THE HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF VIRAL CHRONIC HEPATITIS AND EXTRAHEPATIC CHANGES IN MUSCULO-SKELETAL JOINT

- THESIS RESUME -

Scientific Supervisor,
Cristiana Simionescu, Ph.D.

Ph. D. Candidate,
ANAMARIA KESE

CRAIOVA
2010
INTRODUCTION

CHAPTER I – Normal liver histophysiology

CHAPTER II- Epidemiology and Etiology of chronic viral hepatitis

CHAPTER III - Clinical evaluation and histopathological chronic viral hepatitis

CHAPTER IV – Extrahepatic changes in viral chronic hepatitis

CHAPTER V – Materials and method

CHAPTER VI - Results

CHAPTER VII - Discussion

CONCLUSIONS

BIBLIOGRAPHY
INTRODUCTION

The paper addresses a topic of constant current in liver pathology, that of chronic viral hepatitis, which is a major health problem worldwide and in Romania, and also watched musculoarticular changes in these patients. Globally, it is estimated that over 2 billion people worldwide have been infected with hepatitis B, only appeared in Europe over a million cases annually in November. It is estimated that currently exist throughout the entire globe over 350 million chronic carriers of HBV, with a tendency to increase to 400 million. It is also estimated that, worldwide, are infected with HCV 170 million people, with a prevalence 3%. In Romania, the probable prevalence is between 4 and 8%.

In the current state of knowledge dedicated watched some issues regarding histophisiology of liver, recent data on epidemiology and etiology of chronic viral hepatitis, clinical aspects and histopathology of chronic viral hepatitis, extrahepatic manifestations and association of concepts on chronic viral hepatitis.

In particular the paper I presented the material and methods used, results of personal observations, their discussion in the context of similar research literature and I set out the findings of the study. The study comprised 532 cases of chronic viral hepatitis, which after serological examination were divided into four groups: viral hepatitis C 402 cases, 116 hepatitis B cases, eight B + C co-infected cases and B + D 6 cases. The cases examined were selected in a period of four years (2006-2009) from patients hospitalized in Clinic Emergency Hospital Craiova. Also, I followed these patients and associated changes in muscle and joints.

Histopathological study of liver biopsies followed necroinflamatory activity and fibrosis development patterns and their classification into a scoring system (HAI and Metavir). For cases of chronic hepatitis C (402 cases) is highlighted predominance of moderately active chronic hepatitis, while for chronic hepatitis B (116 cases) depending on the severity of lesion distribution showed predominance of minimally active chronic hepatitis and for co-infections included in study (C and B + B + D) (14 cases) showed the predominance of minimal chronic active hepatitis.

Immunohistochemical study of inflammatory infiltrate of chronic viral hepatitis showed the presence of numerous T lymphocytes, unlike B lymphocytes were reduced in number. Kupffer cells marking with CD68 and portal and intralobular presence and star marking cell alpha-actin shows the role of these cells in the fibrogenesis.

Analysing changes in musculoskeletal joints in patients with chronic hepatitis, we found 11 cases non-specific muscular dystrophy and rheumatoid arthritis in nine cases. For cases with rheumatoid arthritis have pursued in relation to severity of histopathological parameters (sinoviciotitary proliferation, inflammatory infiltration, fibrinoid necrosis, proliferation of fibroblasts and vascular changes). The immunohistochemical investigations have pointed to the predominance of the inflammatory process and the possibility that citrulline B lymphocytes represent a diagnostic marker of rheumatoid arthritis on fragments biopsy.
MATERIAL AND METHOD

MATERIALS RESEARCH

The material studied in the paper, the human liver was obtained by liver biopsy or puncture, and the fragments obtained from muscle and joint biopsies, from patients hospitalized in the Medical Clinics Emergency Hospital Craiova. In a period of four years between 2006-2009, were investigated with 532 liver biopsies collected by puncture of the hepatic lesions have been excluded tumor dystrophic lesions and cirrhosis. It also had muscle biopsies taken 11 and 9 synovial biopsies.

