

## A CLINICO-PATHOLOGICAL STUDY OF 163 UNTREATED CASES OF CHRONIC HEPATITIS C

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*We performed a clinico-pathological study of 163 untreated cases of chronic hepatitis C. Eighty five percent of the patients were clinically asymptomatic and their physical examinations showed unremarkable or minimal changes at the time of the liver biopsy. Liver function tests tended to present slight abnormalities, involving mild elevations of the activity of the aminotransferases and gamma-glutamyl transferase levels. In spite of these mild abnormalities, advanced chronic liver disease was histologically detected in eighty nine percent of the patients, mainly showing chronic active hepatitis. The most characteristic histological finding was an interlobular bile duct damage, which correlated with the presence of lymphoid aggregates in the portal tracts and with the development of fibrosis.*

*Key-words: Chronic hepatitis C. Bile duct damage. Lymphoid aggregate. Histopathology.*

Recently the genome of the blood-borne non-A, non-B virus, designated hepatitis C virus (HCV), has been cloned and specific assays have been developed to detect anti-HCV antibodies<sup>2,7</sup>. Since the introduction of routine blood screening for hepatitis B surface antigen (HBsAg), the incidence of hepatitis B virus infection has decreased and hepatitis C has been the major cause of posttransfusional hepatitis. The clinical presentation of chronic hepatitis C as reported in the literature suggests that the disease often runs a silent course, with few symptoms and signs, and mild biochemical abnormalities<sup>10,11</sup>. Nonetheless, severe liver damage have been reported to occur with high frequency<sup>10,11,14</sup>. The aim of this study is to report the results of the clinical, biochemical and histopathological examinations of 163 untreated cases of chronic hepatitis C, analyse the correlations and relationships among these findings and discuss the nature of chronic hepatitis C.

### MATERIAL AND METHODS

We reviewed the clinical data and histological specimens from 1991 through 1994

pertaining to 163 untreated patients with chronic hepatitis C in the Department of Pathology, Institute of Tropical Medicine, Nagasaki University. The main reason for the biopsy procedure was to evaluate chronic alterations of serum aminotransferases levels and to have a histological diagnosis in serologically defined cases of hepatitis C. From 224 cases initially recorded, 61 were excluded on the basis of the presence of positive testing for HBsAg, presence of hepatocellular carcinoma, previous treatment with interferon, evidence of autoimmune hepatitis or alcoholic liver damage.

The sera from the patients was assayed for the presence of HBsAg and anti-C100-3 HCV using a radioimmunoassay kit (Dainabot, Japan and Ohtsuka assay Co. Ltd., Japan, respectively). Sera negative for both HBsAg and anti-C100-3 HCV was retested using a second generation assay which detects in addition to C100-3 antigen also the C22-3 and C33-c antigen by radioimmunoassay (Ohtsuka assay Co. LTD., Japan). Additional cases of HCV infection included those positive sera for HCV-RNA as detected by the polymerase chain reaction technique.

The liver biopsy specimens were fixed in buffered formalin, embedded in paraffin and stained with hematoxylin and eosin, Azan-Mallory and silver impregnation for reticulin fiber. All biopsy specimens were classified according to the type of chronic hepatitis, using conventional criteria, as having chronic persistent hepatitis (CPH), chronic active

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hepatitis (CAH) with mild or moderate degree of inflammation and with or without cirrhosis. The specimens were graded with respect to the degree of piecemeal necrosis, portal and lobular inflammation and fibrosis according to the histological activity index (HAI) scoring system designed by Knodell et al<sup>6</sup>. Any evidence of the presence of piecemeal necrosis was sufficient to rule out CPH, and to categorize the biopsy as, at least, mild CAH. Cirrhosis was diagnosed when the presence of bridging fibrosis and nodule formation could be established.

