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PHD THESIS

ABSTRACT

**Clinical, paraclinical, histological and immunohistochemical
study on the evolution of postviral chronic hepatites after the
antiviral treatment**

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PhD Thesis Abstract

Introduction

Chronic C-virus hepatitis (CVH) and B-virus hepatitis (BVH) represent important public healthcare problems at a worldwide level. It is estimated that approximately 400 million individuals are BVH carriers worldwide, 75% of these living in Asia and West Pacific. Also, CVH infection is estimated at approximately 170 million people all over the world (Crockett SD, Keeffe EB, 2005; Williams R, 2006; Bini EJ, Perumalswami PV, 2010) and there is a marked geographical variation, with infection rates varying from 1.3-1.6% in the USA, up to 15% in Egypt (Karoney MJ, Siika AM, 2013).

C hepatitis virus infection appears mainly in the patients who have undertaken blood transfusions, users of intravenous drugs, patients subjected to kidney dialysis, individuals with various tattoos and in heterosexuals that practice unprotected intercourse. (Rana D, Chawla Y, Arora SK, 2013). Most often, the acute infection is spontaneously solved in a few weeks, with no therapeutic intervention, but more persons infected with C-virus hepatitis will develop a chronic hepatitis. Chronic infection with CVH is defined when the CVH RNA blood persistence lasts for at least 6 months.

Approximately 75% of the infected patients with acute CVH develop a chronic infection; of these, 20% will develop liver cirrhosis during the following twenty years since infection if the disease is left untreated (Liang TJ, Rehermann B, et al, 2000; Lauer GM, Walker BD, 2001; Thein HH, Yi Q, Dore GJ, Krahn MD, 2008), and 1-4% will develop a hepatocellular carcinoma (Alter MJ, 2007).

Similar to chronic C hepatitis, chronic BVH hepatitis represents one of the main causes of cirrhosis worldwide.

The BVH prevalence is higher among the individuals that migrated in endemic regions (e.g. China), as well as in the populations with a high risk of transmission, such as HIV infected individuals, consumers of parenteral administration drugs, where the prevalence of chronic infection with B virus hepatitis is approximately 10%.

B virus hepatitis infection (BVH) may occur at any time, starting with intrauterine life and up to the oldest age.

Chronic B hepatitis and chronic C hepatitis are considered to be the main etiological factors of the hepatocellular carcinoma, in approximately 70-80% of the cases (El-Serag HB, Rudolph KL, 2007; Xu J, Li J, Chen J, Liu ZJ, 2015).

The antiviral treatment with interferon and ribavirin, recently used all over the world, has changed some aspects of the liver disease epidemiology secondary to some hepatitis virus infections.

Starting from the data regarding the severity of chronic viral hepatitis, and the high social resources intake, we proposed to evaluate a group of patients suffering from chronic viral hepatitis, who received a treatment with interferon, PEG-interferon and ribavirin, between 2001 and 2005.

Chapter I. Liver histophysiology

The liver is the largest gland in the human body, having an exocrine and endocrine function. The exocrine function is represented by the bile, and the endocrine one is represented by the numerous biochemical substances synthesized in the liver and released in the internal environment.

Reported to the organs of the abdominal cavity, the liver is the largest parenchymatous intraabdominal organ, in adult, healthy individuals, weighing approximately 1500 grams, representing approximately 1/40 of body weight. It contains about 800-900 ml of blood.

The liver is composed of various types of cells, which interact one with the other in order to carry out specific functions. The main type of cell in the liver parenchyma is the hepatocyte, representing 60% of the total of the cell population and 80% of the organ volume. The hepatocytes are organized in cordons or laminas, which are interconnected in order to form a plexiform, tridimensional, continuous structure.

A particularity of the liver is that the two functions, exocrine and endocrine, are carried out by the same cell, the hepatocyte, which, through the bile pole, secretes in the external environment, while through the vascular, opposed, pole, it secretes in the internal environment, thus the liver is a gland whose cells have an amphicrine function.

The structural and functional unit of the liver is the hepatic lobe, described by Malpighi in 1866 and whose hexagonal shape on a transversal sectioning was remarked in 1833 by Kiernan. The classical hepatic lobe is a polygonal structure, having a centrolobular venule as its central axis, with the portal tracts distributed alongside, at the periphery.