METHODS RESEARCH

Liver puncture. New technique was used technique vise. Given that the risk of an accident in the liver puncture-biopsy is maximum while the needle is in the liver substance, Vice imagined and implemented a technique in which the needle remains live only for about one second, ensuring is relatively thick tissue fragments. The needle used was 1.2 mm, provided with a brief stopping point form a blunt tip and is 8 inches long. Clinical value of liver puncture biopsy is helpful in 72-85% of cases. Liver biopsies are the limits to obtain very small fragments, insufficient development of pathological diagnosis, sometimes fatal complications of puncture biopsy, unlike laparatomy where passage may be particularly liver, biopsy may be done under ultrasound at best.

Liver biopsy. Snippets harvested are fixed in 10% formalin, pH neutralization being made by calcium carbonate. After fixation at room temperature for 36-48 hours, selected fragments were processed by usual histopathological technique for inclusion in paraffin and sections obtained were stained haematoxylin-eosine.

RESULTS

The histopathological study in chronic viral hepatitis

Statistical study conducted during the 4 year period (2006-2009) revealed the existence of 532 punctures Liver biopsies performed for diagnosis of chronic hepatitis. Regarding age, it ranged between 16 and 68 years (mean 46.96 years). Most cases were between 51-60 years - 207 cases, and other extreme is the range 11 to 20 years with five cases. Serological examination identified the presence of viral subtype causing dividing the study group in four categories: infection with hepatitis C (402 cases), hepatitis B virus (116 cases) and presence of coinfection B + C (8 cases) and B + D (6 cases).
The histological sections were followed to investigate several parameters: diagnosis, diagnosis of incidental lesions, evaluation of histological types of necrosis assessment, evaluation of structural changes, for changes suggestive of etiology.

**Chronic hepatitis C.** In the subgroup of patients infected with hepatitis C, age ranged between 22 and 68 years (average 49.18 years =). Sex ratio F: B was 2,16:1. Age group in most cases has remained between 51-60 years - 181 cases. Microscopic examination of sections followed patterns necrosis, inflammation and fibrosis. Hepatocite damage identification and presence of inflammatory activity was known.

Distribution of inflammatory cells varied from case to case, but all cases were characterized by the presence of a dense infiltrate the portal area monocitary.

Inflammatory infiltrate consisted of lymphocytes and plasma cells. There have also been identified in the portal area necrotic macrophages with intracellular debris. Using a scale with four degrees inflammatory quantification revealed the following results: Grade 1-116 cases (mild inflammation), grade 2-126 cases (moderate inflammation), grade 3-119 cases (moderate-marked inflammation), grade 4 - 41 cases (marked inflammation).

The amount of fibrosis found in the lesions ranged from absent (4 cases) to injuries consistent with a diagnosis of liver cirrhosis (30 cases). Fibrosis was characterized by the presence of a fibrous expansion of portal area without formation of septa in 79 cases and the formation of septa in 178 cases (Table 6.1). Fibrosis septal cirrhosis without the presence of 111 cases were identified.

| Table No. 6.1 Distribution of fibrosis in hepatitis C virus |
|---|---|---|---|---|
| Fibrosis | portal | septal | the bridges | Total |
| No cases | 79 | 178 | 111 | 368 |
| % | 21 | 49 | 30 | 100 |

Steatosis was found in the cytoplasm of hepatocytes in the presence of optically empty because of dissolved lipid vesicles during inclusion in paraffin, with net limits for various sizes. It was evaluated as absent (153 cases), mild (<30% of hepatocytes) (133 cases), moderate (30-60% of hepatocytes) (62 cases) and severe (> 60% of hepatocytes) (54 cases).

**Chronic hepatitis B.** In the subgroup of patients with hepatitis B virus infection, age ranged between 18 and 62 years (mean = 40.58 years). Sex ratio F: B was 2,41:1.

Age group in most cases ranged between 31-40 years - 35 cases (table 6.2).