The presence or absence of the following histological findings were registered for each biopsy; bile duct damage and bile duct proliferation, plasma cell infiltration in portal tracts, acidophilic bodies, sinusoidal lining cell activation, fatty metamorphosis and dysplasia of hepatocytes. Bile duct damage was considered present when the following features were observed alone or in combination; multilayered or stratified epithelium with swollen epithelial cells, inflammatory cell infiltration in epithelium, vacuolization of epithelial cells, loss of polarity and degeneration of epithelial cells.

The statistical significance of the results was evaluated by the Chi-square test. A p-value < 0.05 was considered significant.

## RESULTS

*Clinical characteristics.* Table 1 presents demographic data of the 163 patients with chronic hepatitis C. The mean age of the patients was 54 years and the male to female ratio was 2:1. Most of the patients (70%) were between 50 and 70 years old. Thirty nine out of 105 males (38%) and 24 out of 58 (41%) females had a history of blood transfusion.

Table 1 - Demographic data and blood transfusions in patients with chronic Hepatitis C.

	Number	Mean age (mean/range)	Blood Transfusion
Male	105	54 (24-72)	38%
Female	58	55 (27-75)	41%
Total	163	54 (24-75)	85%

Eighty five percent of the patients were asymptomatic at the time they were submitted to liver biopsy, and the most frequent symptom was general fatigue (11%) followed by vague abdominal discomfort (3%) and itching (1%). Sixty percent presented no

abnormalities at the physical examination and 36% presented hepatomegaly, while only 2% had splenomegaly, and 2% hepatosplenomegaly. Among the patients with hepatomegaly, there was a male predominance (43/105; 41%) when compared to the female group (15/58;25%), and this trend occurred more frequently in the age groups older than 50 years. No statistical significance could be established between either age and gender, and any biochemical abnormalities or histological finding, nor between clinical complaints and abnormal physical finding, and any biochemical abnormalities or histological findings.

*Biochemical Findings.* The biochemical abnormalities concerning the aminotransferases and gamma-glutamyltranspeptidase tended to be mild. The mean levels, and ranges of AST, ALT and GGT observed were 61IU/l(13-421), 77IU/l(15-358) and 63IU/l(11-241), respectively and no correlation could be established among any biochemical determination and specific histological findings.

*Histological Findings.* The general histological findings in chronic hepatitis C are indicated in Table 2. A hepatitis-associated bile duct damage, first described by Christoffersen-Poulsen<sup>8</sup>, is shown in Figure 1. The lesion depicted is of the type 2 hepatitis-associated bile duct damage and was seen in 57% of the patients. Bile ductular proliferation was observed in 47% of the patients. Lymphoid follicle formations with or without germinal center in portal tracts (Figure 2) were seen in 42% of the patients. The majority of the cases (85%) showed mild degree of piecemeal necrosis. Fatty metamorphosis of the

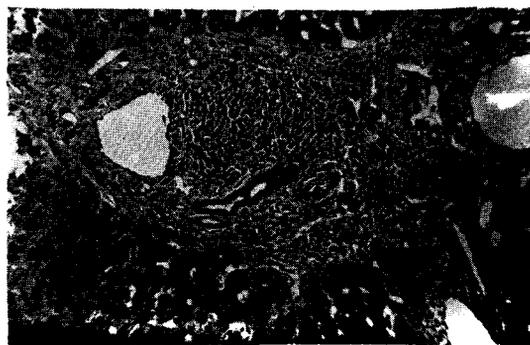


Figure 1. Interlobular bile duct lesion in chronic hepatitis C.

