patients without HS ($182\,578.9 \pm 46\,446.28/\text{mm}^3$). Thrombocytopenia ($\text{PLT} < 150\,000/\text{mm}^3$) was found in both patients with HS and patients without HS (35.71% vs 26.31%). The behavior of metabolic parameters was different in patients with CHC, depending on the presence or absence of HS (Table 3). Thus, the mean blood glucose value was significantly higher ($p=0.05$) in patients with HS (97.14 ± 8.24 mg/dL), compared to patients without HS (89.84 ± 12.37 mg/dL). The mean TG value in patients with HS was higher, but not statistically significant, compared to that recorded in patients

without HS (189.64 ± 36.45 mg/dL vs 176.05 ± 34.78 mg/dL), increased TG values (>150 mg/dL) registering both in patients with HS (71.42%) and in those without steatosis (68.42%). The mean cholesterol value in patients with HS was insignificantly higher (226.07 ± 56.16 mg/dL) compared to the mean value in patients without HS (205.78 ± 40.04 mg/dL), elevated serum cholesterol values (>200 mg/dL) registering both in patients with HS (64.28%) and in those without HS (52.63%). This research showed that only the parameters of carbohydrate metabolism were correlated with the presence of HS in the analyzed patients.

Table 3 – Mean values and SDs of hematological and metabolic parameters recorded in patients with CHC, with or without HS, as well as the statistical significance of these parameters in anticipation of HS

Parameter	Mean value and SD in patients with CHC	Mean value and SD in patients with CHC with steatosis	Mean value and SD in patients with CHC without steatosis	*Statistical significance p-value
Hb [g/dL]	12.51±1.11	12.77±0.88	12.12±1.23	0.19
WBC count [No./mm ³]	5050±1127.5	5176.32±1045.67	4878.57±1249.11	0.50
PLT count [No./mm ³]	174 303±44 676.3	163 071±41 112.3	182 578.9±46 446.28	0.22
Glycemia [mg/dL]	92.93±11.27	97.14±8.24	89.84±12.37	0.05
TG [mg/dL]	181.81±35.59	189.64±36.45	176.05±34.78	0.31
TC [mg/dL]	250.9±27.65	226.07±56.16	205.78±40.04	0.24

CHC: Chronic hepatitis C; Hb: Hemoglobin; HS: Hepatic steatosis; n: No. of cases; PLT: Platelet; SD: Standard deviation; TC: Total cholesterol; TG: Triglycerides; WBC: White blood cell. *The statistical significance of the comparison of the mean values recorded in the patients with CHC and steatosis versus the patients with CHC without steatosis.

The hepatic functions' study

Analyzing the mean values obtained from the biochemical parameters, differences were found in patients with steatosis, compared to those who did not develop steatosis (Table 4). TB showed a higher mean value in patients with steatosis (1.21 ± 0.28 mg/dL) compared to patients without steatosis (1.03 ± 0.24 mg/dL), with not statistically significant differences. TB was elevated in half (50%) of the patients with HS and only in two (10.52%) patients without HS, with not statistically significant differences between the two groups. The cholestasis enzymes had a significantly higher mean plasma activity ($p=0.02$) in patients with CHC and HS (57.42 ± 15.59 U/L) compared to patients with CHC without HS (43.73 ± 13.94 U/L) in the case of GGT and significantly lower mean plasma activity ($p=0.01$) in the patients with HS (205.14 ± 52.51 U/L) compared to patients without HS (156.47 ± 45.76 U/L) in the case of ALP. GGT was elevated in eight (57.14%) patients with HS and in only three (15.78%) patients without HS, while ALP had elevated plasma activity in most patients with HS, 13 (92.85%) patients and in 12 (63.15%) patients without HS. The behavior of hepatic cytolysis enzymes was similar, with a higher mean plasma activity in patients with CHC and HS (117.78 ± 88.77 U/L) compared to patients with CHC without HS (107.73 ± 55.13 U/L) in the case of ALT and higher mean plasma activity in patients with HS (121.85 ± 105.45 U/L) compared to patients without HS (87.26 ± 47.28 U/L) in the case of AST, with not statistically significant differences between the two groups. Elevated ALT values were found in 11 (78.57%) patients with HS and in 17 (89.47%) patients without HS, and AST was elevated in most patients, 10 (71.42%) patients with HS and 15 (78.94%) patients without HS. In patients with CHC who presented with HS, the mean value of PT was 13.71 ± 1.37 s, a slightly longer value than that recorded in