<p>| Table 6.2 Distribution of cases of chronic hepatitis according to age group and gender in patients with hepatitis B virus infection |
|---|---|---|---|---|
| Age Group | Male | Female | Total |
| 11-20 years | 5 | 0 | 5 |
| 21-30 years | 15 | 4 | 19 |
| 31-40 years | 22 | 13 | 35 |</p>
<table>
<thead>
<tr>
<th>Age Group</th>
<th>Cases</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>41-50 years</td>
<td>23</td>
<td>6</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>51-60 years</td>
<td>15</td>
<td>10</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>61-70 years</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>34</td>
<td>116</td>
<td></td>
</tr>
</tbody>
</table>

Most port facilities presented in varying amounts, a limfoplasmocitar infiltrated. Although its layout is more common aggregates and follicles in chronic hepatitis C was found in group of patients with chronic hepatitis B in 17 cases. Inflammatory infiltrate was most frequently localized in portal area, but also intralobular, arranged in small outbreaks in spots, often around altered hepatocytes or sinusoidal take the form of rows. Quantification of inflammatory revealed the following results (table 6.3): grade 1-43 cases (mild inflammation), 37 cases grade 2 (moderate inflammation), grade 3 to 25 cases (moderate-marked inflammation), grade 4-10 cases (inflammation marked).

Table 6.3 Distribution of necroinflammatory activity in hepatitis B

<table>
<thead>
<tr>
<th>Necroinflammatory Activity</th>
<th>Light</th>
<th>Moderate</th>
<th>Moderate / marked</th>
<th>Marked</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cases</td>
<td>43</td>
<td>37</td>
<td>25</td>
<td>10</td>
<td>115</td>
</tr>
<tr>
<td>Percentage%</td>
<td>37</td>
<td>32</td>
<td>22</td>
<td>9</td>
<td>100</td>
</tr>
</tbody>
</table>

Injury to segmental bile canals characterized by vacuolization, necrosis and lymphocytic inflammatory infiltrate was identified in five cases. Hepatocyte necrosis was present in several aspects (table 6.11): focal or spots (in spots) affecting cell or a small group of cells - one goal or less with hotbed 10x - 12 cases, 2-4 foci with 10x objective -96 cases, 5-10 foci with 10x objective – 7 cases; either as confluent necrosis in liver affected large areas of necrosis present and connective tissue containing inflammatory cells including macrophages and cellular debris - six cases.

Table 6.4 Distribution of necrosis in viral hepatitis B

<table>
<thead>
<tr>
<th>Hepatocyte necrosis</th>
<th>0 or 1 outbreak</th>
<th>2-4 foci</th>
<th>5-10 outbreaks</th>
<th>confluent necrosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cases</td>
<td>12</td>
<td>96</td>
<td>7</td>
<td>6</td>
<td>121</td>
</tr>
<tr>
<td>Percentage%</td>
<td>10</td>
<td>79</td>
<td>6</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

Identification of fibrotic changes was facilitated by the use of special stains. Fibrosis stage was assessed by the following criteria (table 6.6) pericelular fibrosis (focal, or extensive) - three cases pericelular fibrosis (focal, or extensive) plus portal fibrosis - 11 cases (Fig. 6.18), fibrosis of the bridges (in thin bridge port portals PP) - 48 cases and portal fibrosis in Porto thick bridges (PP) and portocentral (PC) - 21 cases.
Table 6.6 Distribution fibrosis in hepatitis B virus

<table>
<thead>
<tr>
<th></th>
<th>pericellular fibrosis</th>
<th>pericellular fibrosis</th>
<th>fibrosis in thin decks PP</th>
<th>thick fibrosis in PP and PC bridges</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cases</td>
<td>3</td>
<td>11</td>
<td>48</td>
<td>21</td>
<td>83</td>
</tr>
<tr>
<td>Percentage%</td>
<td>4</td>
<td>13</td>
<td>58</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

Steatosis was characterized by accumulation of fat droplets in hepatocyte steatosis to quantify invasion have taken into account in the fatty liver parenchyma so (Table 6.14): absent (40 cases), mild (<-30% of the hepatocyte) (26 cases), moderate (30-60% of hepatocytes) (31 cases), severe (> 60% of hepatocytes) (19 cases).

Table 6.7 Distribution of cases of chronic hepatitis B associated steatosis

<table>
<thead>
<tr>
<th>Steatosis in hepatitis B</th>
<th>absent</th>
<th>slight</th>
<th>moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cases</td>
<td>40</td>
<td>26</td>
<td>31</td>
<td>19</td>
<td>116</td>
</tr>
<tr>
<td>Percentage%</td>
<td>35</td>
<td>22</td>
<td>27</td>
<td>16</td>
<td>100</td>
</tr>
</tbody>
</table>

Using HAI scores and Metavir the 402 punctures liver biopsies of patients with C virus infection were distributed as follows: 148 cases were minimal chronic active hepatitis, 178 cases were moderately active chronic hepatitis, 46 cases with chronic hepatitis severely active, 30 cases with cirrhosis.