In the structure of a liver lobule, there are four fundamental components: the epithelial component, the vascular component, the biliary component and the stromal one.

The epithelial component is represented by the epithelial cordons of the liver lobe, made up of hepatocytes.

The hepatocytes represent 80% of the cellular population of the liver, the rest of 20% being represented by the epithelial cells of the biliary ducts - 1%, the endothelial cells of the liver sinusoids - 10%, the Kupffer cells - 4% and lymphocytes 5% (B and T lymphocytes of the immune system and inborn lymphocytes of the immune system, such as natural killer-NK lymphocytes and T-NKT natural killer cells).

The vascular component. Among the hepatocyte cordons of the liver lobe there are found blood capillaries, with a sinusoidal aspect, at this level taking place massive bidirectional changes between blood and hepatocytes.

The liver sinusoid capillaries contain a mixture of portal blood (75%), coming from the digestive tract and of arterial blood (25%), coming from the liver artery.

Chapter II. Chronic hepatitis

Chronic hepatitis represents a localized inflammatory process of the liver lasting for more than six months. Most often, it is caused by a group of viruses, known as hepatitis viruses: A, B, C, D and E. Other viruses, such as the ones causing mononucleosis (the Epstein-Barr virus), the simple herpes virus (SHV), cytomegalovirus or varicella virus, may also cause chronic hepatitis. Besides microorganisms, chronic hepatitis may be caused by drug intake, excessive alcohol intake, autoimmune reactions or by some toxins in the environment (industrial organic solvents, toxic plant substances, etc.).

The B hepatitis virus is transmitted through skin or permucosal contact, through direct blood contact, infected biological liquids or sanitary materials. The main way of transmission of BVH is also a geographical one.

The natural history of BVH infection is determined by the interaction between the ability to replicate of the virus and by the intensity of the immune response. Liver damage is mainly caused by a protector immune response, which destroys the hepatic cells infected by the virus. In the absence of an insufficient immune response that eliminates the virus, the infection becomes chronic.

C hepatitis is an infectious disease caused by the C hepatitis virus (CVH). The infection often presents no symptoms, the virus persistence in the liver leading to progressive, fibrous lesions of the liver and, ultimately, after many years of activity, may lead to cirrhosis. In some cases, the ones suffering from cirrhosis will continue to develop phenomena of liver failure or liver cancer (Ryan KJ, Ray CG, 2004; Gravitz L, 2011).

CVH is mainly transmitted through a direct contact with the infected blood, including transfusions, through injection of intravenous drugs, through the use of infected materials or through the use of insufficiently sterilized medical equipment. The source of CVH infection remains unclear in many cases.

Chapter III. Interferons used in hepatitis treatment

The purpose of the treatment of chronic viral C hepatitis is to provide a sustained viral response defined as the absence of CVH RNA (undetectable) after 6 months since treatment stopped. The sustained viral response (SVR) is associated to an improved histological result and to a reduction of morbidity and mortality caused by liver diseases (Wilkins T, Malcolm JK, 2010).

In the last two decades, combined therapy with pegylated interferon and ribavirin has represented the optimal treatment for the patients suffering from chronic C hepatitis and it represents a significant progress in comparison to previous treatments.

Interferons belong to the large class of glycoproteins known as cytokines, synthesized molecules for intercellular communication, triggering a defence protection of the immunitary system that helps to eradicate pathogens (Cohen B, Parkin J, 2001). They interfere with the immune response through inhibiting the viral replication in the host cells, activation of natural killer cells and macrophages, increase of antigen cells number, increase of host cells resistance to viral aggression. Soon after the discovery of the interferone role in the protection of human body against viruses, there were also discovered its effects. There was observed that the interferon inhibits some tumors growth and it represented a chance in cancer treatment.

Their antiviral effects exert upon a higher number of viruses, unrelated among them, which makes the use of interferons in viral diseases to represent a major interest.

Ribavirin represents an altered nucleoside with an antiviral activity against various different viruses.