patients without HS (13.47 ± 1.12 s). The index of prothrombin (IP) showed a slightly lower mean value in patients with CHC who had HS ($75 \pm 16.2\%$ vs $83 \pm 12.14\%$), without this difference being statistically significant. The mean values of albuminemia found in the two groups of patients were approximately equal (3.74 ± 0.37 g% vs. 3.79 ± 0.5 g%), without any statistically significant difference between the patients of the two groups of patients.

The evaluation of the inflammatory response

The evaluation of the inflammatory response of the hepatic mesenchymal cells (portal and intralobular lymphoplasmacytic infiltrate cells) by so-called "mesenchymal hyperreactivity" functional tests or mesenchymal inflammation tests showed some differences in these tests depending on the presence or absence of HS (Table 5).

Thus, the mean value of erythrocyte sedimentation rate (ESR) in one hour (ESR/1 h) was significantly ($p=0.002$) higher in the patients with CHC and HS (10.14 ± 2.68 mm/1 h), compared to the patients with CHC without HS (6.95 ± 2.09 mm/1 h). Also, the mean C-reactive protein (CRP) value in the patients with HS was significantly ($p=0.05$) higher (0.65 ± 0.29 mg/dL) than the mean value of CRP in the patients without HS (0.49 ± 0.17 mg/dL). The mean value of α_2 -globulins in patients with CHC and HS (11.64±1.49%) was statistically significantly ($p=0.0001$) higher compared to the value of patients with CHC without HS (8.79±2.12%). The mean value of the serum γ -globulins concentration was higher in the patients with CHC who had HS (1.66 ± 0.35 g/dL) compared to the mean value of those who did not develop HS (1.6 ± 0.37 g/dL), without this difference being statistically significant. Patients with CHC and steatosis showed higher mean values (432.36 ± 295.45 IU/mL) of immunoglobulin G (IgG) compared to the group of patients with CHC without steatosis (307 ± 187.57 IU/mL). Abnormal IgG values were

found in 78.57% (11/14) of patients with CHC and HS and in 73.68% (14/19) of patients with CHC without HS. In patients with CHC and HS the mean serum immunoglobulin M (IgM) concentration was 234 ± 125.28 IU/mL, the increased values being found in 71.42% of patients, while in patients with CHC without HS the mean IgM value was lower (228.16 ± 102.94 IU/mL), with a lower

percentage of those who showed increased values (63.15%). The group of patients with steatosis, 28.57% had increased values of immunoglobulin A (IgA), the average value being 241.14 ± 120.95 IU/mL, while the group of patients without steatosis had a lower average of IgA of 195.26 ± 45.16 IU/mL, and the percentage of patients with high values was lower (26.31%).

Table 4 – Mean values and SDs of biochemical parameters recorded in the patients with CHC with or without HS, as well as the statistical significance of these parameters in assessing HS

Parameter	Mean value and SD in patients with CHC	Mean value and SD in patients with CHC and steatosis	Mean value and SD in patients with CHC without steatosis	*Statistical significance p-value
TB [mg/dL]	1.1±0.27	1.21±0.28	1.03±0.24	0.10
GGT [U/L]	49.54±15.98	57.42±15.59	43.73±13.94	0.02
ALP [U/L]	177.12±53.80	205.14±52.51	156.47±45.76	0.01
ALT [U/L]	112±70.25	117.78±88.77	107.73±55.13	0.55
AST [U/L]	101.93±77.61	121.85±105.45	87.26±47.28	0.21
PT [s]	13.71±1.37	14.03±1.64	13.47±1.12	0.37
IP [%]	79±14.28	75±16.2	83±12.14	0.20
ALB [g/dL]	3.76±0.42	3.74±0.37	3.79±0.5	0.76
ALB [%]	52.73±6.97	51.22±6.87	53.85±7.02	0.64

ALB: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CHC: Chronic hepatitis C; GGT: γ -Glutamyltransferase; HS: Hepatic steatosis; IP: Index of prothrombin; PT: Prothrombin time; SD: Standard deviation; TB: Total bilirubin. *The statistical significance of the comparison of the mean values recorded in the patients with CHC and steatosis versus the patients with CHC without steatosis.