Distribution of cases in chronic hepatitis C according to the lesions described by HAI and Metavir shown in Table 6.8.

Table 6.8 Distribution of cases of chronic hepatitis according to HAI and score Metavir

<table>
<thead>
<tr>
<th>Hepatitis type</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal chronic active hepatitis</td>
<td>51 cases</td>
<td>97 cases</td>
<td>148 cases</td>
</tr>
<tr>
<td>Moderate chronic active hepatitis</td>
<td>56 cases</td>
<td>122 cases</td>
<td>178 cases</td>
</tr>
<tr>
<td>Severe chronic active hepatitis</td>
<td>11 cases</td>
<td>35 cases</td>
<td>46 cases</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>11 cases</td>
<td>19 cases</td>
<td>30 cases</td>
</tr>
<tr>
<td>Total</td>
<td>128 cases</td>
<td>274 cases</td>
<td>402 cases</td>
</tr>
</tbody>
</table>
piecemeal necrosis absent or altered focal periportal plate, only in some areas wear inflammation, steatosis.
- F0, F1, F2 or F3 - absent fibrosis / portal without septa / portal with rare septa.

Metavir score of active lesions of hepatitis minimum is shown in table no. 6.9

**Table 6.9 degree and state cases of active chronic hepatitis C according to the score minimum Metavir**

<table>
<thead>
<tr>
<th>Metavir Score</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1F0</td>
<td>4</td>
</tr>
<tr>
<td>A1F1</td>
<td>69</td>
</tr>
<tr>
<td>A1F2</td>
<td>60</td>
</tr>
<tr>
<td>A1F3</td>
<td>15</td>
</tr>
</tbody>
</table>

Moderate chronic active hepatitis was present in 178 cases, representing 44.27% of 402 chronic hepatitis C. Sex female dominated with 122 cases, patients with ages between 27 and 64 years.

HAI score was between 9:15 (scoring: grade 7-9 and stage: 2-5) and the score was Metavir A2 and F1, F2 or F3.

Metavir score was A2 or 3, F2 or 3 and presented the following histological changes (table 6.10):
- A2 - all piecemeal necrosis around portal area several inflammatory necrotic foci or diffuse alteration periportal plate in all portal area;
- F2, or 3 - portal fibrosis with rare septa and numerous septa.

**Table 6.10 Degree and stage chronic hepatitis C cases according to the score moderately active Metavir**

<table>
<thead>
<tr>
<th>Metavir Score</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2F1</td>
<td>10</td>
</tr>
<tr>
<td>A2F2</td>
<td>109</td>
</tr>
<tr>
<td>A2F3</td>
<td>59</td>
</tr>
</tbody>
</table>

Severe chronic active hepatitis was present in 46 cases, representing 11.44% of the total of 402 liver biopsy punctures.

Sex distribution for females was represented with 35 cases. HAI score ranged between 12 and 20 (grade 10 to 15, stage 2-5) and the score was Metavir A3 A3 F2 or F3 and presented the following histological changes (Table 6.11):
- A3 - piecemeal necrosis around the portal area of all necrotic foci and more diffuse inflammatory or altering the plate in the periportal spaces porte;
- F2, or 3 - portal fibrosis with rare septa and numerous septa.
Table 6.11 Degree and state cases of severe active chronic hepatitis C according to the score Metavir

<table>
<thead>
<tr>
<th>Metavir Score</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3F2</td>
<td>9</td>
</tr>
<tr>
<td>A3F3</td>
<td>37</td>
</tr>
</tbody>
</table>

The presence of cirrhosis was identified in 30 cases because due to fibrosis and architectural changes were accompanied by varying degrees of activity (table 6.12). In three cases was accompanied by a substantial inflammatory necrotic. In all cases of cirrhosis, lobular architecture was removed with the formation of fibrosis surrounding cirrhotic nodules.