Pegylated interferon was created with the intent of improving the pharmacological and pharmacodynamic properties of the interferon. Polyethylene glycole (PEG) represents a polymer consisting in a low quantity of ethylenglycol bound to form a variable-shaped chain. The covalent bound of PEG with interferon results in an improved physical and thermic stability, protected against rapid enzymatic degradation. Therefore, pegylated interferon has a longer life duration and a more reduced distribution volume, thus allowing a single weekly dosage (Xu ZX, Patel I, Joubert P, 1998; Reddy KR, 2000).

Combine therapy with pegylated interferons was accepted as the optimal treatment for chronic infection with CVH, from a given perspective of a viral eradication in a substantial percentage of the treated patients.

Treatment of chronic hepatitis with B virus. The main purpose of the therapy is to permanently eliminate or suppress the BVH infection, in order to reduce the severity of hepatitis and to slow down or limit the progression of the liver disease towards chronicization. The long-term final objectives of the treatment are to achieve a sustained viral response (SVR) and to remove the HBs antigen (AgHBs) from the body, thus preventing or reducing the development of liver decompensation, its evolution towards cirrhosis or hepatocellular carcinoma (HCC) and prolonging the survival (Liaw YF, et al, 2012).

Chapter IV. Importance and objectives of the study

Taking into consideration the complexity of the liver lesions caused by the hepatitic viruses C and B, in the present PhD thesis we proposed the following objectives:

- the clinical, biological and evolutive analysis of a group of patients diagnosed with B and C chronic hepatitis, who received a treatment with interferon and ribavirin according to the standards established by the Ministry of Health;
- the assessment and staging of the histological lesions present in the liver in all the patients included in the study before initiating the treatment with interferon and ribavirin;
- the immunohistochemical study of hepatocyte lesions, with highlighting the severity of the hepatocyte necrosis lesions, the inflammatory cell reaction, especially of T and B lymphocytes;
- the highlighting of Kupffer cell and dendritic cell reactions;
- the assessment of the response to the applied treatment through obtaining a sustained viral response;
- the assessment of seric transaminases changes and of other biological parameters during treatment duration;

- the assessment of adverse effects of the treatment with pegylated interferon and ribavirin;

Chapter V. Clinical and statistical study on viral chronic hepatitis

The clinical and statistical study included patients that registered records at CAS Dolj for the approval of the treatment with interferon, peg-interferon, ribavirin and lamivudine by the Expert Board of CNAS for postviral chronic hepatitis, between 2001 and 2005. The patients were diagnosed with postviral chronic hepatitis in the Clinics of Gastroenterology and Internal Medicine within the Emergency Clinical Hospital of Craiova, in the Clinic of Infectious Diseases within the "Victor Babes" Infectious Diseases Hospital of Craiova and in the Medical Clinic of "Filantropia" Municipal Hospital of Craiova.

The inclusion of the patients in the treatment was based on clinical, laboratory and histopathological criteria (liver biopsy), established by the Expert Board of CNAS. The minimum age of the patients who could benefit from the treatment with interferon was 18 years old, and the maximum age was of 65 years old, at first, but this limit was subsequently discharged.

The duration of the treatment the patients underwent varied between 3 months (a minimum of 12 cures of treatment with interferon) and 12 months (a maximum of 48 cures of interferon). The treatment with interferon was performed rhythmically, a phial of interferon/ week subcutaneously, to which ribavirin was associated (in C hepatitis). The initiation of interferon treatment was made in the hospital, under medical surveillance, and the rest of the treatment was performed through ambulatory care, under the surveillance of the GP or the Internist or the Gastroenterologist.

All the patients, after the 12th dosis of interferon (after 3 months) mandatory performed the control viremia, as well as the biochemical analyses regarding the liver and blood activity. After the administration of 12 therapeutical doses (after 3 months), the level of viremia significantly decreased both in women and in men. Of a total of 105 women, after 12 doses, only a number of 18 (17.1%) still presented detectable viremia, compared to seven men (15.9%) of a total of 44. After six months of therapy, in a number of 3 men (6.81%), namely in 4 women (3.8%), there were recorded detectable viremias. Two men and 4 women were declared non-responders to the administered antiviral treatment.

The average values of seric transaminases significantly dropped after administering the 12 doses of antiviral medication; the effect also maintained after eight months of antiviral treatment.