Table 5 – Mean values and SDs of inflammation and immunological parameters recorded in the patients with CHC, with or without HS, as well as the statistical significance of these parameters in the assessment of HS

Parameter	Mean value and SD in patients with CHC	Mean value and SD in patients with CHC and steatosis	Mean value and SD in patients with CHC without steatosis	*Statistical significance p-value
ESR [mm/1 h]	8.3±2.82	10.14±2.68	6.95±2.09	0.0024
CRP [mg/dL]	0.56±0.24	0.65±0.29	0.49±0.17	0.05
α_2 -Globulins [%]	10±2.34	11.64±1.49	8.79±2.12	0.0001
γ -Globulins [g/dL]	1.63±0.36	1.66±0.35	1.6±0.37	0.51
γ -Globulins [%]	23.52±4.52 %	25.59±4.77	23.46±4.46	0.41
IgG [IU/mL]	360.73±203.35	432.36±295.45	307±187.57	0.52
IgM [IU/mL]	230.64±111.11	234±125.28	228.16±102.94	0.96
IgA [IU/mL]	230.64±87.31	241.14±120.95	195.26±45.16	0.21

CHC: Chronic hepatitis C; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; HS: Hepatic steatosis; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M; SD: Standard deviation. *The statistical significance of the comparison of the mean values recorded in the patients with CHC and steatosis versus the patients with CHC without steatosis.

The pathological study

The pathological study on the liver fragments obtained from the patients with CHC, in addition to identification of HS, analyzed and quantified the scores of necro-inflammatory and fibrotic processes encountered in these patients, using the Ishak score. The assessment of necro-inflammatory and fibrotic processes indicated specific aspects. HS revealed on the bioptic liver fragments had a macrovesicular appearance and was periportal located, with the presence of interface hepatocytes with vacuolated cytoplasm, which contains large vacuoles that pushed the nucleus to the periphery. The appearance of steatosis was frequently associated with moderate/severe portal inflammatory activity, with chronic inflammatory elements with lymphoid disposition, with moderate/severe interface hepatitis and/or variable portal and septal fibrosis (Figure 1, A–D).

The analysis of histopathological aspects encountered in patients with CHC highlighted the following features related to necro-inflammatory activity and the process of

liver fibrosis. Thus, in patients studied with CHC, the histological activity index (HAI) showed an average value of 7.93 ± 2.46 while the score of hepatic fibrosis (degree of fibrosis) showed a value of 2.63 ± 1.45 (Table 6).

Research on hepatic necro-inflammatory activity in the two groups of patients with CHC revealed a number of differences. Thus, in patients with CHC and HS the mean value of HAI was higher (8.28 ± 3.04) than in patients with CHC without HS (7.68 ± 1.97). It was also observed that patients with CHC and HS had mild liver activity in 35.71% of patients, moderate in 28.57% of patients and severe in 28.57% of patients. Patients with CHC without HS had aspects of chronic hepatitis: mild in 26.31% of patients, moderate in 68.42% of patients and severe in 5.26% of patients. In conclusion, patients with CHC and HS had an almost equal incidence of the three histopathological types of chronic hepatitis, while patients with CHC without HS had moderate liver activity in most cases (68.2%). The independent study of the four parameters that make up HAI showed higher average values, but not statistically significant, of the scores of these parameters in

patients with steatosis compared to the group of patients without steatosis. Thus, the mean values of the scores of the four parameters in patients with steatosis and those without steatosis were 2.21 ± 0.63 vs 2.14 ± 1.02 for periportal and periseptal necrosis, 1.18 ± 1.05 vs 0.94 ± 0.81 for confluent necrosis (bridging necrosis), 1.92 ± 0.99 vs 1.47 ± 0.69 for focal/intralobular necrosis and 3.15 ± 0.6 vs 3 ± 0.67 for portal inflammation. Regarding the analysis of the fibrotic process, the fibrosis score showed a statistically significant average value ($p=0.05$) higher in patients with steatosis (3.15 ± 1.65) compared to the group of patients without steatosis (2.12 ± 1.25). The patients