If the minimum active hepatitis, extreme ages were between 18 years (male) and 56 years (female), in moderately active hepatitis them were between 19 and 62 years, both male owned. Severe active hepatitis was found only in male patients with ages ranging between 20 and 59 years.

Chronic viral hepatitis in our study were investigated by special methods to obtain information regarding viral antigens, lymphocyte subsets and other structural changes in liver tissue. Data was recorded and expressed semiquantitatively.

Immunohistochemical study was performed on 30 selected cases. Of these cases, five had viral hepatitis B and chronic hepatitis C, 25. The five cases with a diagnosis of chronic hepatitis B were investigate using immunohistochemical methods to detect the presence of infection in hepatocytes. In three cases cytoplasmic positivity was noted. Of these, one case presented.

Immunomarkers membrane. Immunomarkers intensity in cells varied from a slight perinuclear positivity to intense positivity throughout the cytoplasm.

Two cases were positive HBc, with positive Immunomarkers intense in the nuclei as well as the cytoplasm Immunomarkers weak positive.

Pathogenesis and perpetuation of hepatocellular injury in viral hepatitis infection remains unclear. Existing hypotheses include a direct viropatic effect, the host immune response, or both. Therefore we included in our study to investigate inflammatory immunophenotype. They used markers to identify B-cell phenotype (CD20) and T (CD45RO).

To assess the role of mononuclear inflammatory cells, including T lymphocytes in them, B and Kupffer cells on the 30 selected cases of liver biopsy punctures, we used the following markers: CD45RO, CD4, CD8, CD20 and CD68.

In the cases studied, we observed that CD20 positive B lymphocytes were reduced in number in both port and intralobular space.

B cell infiltration were observed mainly in the germinal centers of lymphoid follicles in portal area (24 cases) were positive diffusely and in rare lymphocytes in six cases.

T cells were the predominant cell type in both types of chronic hepatitis, but the intensity of T cell infiltrate differ. Infiltrate was CD4 positive was present in rare cells in the port area (5 cases) and intralobular (4 cases). CD4 lymphocytes were present in...
moderate 11 cases and intralobular space port in four cases, they were numerous in the port area in three cases and intralobular in three cases.

**Histological and immunohistochemical study in musculo-articular changes**

Histopathological study of musculoskeletal joint changes included a number of 11 cases diagnosed with muscular dystrophy muscle biopsy nonspecific, and nine cases of synovial biopsies diagnosed with rheumatoid arthritis.

*Histopathological study on muscle biopsies.*

The 11 cases of muscle biopsy was taken from patients with viral hepatitis C virus in eight cases and in patients with B virus in three cases. Histopathology in 8 of 11 patients noted the appearance of microscopic non-specific muscular dystrophy that is characterized by the presence of muscle fibers of various sizes, uneven endomisial. Endomisial fibrosis was willing focal areas in five cases and diffuse in 4 cases. Hypertrophic muscle fibers were observed in 3 cases and around them stood endomisial fibrosis and atrophic muscle fibers lipomatosis infiltration was observed in nine of 11 cases. Inflammatory infiltrate was absent in eight cases and reduced in three cases. In two cases the inflammatory infiltrate was perivascular and ordered a case of rare lymphocytes were noted periendoimisial arranged (similar to the polymyositis).

*Synovial biopsies histopathological study.*

We studied 20 cases of musculo-articular manifestations in patients with chronic hepatitis, muscle biopsies were taken 11 and 9 synovial biopsies. In all the nine cases biopsies of cases of joint appearance was rheumatoid arthritis, for which we intend pursuing several elements: sinoviocitary proliferation, inflammatory infiltration, fibrinoid necrosis, proliferation of fibroblasts and vascular changes. Profiling of aggression and response prediction therapy are essential in diagnosis and therapeutic management in the early stages of disease preventing installation destructive synovial lesion.

A first issue that concerned us was related to the extent of sinoviocitary proliferation, quite characteristic feature of rheumatoid arthritis. Depending on the intensity of proliferation of synovial cells for the cases investigated we found (Table 6.15).