Table 2 - General histological findings in chronic hepatitis C.

	n = 163 (%)
<b>Portal Tract Lesion</b>	
Lymphoid Infiltrate	
none	3 (2)
loose condensation	92 (56)
lymphoid follicle without germinal center	62 (38)
lymphoid follicle with germinal center	6 (4)
Piecemeal Necrosis	
none	11 (7)
mild	139 (85)
moderate	12 (8)
marked	1 (1)
Bile Duct Damage	93 (57)
Bile Ductular Proliferation	68 (47)
Plasma Cell Infiltration	48 (29)
<b>Parenchymal Lesion</b>	
Sinusoidal lining cell activation	149 (91)
Spotty necrosis	158 (97)
Acidophilic bodies	50 (31)
Fatty metamorphosis	47 (29)
Dysplasia of hepatocytes	16 (10)
<b>Fibrosis</b>	
Limited in portal tract	9 (6)
Portal fibrous expansion	60 (37)
Bridging fibrosis	74 (45)
Cirrhosis	20 (12)

hepatocytes was detected in 29% of all the cases. Most cases presented more advanced fibrosis, as in the cases of fibrous portal expansion and bridging fibrosis.

4. Bile duct damage significantly correlated with advanced fibrosis ( $p < 0.05$ ).

Table 3 - Bile duct damage (BDD) and lymphoid infiltrate in portal tract.

Lymphoid infiltrate	BDD	
	present	absent
None (n = 3)	1 (33%)	2 (67%)
Loose condensation (n = 92)	42 (46%)	50 (54%)
Follicle without germinal center (n = 62)	44 (71%)	18(29%)*
Follicle with germinal center (n=6)	6 (100%)	0 (0%)*

\* Statistically significant,  $p < 0.05$

Table 4 - Bile duct damage (BDD) and fibrosis.

Fibrosis	BDD	
	present	absent
Limited to portal tract (n = 9)	3 (33%)	6 (67%)*
Portal fibrous expansion (n = 60)	36 (30%)	24 (40%)*
Bridging fibrosis (n = 74)	50 (68%)	24 (32%)*
Cirrhosis (n = 20)	15 (75%)	5 (25%)*

\* Statistically significant,  $p < 0.05$ .

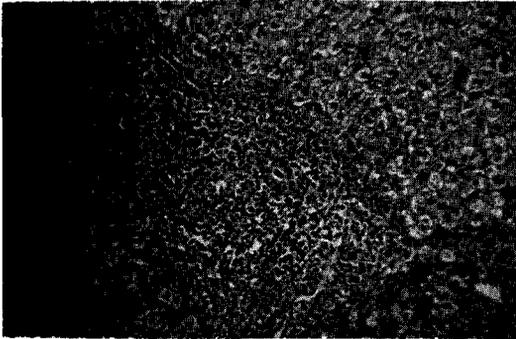


Figure 2. Lymphoid follicle in portal tract in chronic hepatitis C.

Table 3 relates the bile duct damage to portal inflammation in chronic hepatitis C. Bile duct damage significantly correlated with the degree of portal inflammation and especially all cases of lymphoid aggregates with germinal center in portal tracts showed bile duct damage ( $p < 0.05$ ).

The correlation between bile duct damage and the degree of fibrosis is indicated in Table

The final histological diagnosis of the 163 cases of hepatitis C included 19 (12%) of chronic persistent hepatitis, 113 (69%) cases of chronic active hepatitis (CAH), with mild activity and without cirrhosis, 16 (10%) cases of CAH with mild activity and with cirrhosis, 15(9%) cases of CAH with moderate activity and without cirrhosis, and 1 (1%) case with CAH with moderate activity and with cirrhosis.