with CHC and HS presented mild liver fibrosis in 28.57% of cases, moderate in 14.28% of cases, severe in 35.71% of patients and very severe (liver cirrhosis) in 14.28% of patients. The patients with CHC without HS had mild fibrosis in 26.31% of cases, moderate in 31.57% of cases, severe in 31.57% and very severe in 10.52% of cases. The analysis of the severity of the fibrotic process in the two groups of patients with CHC showed some differences regarding the degree of liver fibrosis. Thus, the patients with CHC and HS presented in most cases severe and very severe fibrosis, while the patients with CHC without HS presented moderate and severe hepatic fibrosis.

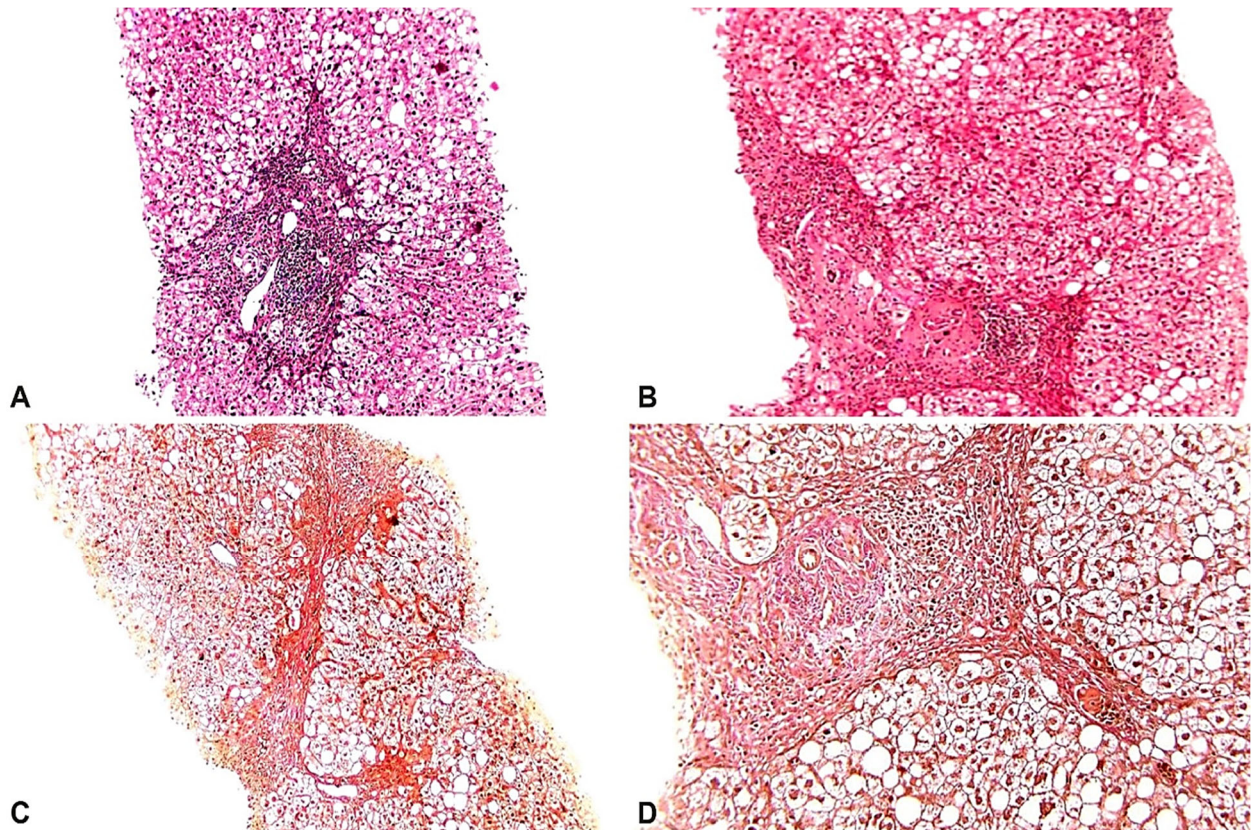


Figure 1 – Hepatic steatosis in patients with chronic hepatitis C: (A) Periportal macrovesicular steatosis associated with moderate/severe portal follicular inflammation (3), moderate interface hepatitis (3); (B) Macrovesicular steatosis associated with moderate/severe lymphocytic follicular inflammation (3), moderate/severe interface hepatitis (3), portal and septal fibrosis (F3); (C) Periportal macrovesicular steatosis associated with moderate lymphocytic follicular inflammation (2), moderate interface hepatitis (2), portal and septal fibrosis (F3); (D) Periportal steatosis associated with moderate follicular lymphocytic inflammation, with moderate interface hepatitis (2) and mild lobular hepatitis (1), and septal fibrosis (F3) (Professor Crisțiana Eugenia Simionescu collection). Hematoxylin–Eosin (HE) staining: (A and B) $\times 100$. van Gieson staining: (C) $\times 100$; (D) $\times 200$.

Table 6 – Mean values of the scores of necroinflammatory and fibrotic processes found in patients with CHC and the statistical significance of each histological parameter, depending on the presence and absence of HS

Histological parameter/score	Mean values and SD in patients with CHC	Mean values and SD in patients with CHC with steatosis	Mean values and SD in patients with CHC without steatosis	*Statistical significance p-value
HAI	7.93 ± 2.46	8.28 ± 3.04	7.68 ± 1.97	0.53
Periportal necrosis	2.18 ± 0.8	2.21 ± 0.63	2.14 ± 1.02	0.86
Confluent necrosis	1.06 ± 0.93	1.18 ± 1.05	0.94 ± 0.81	0.76
Focal/intralobular necrosis	1.66 ± 0.85	1.92 ± 0.99	1.47 ± 0.69	0.18
Portal inflammation	3.09 ± 0.63	3.15 ± 0.6	3 ± 0.67	0.3
Score of liver fibrosis	2.63 ± 1.45	3.15 ± 1.65	2.12 ± 1.25	0.05

CHC: Chronic hepatitis C; HAI: Histology activity index; HS: Hepatic steatosis; SD: Standard deviation. *The statistical significance of the comparison of the mean values recorded in the patients with CHC and steatosis versus the patients with CHC without steatosis.

Discussions

The appearance of steatosis in patients with CHC is the result of a complex interaction between the particularities of the host and viral factors [18].

The risk factors of the host