Regarding the evolution of the average values of haemoglobin, we identified a moderate decrease during the antiviral treatment, from average values of 14.2 g/dl for men, and 13.4 g/dl, respectively, for women, up to 11.2 g/dl and 11.12 g/dl, respectively, after eight months of antiviral treatment.

Most of the patients (14 men – 66.6%, and five women – 55.5%) received treatment with Pegasys 180. Four men and two women received treatment with Ronferon 9 MU (20% of the whole group), while five patients (three men and two women) received treatment with Intron 10 MU. There was not recorded any non-responder to antiviral treatment, at the end of the study. Under treatment, seric transaminases significantly dropped, reaching normal values after 8 months since treatment initiation.

Regarding the evolution of the average values of haemoglobin, we identified a mild decrease during the antiviral treatment, from average values of 14.3 g/dl in men, and 13 g/dl in women, respectively, up to 13.6 g/dl and 12.1 g/dl, respectively, after 12 doses of antiviral treatment.

The clinical and statistical study highlighted a favorable progress of postviral chronic hepatitis after the applied antiviral treatment.

Chapter VI. Histological aspects of the hepatic lesions in chronic hepatic

The histological assessment of liver alterations is considered "the golden standard" regarding the disease progression. The stage of liver fibrosis is one of the most important diagnosis and prognosis assessments in chronic liver disease.

Our study included a number of 43 liver biopsies sampled from the same number of patients diagnosed with chronic C hepatitis at the beginning of the treatment and 11 biopsies sampled from the same number of patients at the end of the treatment.

The biological material, right after sampling was fixed in a 10% neuter formalin solution for 24 hours, and sent to the laboratory of pathological anatomy for paraffinum inclusion, staining and results interpretation.

The biological material was processed with the classical histological technique of paraffinum inclusion, a technique used in all the laboratories of histopathological diagnosis, as it allows the performance of serial sections of a 3-5 μ thickness on the biological material, thus facilitating a good highlighting of tissular and cellular lesions.

The histological samples were stained with Hematoxylin-Eosine, the most used method of tissue highlighting, with the trichromic based on green light after the Goldner-Szeckell method, in order to electively highlight collagen fibers, and through argentic impregnation, the Gomori technique, for highlighting the reticulin fibers from the liver stroma.

The interpretation of the histopathological data was performed by anatomopathologists according to the Metavir, Knodell and Knodell-Ishak scores.

According to the Metavir system regarding liver fibrosis, anatomopathologists made the following observations: F0, absence of fibrosis; F1, single portal fibrosis; F2, portal fibrosis with rare porto-portal septa; F3, portal fibrosis with multiple septa, including porto-central ones; F4, cirrhosis.

In our study, the assessment of histopathological data was performed after the Knodell-Ishak system that seemed the most complex one, being the one that provides the most detailed data regarding the inflammatory reaction, fibrosis, necrosis and apoptosis that emerge during chronic C hepatitis.

In our study, most often, inflammation, namely the inflammatory processes had a higher intensity in portal areas and less in intralobular ones. The inflammatory cells were most often localized diffusely, but there were also cases when there were lymphoid follicles with germinative centers. We consider that liver inflammation is an essential lesion in chronic hepatitis, as it causes hepatocyte necrosis and the onset of fibrosis, at first in portal areas, and then in the liver lobe, thus leading to severe complications, such as liver cirrhosis with severe liver failure.

In our study, besides the necroinflammatory activity, we also assessed liver fibrosis, one of the most severe lesions found in hepatitis. As we mentioned before, most of the patients with chronic C hepatitis (over 97%) were diagnosed with liver fibrosis. Clarifying the mechanisms that are at the core of liver fibrogenesis is of capital importance for the management and prevention of terminal liver diseases (Sebastiani G, Gkouvatsos K, Pantopoulos K, 2014).

Chapter VII. Immunohistochemical aspects of hepatic lesions in chronic hepatitis

From the numerous methods of molecular medicine, in the present thesis we used the immunohistochemistry techniques in order to highlight the reaction of the cells involved in the etiopathogeny and progression of chronic hepatitis.

From the biological material used for the histopathology studies, for the immunohistochemistry study we selected a number of 29 liver biopsies included in paraffin, leaving out the old biological material or the one that was not in an appropriate quantity or could have been caused artefacts or difficulties regarding the result interpretation.