<table>
<thead>
<tr>
<th>Degree Proliferation</th>
<th>1 (≤ 3 lines)</th>
<th>2 (3-4 lines)</th>
<th>3 (5-6 lines)</th>
<th>4 (≥ 7 times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cases</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Percentage%</td>
<td>22.2%</td>
<td>44.4%</td>
<td>22.2%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

It may be noted that in most cases sinoviocitary proliferation was intense lesions fits over two score. Thus, depending on the extent of sinoviocitary proliferation I found:
- Grade 1 in two cases (22.2%), sinoviocitary proliferation with the ≤ 3 lines;
- Grade 2 in 4 cases (44.4%), with sinoviocitary proliferation on 3-4 occasions;
- Grade 3 in 2 cases (22.2%), with 5-6 rows sinoviocitary proliferation.
- Grade 4 in one case (11.1%), with the sinoviocitary proliferation ≥ six times (Figure 6.52).

Another defining feature is the extent of inflammatory arthritis synovitis. For the cases analyzed in the intensity and distribution methods have established that the inflammatory and sinoviocitary proliferation more severity. Thus, we found (Table 6.16)

<table>
<thead>
<tr>
<th>Grade inflammatory cell infiltration</th>
<th>Grade 1 Absence of infiltrate / inflammatory aggregates</th>
<th>Grade 2 Formation of lymphoid follicles</th>
<th>Grade 3 Massive inflammatory infiltration</th>
<th>Grade 4 The presence of giant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cases</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Percentage%</td>
<td>66.6</td>
<td>22.2</td>
<td>11.1</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Analyzing the inflammatory intensity in order of frequency we find the following:
- Grade 1 in 6 cases (66.6%) were the most numerous cases had lymph-plasmocitar inflammatory infiltrates reduced ordered aggregate form perivascular topography (Figure 6.53).
- Grade 2 in two cases (22.2%) the infiltration were prepared as lymphoid follicles, sometimes even forming germ centers (Figure 6.54)
- Grade 3 in one case (11.1%) were the heavy infiltration with diffuse or follicular available (Fig. 6.55)
- Grade 4 in one case (11.1%) the matters described in addition we observed the presence of multinucleated giant cells rare.

Fibrinoid necrosis was often present in patients with early rheumatoid arthritis. It had spread and depth at different sinoviocites lining. Depending on the severity of the lesion we found the following degrees (Table 6.17)

<table>
<thead>
<tr>
<th>Grade of lesion</th>
<th>Grade 0 Absent</th>
<th>Grade 1 Length</th>
<th>Grade 2 Zonal</th>
<th>Grade 3 Diffuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cases</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>33.3</td>
<td>33.3</td>
<td>22.2</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Analyzing the cases studied in terms of extent of fibrinoid necrosis we observed that they corresponded in order of frequency:
- Grade 0 in 3 (33.3%) in which fibrinoid necrosis was absent,
- Grade 1 in 3 (33.3%) in the necrosis fibrinoid appeared in several areas, the focal character, affecting small groups of sinoviocite, the synovial membrane surface areas seen to sinoviocites desquamation and / or small areas degeneration and necrosis
- Grade 2 in two cases (22.2%) in which fibrinoid necrosis was present in greater scope, with regional character.
- Grade 3 in one case (11.1%) in which fibrinoid necrosis was present in large areas with diffuse character and appearance of "band"-shape necrosis.
Another histological parameter was related to the intensity followed fibroblast proliferation. Depending on the intensity and distribution methods have employed the fibroblast proliferation nine cases of early rheumatoid arthritis in one of the following degrees of severity (table 6.18)

**Table 6.18. Casuistry in the intensity distribution of fibroblast proliferation**

<table>
<thead>
<tr>
<th>Degree fibroblast proliferation</th>
<th>Grade 0 Absent</th>
<th>Grade 1 Poor</th>
<th>Grade 2 Average</th>
<th>Grade 3 Intense</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cases</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Percentage%</td>
<td>22.2</td>
<td>44.4</td>
<td>22.2</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Analysis where the intensity of fibroblast proliferation indicated the following:
- Grade 0 in two cases (22.2%), the proliferation of fibroblasts was absent grade 1 in 4 cases (44.4%), the proliferation of fibroblasts was low intensity (Figure 6.57)
- Grade 2 in two cases (22.2%), the proliferation of fibroblasts was of medium intensity, sometimes with pale layout ISAD (fig.6.58)
- Grade 3 in one case (11.1%) the proliferation of fibroblasts was increased, making pseudo issues.