The main risk factors of the host, which contributed to the appearance of HS were age, sex, body weight, BMI, and personal pathological history. In patients with CHC, in whom insulin resistance plays an important role, HS may be due to high BMI, obesity, hyperlipidemia, metabolic syndrome, and diabetes mellitus [19]. In the CHC studied patients, the mean age of those with HS was significantly higher than the mean age of patients without HS. The importance of age in HS's occurrence was documented in an Iranian study that showed lower age values of patients with CHC and HS compared to the values found in our study [20]. In the CHC studied patients, HS was present especially in men (71.42% vs 28.57% women). The occurrence of HS, especially in men, was also highlighted in the study conducted by Asselah *et al.* on a group of 290 patients with CHC [21]. In CHC studied patients, the mean body weight and BMI were statistically significantly higher in patients with HS than those without HS. The importance of body weight in the determination of steatosis has been studied by Hickman *et al.*, who suggested that weight loss in patients with CHC is associated with both a reduction in steatosis and a reduction in fibrosis, despite the persistence of the virus [22]. Another important anthropometric parameter that was correlated with the appearance of HS was the AC. Patients with CHC who had HS had a significantly higher mean value of the AC than the one in the patients who did not develop HS. Numerous authors have demonstrated the importance of abdominal obesity in the occurrence of steatosis, being an independent risk factor for the occurrence of steatosis in patients with CHC [23]. In the CHC studied patients, the incidence of hypertension was 33.33%, which was more common in the patients with HS compared to the patients without HS. The presence of high values of the blood pressure in Taiwanese patients with CHC and HS was correlated with the presence of liver fibrosis in the univariate analysis, but not in the multivariate one [24]. The incidence of diabetes mellitus in the studied patients was 21.21%, which was more common in patients with CHC and HS, than patients with CHC without HS. Kralj *et al.* have shown that HS's occurrence in patients with CHC involves both insulin resistance caused by the direct action of the virus and insulin resistance caused by abnormal lipid metabolism of the host [25]. Dyslipidemia, encountered in the personal history of 69.69% of patients with CHC, was more common in patients with CHC who had HS compared with patients without HS. The role of dyslipidemia in the determination of HS in patients with CHC genotype 1 was studied by Valkov *et al.* who demonstrated that plasma TG levels in patients with CHC genotype 1 correlated with the presence of steatosis, and in the presence of HS, the ratio of TG/low-density lipoprotein (LDL)-cholesterol above 0.52 is a potential marker for liver cirrhosis [26]. In the CHC studied patients, the incidence of the metabolic syndrome was 36.36%, its incidence was significantly higher