In our study, we proposed to assess the inflammatory reaction present in the liver of patients with CVH. Inflammation represents a physiological, crucial way within the homeostatic response, emerging as a response reaction to a number of exogenous stimuli that tend to influence the internal homeostasis of the body. Although it is a beneficial response, a chronic inflammatory state or an excessive inflammatory response may lead to pathological effects.

The immunohistochemical study highlighted the presence of a more or less intense inflammatory infiltrate in the portal areas with predominating T lymphocytes CD3+ and, in a smaller quantity, B lymphocytes CD20+. The T lymphocytes were also identified in the structure of the liver lobules among the hepatocyte cords. In some cases, in the portal areas, there were identified lymphoid nodules constituted of germinative centers with numerous lymphoblasts, surrounded by a coating of mature B lymphocytes and rare T lymphocytes. B lymphocytes were less numerous in comparison to T lymphocytes, their reappearance in the portal inflammatory infiltrate being completely heterogeneous. Moreover, there were cases when B lymphocytes were well-represented and cases when these cells could be found in very small quantities.

A particular aspect of our study was that of highlighting the lymphoid follicles in the portal inflammatory infiltrate in some patients with chronic C hepatitis. Lymphoid follicles were formed from a central area, intensely reactive to the CD20 antibody, made up of young lymphoblast cells and a peripheral crown with less CD20+ cells. The presence of these lymphoid follicles did not correlate with the intensity of hepatocyte lesions, denoting the fact that their emergence is subjected to other immunological mechanisms.

Another immune reaction aspect under study in the present PhD thesis was the reaction of the monocyte-macrophage system cells. The immunohistochemical study of the macrophages was performed by using the CD68 antibody.

Our study, through the use of immunohistochemistry techniques highlighted the presence of numerous T lymphocytes (CD3+) and B lymphocytes (CD20+) in the portal inflammatory infiltrate, from the porto-portal and porto-central bridges resulted after fibrillogenesis, as well as from the hepatocyte cordons. These microscopic aspects emphasize the importance of lymphocytes in the process of disease cure and also in the pathological, progressive processes of the disease.

Lately, both clinicians and researchers made an important emphasis on liver fibrosis, considered to be the main lesion that alters the architecture of the hepatic lobe, causes the pressure increase in the portal vein and facilitates the lesion progression towards liver cirrhosis with various stages of hepatocyte failure.

In our study, we observed that the liver lesions overlap, namely where the inflammatory processes were accentuated, the process of liver fibrosis was well-represented. According to the histological and immunohistochemistry aspects highlighted by us, we believe that the hepatocyte necrosis and liver inflammation are followed, when an efficient treatment lacks, by processes of fibrosis, as a normal response reaction of the body to an aggression.

The histopathological and immunohistochemical aspects observed makes us believe that, alongside starved hepatic cells, the fibroblasts and myofibroblasts that appear in the porto-biliary area intensely participate in the liver fibrogenesis. The latter cells may also be found in the portal area, through the mobilization of mesenchymal cells from the bone marrow and their transformation into fibroblasts and myofibroblasts, representing some relatively recent described aspects.

Chapter VIII. Conclusions

In the patients with chronic C hepatitis, the architectural changes found in the liver parenchyma had average values (score 3), the score of lytic necrosis highlighted the predominance of transition changes, with values of 1 and 2, respectively, while the fibrosis staging highlighted the predominance of values F2 and F3. After the administration of 12 therapeutic doses (after 3 months), the level of viremia dropped significantly, both in women and in men. Of a total of 105 women, after 12 doses, only 18 (17.1%) still presented detectable viremia, in comparison to seven men (15.9%) of a total of 44. After six months of treatment, in 3 men (6.81%), and 4 women (3.8%), respectively, there were recorded detectable viremias. Two men and 4 women were recorded as non-responders to the administered antiviral treatment.

In the patients with chronic viral B hepatitis, the changes of the liver architecture were included in stages 1 and 2, in most of the patients the HAI score was 0, in most of the patients with chronic viral B hepatitis the lytic necrosis score was 2. Under treatment, serum transaminases significantly dropped, reaching normal values after 8 months of treatment.

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