For the cases analyzed we quantify the extent of vascular change depending on the presence of one or several related changes, such as congestion, increasing surface ships located in the lining layer sinoviocites , erythrocyte extravasation, endothelial proliferation and postcapilar arterioles. Therefore, we classified the cases analyzed in one of the following four grades of severity (table 6.19).

**Table 6.19 Vascular changes**

<table>
<thead>
<tr>
<th>Degree vascular changes</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cases</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Percentage%</td>
<td>44.4</td>
<td>22.2</td>
<td>22.2</td>
<td>11.1</td>
</tr>
</tbody>
</table>

It is apparent that most cases showed extensive vascular changes, involving all the criteria mentioned. Depending on the severity of these changes I found:
- Grade 1 in 4 cases (44.4%) in which there was pronounced vasodilation, particularly in blood vessels subsinoviocites
- Grade 2 in two cases (22.2%), which existed alongside vasodilation of or increase subsinoviocites
- Grade 3 in 2 cases (22.2%), which existed alongside vasodilation, and increased hematic extravasation subsinoviocitary vessels (Fig. 6.60)
- Grade 4 in one case (11.1%) that existed along the vasodilation, hematic extravasation, increasing subsinoviocitar vessels, vascular endothelial proliferation and microtrombosis.

**Immunohistochemistry study of synovial biopsies**
IHC study included nine cases of synovial biopsies, clinically and histopathologically diagnosed with rheumatoid arthritis. We used markers that have addressed the
inflammatory process, namely CD20 to identify B lymphocytes, T cells and CD45RO to identify citrulline.

Immunoreactive CD20cy was present in all cases included in the study marking the apical membrane and cytoplasmic. B lymphocytes were diffuse in distribution. Five cases of rare cells marked (grade 1). The two cases were disposed perivascular lymphocytes, are present a moderate number of labeled cells (grade 2). In one case B lymphocytes were present in large numbers, perivascular, with the formation of follicular structures (Grade 3).

Immunohistochemical reaction for CD45R0 was positive in all cases, T lymphocytes were marking the apical membrane and cytoplasmic. In five cases labeled cells were rare and diffuse arranged without relation to the vessels (grade 1). In other cases perivascular T cells were present in a number of moderate (2 cases, grade 2) or solid-looking follicular (1 case, grade 3). In two cases the appearance of follicular distribution was no close relationship with vessels.

Immunohistochemical study of citrulline in the investigated cases showed negative reaction to the two control cases and 72.7% positivity in cases diagnosed with rheumatoid arthritis. Citrulina was present both intra and extracellular Endothelial cells, fibroblasts and mononuclear cells were also positive for citrulline protein and extracellular citrulina was positive in the amorphous area of fibrin. However, unlike sinoviocite, mononuclear cells were present only Citrulline proteins isolated, and a small number of endothelial cells and fibroblasts.

CONCLUSIONS

Our study was made on a number of 532 hepatic biopsies from 2006-2009 period and on number of 20 synovial and muscular biopsies.

The serologic exam allowed the identification of the hepatic virus type: the most frequent was hepatitis C (402 cases), followed by hepatitis B (116 cases) and the co-infection B+C (8 cases) and B+D (6 cases).

For the 532 hepatic biopsies, the histopathological exam aimed to follow the patterns of necrosis, inflammation and fibrosis activity development and to measure these one by using a score system (HAI and METAVIR).

For the hepatitis C, the most frequent affected age group was between 40-60 years, being more frequent at women.

The necrosis and inflammation activity was classified as follows: grade 1 – 116 cases (slight inflammation), grade 2 – 126 cases (moderate inflammation), grade 3 – 119 cases (moderate-important inflammation), grade 4 – 41 cases (important inflammation).

The fibrosis stage emphasized: the presence of a fibroses expansion of the portal spaces without septs formation at 79 cases and with septs formation at 178 cases. The bridges fibrosis without cirrhosis presence was identified at 111 cases.
The hepatic steatosis was evaluated as being absent (153 cases), slightly (<-30% of the hepatic cells) (133 cases), moderate (30–60% from the hepatic cells) (62 cases) and severe (>60% hepatic cells) (54 cases).