in patients with HS than patients without HS. The results of a Brazilian study showed a lower incidence (21.6%) of the metabolic syndrome in patients with CHC infected with genotype 1. The presence of metabolic syndrome was significantly associated with the co-existence of hypertension, insulin resistance, abdominal obesity and overweight [27]. The overweight was found predominantly in patients with HS (71.24%), compared to patients without HS (21.05%). The role of overweight or obesity in the occurrence of HS was analyzed in an American study, which showed that obesity is an independent risk factor for the occurrence of HS, which in turn increases the activity and progression of chronic hepatitis and is independently associated with stages III/IV fibrosis [23].

The virus-related factors

In CHC studied patients, viremia had a statistically significantly higher mean value ($p=0.001$) in the group in which the liver biopsy puncture showed HS, compared to the mean value of viremia in the group in which the puncture of liver biopsy did not demonstrate HS. Genotype 1 was found in the majority (92.85%) of patients with HS and without HS (94.73%), while genotype 2 was present in one (7.15%) patient with HS and in one (5.27%) without HS. The results of an Iranian study of 80 patients with CHC showed that only for HCV genotype 3 was there a correlation between the value of viremia and the severity of steatosis, while for genotype 1 this correlation was no longer valid [28]. Patel & Harrison also stated that in patients infected with HCV genotype 1, no correlation was found between the degree of steatosis and viral load, and that the sustained virological response (SVR) was not accompanied by an improvement in steatosis induced by HCV infection. Our study's different conclusion from the other studies probably comes from the relatively small number of patients in our group [29].

The hematological and metabolic constants

Analyzing the mean values of the metabolic and hematological constants, the following differences between the behavior of the constants were highlighted in patients who presented with HS, compared to those who did not develop HS. The mean Hb value in patients with HS was insignificantly higher compared to patients without HS. The Iranian study of 115 patients with CHC, of whom 68 (58.2%) patients associated HS, showed a slightly higher mean value ($p>0.05$) of Hb in patients with HS compared to patients without HS (14.63 ± 2.18 g/dL vs 14.02 ± 2.50 g/dL) [20]. Regarding the number of WBC, the patients with CHC and HS did not register statistically significant differences. In these patients, the average value of the number of WBC is slightly higher than that reported in the patients with CHC without HS. The same WBC count behavior was recently described in a study of a group of 197 Thai patients with previously untreated CHC, with the mean WBC being slightly higher ($6530/\text{mm}^3$) in patients with HS compared with patients without HS ($6110/\text{mm}^3$). This study showed an incidence of steatosis of 26.9% among patients with CHC and an association of steatosis with host metabolic factors, especially obesity and diabetes [30]. The mean PLT in patients with CHC and HS was insignificantly