## DISCUSSION

The results of our study confirm the general impression that chronic hepatitis C runs a protracted course with few nonspecific symptoms and signs, along with mild biochemical abnormalities, despite the presence of, or progression to, advanced chronic liver disease. The majority of the patients were asymptomatic at the time of diagnosis, and were referred for liver biopsy to elucidate chronic serum elevation of aminotransferases or to provide histological information for further interferon therapy. Physical examination was unremarkable in most of the patients. When present, the most common physical abnormality was hepatomegaly, more prevalent among older male patients. It has been reported that older patients present more severe manifestations of the disease<sup>10</sup>. Among the biochemical tests, abnormalities involved mainly serum levels of AST, ALT and GGT, but no correlation could be established with the degree of piecemeal necrosis or portal or intralobular inflammation. This lack of correlation with hepatic histology has already been pointed out<sup>10,11</sup> and could be a result of sampling error due to the fluctuating course of aminotransferases and advanced fibrosis. In this regard, the clinical severity cannot be assessed by the serum aminotransferases level alone, and liver biopsy is essential in assessing the extent of liver damage. Fluctuation in aminotransferase levels may represent alteration in viral replication, host immunity, or both<sup>11</sup>. These fluctuant levels correlate to episodes of lobular necrosis, thought to be responsible for the progression to chronic disease<sup>15</sup>.

The most conspicuous histological finding in our study was bile duct damage (BDD), observed in 57% of the cases, while the incidence rates in the literature differs from 22%<sup>15</sup>, 25%<sup>13</sup>, 30%<sup>8</sup>, 31%<sup>6</sup>, 90%<sup>3</sup> to 91%<sup>1</sup>. The affected bile ducts are small or medium sized interlobular ducts. The BDD appears to involve immunological mediated reaction to antigens on bile duct epithelium, possibly histocompatibility antigens (HLA) or HLA antigens displayed with virus-related antigens<sup>19</sup>. An association between BDD and a better response to interferon therapy in patients with chronic hepatitis C has been documented and may be of prognostic significance<sup>9</sup>.

The occurrence of BDD had a significant correlation with the degree of portal/periportal inflammation in our cases. The affected bile ducts were usually surrounded by a dense lymphoid infiltrate, sometimes with a germinal center, close to the damaged duct. Lymphoid follicles have been reported to occur in frequencies varying from 49%<sup>19</sup>, 52%<sup>8</sup>, 63%<sup>13</sup> to 78%<sup>17</sup>. In our study, 42% of the patients displayed lymphoid follicles and among these 73% had BDD. Lymphoid follicle is a feature of a variety of chronic inflammatory diseases, often of autoimmune nature and their presence in hepatitis C may reflect an ongoing immunologic reaction. Lymphoid follicles may be formed early in the acute stages of hepatitis<sup>16</sup>. The appearance or disappearance of lymphoid follicles during the course of the illness does not seem to correlate with either improvement or deterioration of the histological activity index<sup>12</sup>.

A significant correlation between the occurrence of BDD and the degree of hepatic fibrosis in non-cirrhotic livers, which has been described in several reports<sup>17</sup> could also be detected in our cases. While 60% of the cases presenting fibrous portal expansion and 68% of those with bridging fibrosis displayed bile duct damage, only 33% of the cases involving limited fibrosis in the portal tracts showed BDD.

In conclusion, chronic hepatitis C is a relentlessly chronic progressive disease, frequently running a silent course, with mild or unremarkable biochemical abnormalities, which do not correlate with the histological activity of this disease. Liver biopsy is the only method to assess the degree of liver damage. The single most characteristic histological feature of chronic hepatitis C is a bile duct damage, surrounded by a lymphoid aggregate.

## RESUMO

*Realizou-se estudo clinico-patológico de 163 casos não tratados de hepatite crônica C. A maioria dos pacientes era clinicamente assintomática e seus exames físicos demonstravam alterações inespecíficas ou mínimas por ocasião da biópsia hepática. As provas de função hepática tendiam a apresentar alterações discretas, envolvendo especialmente os níveis séricos das aminotransferases e gama-glutamil transpeptidase. Apesar destas alterações discretas, detectou-se doença hepática crônica histologicamente avançada,*

consistindo principalmente de hepatite crônica ativa. O achado histológico mais característico foi lesão de ducto biliar interlobular, que se correlacionou com a presença de agregados linfóides nos tratos portais e com o desenvolvimento de fibrose.

*Palavras-chaves:* Hepatite crônica C. Lesão de ducto biliar. Agregado linfóide. Histopatologia.

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