For the patients subgroup with hepatitis B, the age varied between 18 and 62 years (average = 40.58 years), the age group with the most cases was 31-40 years – 35 cases and the ratio on genders females: males was 2.41:1.

The necrosis and inflammation activity emphasized a high frequency of slight and moderate inflammation: 43 cases (slight inflammation), 37 cases (moderate inflammation), 25 cases (moderate-important inflammation) and only 10 cases with important inflammation.

The fibrosis stage emphasized as being the most frequent variety of fibrosis the septa and bridges aspect.

The moderate and slight hepatic steatosis were the most frequent ones, met in 31, respectively 26 cases.

The chronic hepatitis immunohistochemical study was made on 30 selected cases, from which 5 cases presented hepatitis B and 25 cases hepatitis C.

The inflammatory infiltrate was rich in T cell lymphocytes, B lymphocytes were few.

The Kupffer cells were marked with CD68 and their portal and intra-lobar presence, as well as the star cells marked with alpha actine showed these cells important role in fibrosis genesis process.

Were studied histopathological and immunohistochemical the muscular and joint modifications.

Were been drawing 11 cases of muscular biopsies from the patients with viral hepatitis. The histopathological study emphasized the lipomatosis infiltration at 9 from the 11 cases. The inflammatory infiltrate was absent in 8 cases and little in 3 cases. In 2 cases the inflammatory infiltrate was arranged perivascular and in one case were remarked rare lymphocytes disposed around endomysium. This association suggests that hepatitis C virus may determine an inflammatory myopathy and that the immune mechanisms humoral controlled of the muscular structures, and not directly connected with the hepatitis C viral infection, can sustain the local inflammatory reactions.

The 9 synovial biopsies diagnosed with rheumatoid arthritis analyzed histopathologically different parameters (synovial cells proliferation, inflammatory infiltrate, fibrosis, necrosis, fibroblast cells proliferation, vascular modifications) whom were granted various grades of severity.

Was noticed a great variability of histopathological aspects, the fact that the compose histological score can offer information about the affection severity, without any correlations with the affection beginning period or the patients age.

The immunohistochemical study of the inflammatory infiltrate proved the implication of both B and T lymphocytes types in the rheumatoid arthritis pathogenesis, the developed lesions being also a consequence of activation and collaboration of both lymphocytes types.

The similar distribution of B and T lymphocytes in the inflammatory infiltrate of the rheumatoid arthritis proves the pathogenic mechanisms.
Citrulina, positive in 72.7% of the studied cases, can represent a new diagnosis possibility in order to differentiate the rheumatoid arthritis biopsies fragments at patients with chronic hepatitis from other bone-joint inflammatory affections. The precocious presence of the citrulina proteins, concomitant or after short time from the clinic beginning of clinical symptomatology, can be correlate with the illness severity and with the prognostic, suggesting not only the biomarker role, but also the implication in patho – physiology of the illness.

REFERENCES


Chen Feng, CAI Weimin, CHEN Zhi, Chen Xiangming Liu Ronghua - Dynamic changes in the collagen metabolism of liver fibrosis at the transcript level ion Rabbits with Schistosomiasis japonica in Chinese Medical Journal, 2002, Vol No. 115 11: 1637-1640

Chinh N. Tran, Steven K. Lundy, David A. Fox, biology and synovial T cells in rheumatoid arthritis. Pathophysiology, 2005, 12: 183-189


Franciscus, Alan - An Overview of extrahepatic Manifestations of Hepatitis C in 2009, hcpFACTsheet, 4, 1-5


Ishak KJ - Chronic Hepatitis: morphology and nomenclature. Mod Pathol., 1994, 7, 690-713


McCaughan GW, George J fibrosis progression in chronic Hepatitis C virus infection *Gut* 2004, 53, 318-321

McCuskey, RS: Morphological mechanisms for regulating blood flow through hepatic sinusoids. Liver 2000, 20: 3-7


Senoo, Haruki - Structure and function of hepatic stellate cells: Medical Electron Microscopy, Volume 37, Number 1, March 2004 , pp. 3-15(13);