lower than the mean in patients with CHC without HS. In a study by Sirinawasatien & Techasirioangkun, the mean PLT of patients with HS was higher than the group of patients without HS ($176.49 \pm 61.99 \times 10^9/L$ vs $169.75 \pm 79.57 \times 10^9/L$) [30]. The behavior of metabolic parameters was different in patients with CHC, depending on the presence or absence of HS, in the case of glycemia and similarly in the case of cholesterol and TG. Blood glucose was statistically higher in patients with steatosis than in patients without steatosis. Statistically significant differences in mean blood glucose values in patients with CHC with HS (106.4 ± 37.1 mg/dL) compared with those in patients with CHC without HS (89.7 ± 17.8 mg/dL) were reported and in other clinical trials [31]. Over time, HS has been frequently reported in patients with CHC, and its occurrence has been correlated with the presence of insulin resistance and type 2 diabetes, the severity of the disease and a poor response to antiviral therapy. The chronic infection with HCV genotype 1 and 2 associate irreversible metabolic steatosis with antiviral therapy. However, steatosis may be partly an indirect consequence of viral infection, as HCV induces a metabolic disorder capable of insulin resistance [32]. In the Bugianesi *et al.* study of a group of 264 patients with CHC, half of whom (50%) had HS, insulin resistance in both the liver with metabolic steatosis and the liver with viral steatosis was an independent factor for fibrosis advanced. In contrast, only metabolic steatosis contributes to liver fibrosis, such that in the patients with HCV genotype 3 infection, insulin resistance does not directly promote necroinflammation and liver fibrogenesis [33]. The components of lipid metabolism did not register statistically significant differences, depending on the presence or absence of steatosis, the average values of TG and cholesterol being slightly increased in the group of patients with steatosis. Similar behavior of TG was found in a study of 401 African-American patients known to have CHC, of which 253 (63%) patients developed HS [31]. Also, in a study on 112 Bulgarian patients with CHC, of which 75 (66.96%) patients had HS, the mean serum cholesterol was higher in patients with HS than the group of patients without HS [26].

The biochemical constants

Regarding the analysis of hepatic functions (biochemical study), the study concluded that only the plasma activity of hepatic cholestasis enzymes was correlated with the presence of HS in patients with CHC studied. The mean values of TB as well as the levels of serum activities of GGT, ALP, ALT and AST were higher in the group of patients with steatosis compared to the average values of these parameters in the group of patients without steatosis. Similar behavior of liver tests was observed in the Japanese study performed on 282 patients with CHC which aimed to assess the relationship between HS and long-term progression of hepatitis C. Out of the total number of patients, 170 (60.28%) cases associated with HS, and the mean values of TB, GGT, ALP, ALT and AST in these patients were higher than the mean values in patients without HS [34]. Elevated liver enzymes in patients with CHC and steatosis compared to the group of patients without steatosis were also highlighted by the Egyptian

study led by Ahmed *et al.*, which included 92 patients with CHC of which 50 (54.34%) patients associated with steatosis. The univariate analysis of the study showed no association between the presence of steatosis and elevated levels of cytolysis enzymes or elevated liver necroinflammation scores. In contrast, multivariate analysis has shown that the main predictor of steatosis in patients with CHC is insulin resistance [35]. The hepatic insufficiency syndrome parameters' behavior differed depending on the presence or absence of HS, but the average values of these parameters did not register statistically significant differences. The aforementioned Japanese study, performed on 282 patients diagnosed with chronic hepatitis or liver cirrhosis, showed that before the start of antiviral treatment there were no statistical differences between the group of patients with steatosis and those without steatosis, in terms of average values of total protein, albumin and IP [34].

The systemic inflammation's constants

Analyzing the mean values of the constants that assessed systemic inflammation, a different behavior of these parameters was recorded in patients with CHC, depending on the presence or absence of HS. Thus, patients with CHC and HS had significantly higher mean values of ESR, CRP and α_2 -globulin than the values of these constants in patients without steatosis. Popović-Dragonjić's study of 45 patients with CHC, 23 of whom had HS, showed that high-sensitivity (hs) CRP can be considered a predictor of CHC progression. Thus, patients with CHC showed a higher mean value of hsCRP than patients without CHC, and patients with CHC and HS had a higher average value of this parameter, than those with CHC without HS [36]. The analysis of the average values of the immunological parameters revealed a different behavior of these parameters in the patients with CHC, depending on HS's presence or absence. A study by Czaja *et al.* showed that patients with chronic hepatitis and HS had lower serum γ -globulins ($p=0.01$) and IgG ($p=0.05$) levels compared to patients without steatosis, as well as a lower frequency of antinuclear antibodies ($p=0.01$), showing that steatosis in CHC is mainly the effect of viral infection, and host metabolic factors may potentiate this manifestation. The same author concluded that the storage of TG in the hepatocytes is not associated with the immune response, and this cannot be considered a distinct pathogenic mechanism in the production of steatosis [37].

The pathological constants

Currently, in CHC, liver biopsy is known to be the "gold standard" in assessing the degree of necroinflammation and the stage of fibrosis, which subsequently helps us to choose the treatment and assess the prognosis of the disease [38]. The Ishak score was a complex semiquantitative histological score, which we used in other clinical trials to fully evaluate both liver necroinflammatory activity and the degree of liver fibrosis [39]. HS, with lipid accumulation above 5% of the total cells, contribute to the altered cytoarchitectonics [40]. The histopathological aspects of HS encountered in the studied patients with CHC were similar to those previously described by other authors [9]. The research of the hepatic necroinflammatory activity

in the two groups of patients with CHC revealed several differences. Patients with CHC and HS had higher mean values of necroinflammatory component scores than patients with CHC without HS. The correlations between steatosis, necroinflammatory activity and fibrosis were shown by other authors [41]. In a study performed by Wyatt *et al.* on 233 biopsies sampled from patients with CHC, HS could be seen in 117 (50.2%) patients. In these patients, the univariate analysis showed a statistically significant association of steatosis with age ($p < 0.0001$) and with two of the histopathological components of the HAI score, namely with portal inflammation ($p = 0.001$) and interface hepatitis ($p = 0.005$), but without the association of lobular activity ($p = 0.374$) [42]. Other authors, who have shown that HS is associated with hepatic inflammatory activity, have also pointed out that steatosis may contribute to hepatic fibrosis's accelerated progression [43]. Regarding the process of hepatic fibrosis, patients with CHC with HS had an average score of fibrosis significantly higher ($p = 0.055$) than the one found in the patients with CHC without HS. The histological study of the liver fragments obtained from the patients with CHC and HS revealed the presence of septal and periportal fibrosis, similar to the disposition of fibrosis found in patients with non-alcoholic steatofibrosis, in which the role of metabolic factors in the occurrence of fibrosis is significant. Multiple other studies on liver biopsy fragments sampled from patients with CHC have shown that moderate or severe HS has been correlated with the degree of liver fibrosis. Thus, in patients infected with HCV genotype 3, in whom HS is mainly due to viral multiplication, moderate and severe steatosis was associated with hepatic fibrosis [44]. In a multi-year clinical study focusing on liver biopsies obtained over time, it was reconfirmed that the progression of steatosis was the only factor independently associated with the evolution of liver fibrosis [45]. Other researchers have shown that advanced steatosis is associated with worsening fibrosis, suggesting a possible role for steatosis in the progression of liver disease in patients with chronic hepatitis, and steatosis control measures may play an important role in stopping the progression of chronic liver disease [46]. Wyatt *et al.* study of 233 biopsies collected from patients with CHC, in which HS could be seen in 117 (50.2%) patients and liver fibrosis was found in 148 (63.5%) patients a statistically significant association ($p < 0.01$) between steatosis score and degree of fibrosis. This association was even stronger after excluding patients with liver cirrhosis ($p < 0.0001$) [42]. Finally, there are authors who have mentioned that in patients with HCC, the clinical significance of HS involves an association between liver fibrosis and an unsatisfactory response to combination antiviral therapy with Pegylated Interferon (PEG-IFN) and Ribavirin [19].

☒ Conclusions

HS is a common finding in patients with chronic infection with HCV genotypes 1 and 2. Host metabolic factors, such as obesity and diabetes, were the major etiological factors for HS in patients with CHC. The pathogenic mechanism involved in steatosis in these patients with CHC appears to be a non-immune inflammatory

mechanism. Early detection of HS by invasive or non-invasive methods is an important objective of monitoring patients with CHC, because HS is correlated with a high degree of fibrosis. In these patients, early correction of metabolic factors and early introduction of antiviral therapy are essential targets for treating patients with CHC.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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