UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL

Liver Fibrosis Noninvasive Assessment in Chronic Viral Hepatitis C Patients and Chronic Kidney Disease treated with Hemodialysis

Ph. D. Thesis Abstract

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2019

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Key Words: *chronic viral heopatitis, end stage chronic kidney disease, liver fibrosis*

STATE OF KNOWLEDGE

1. Introduction

Chronic Viral Hepatitis C (HCV) is considered a worldwide health burden which affects around 120-125 millions of patients and represents the main cause of cirrhosis being directly involved hepatocellular carcinoma (CHC) pathogenesis. The virus is transmitted after exposure to infected blood, which was confirmed in the 1980's when the main way of transmission was through blood transfusions.

A special subgroup of patients of HCV infected patients is the one composed by patients with end stage kidney disease (ESRD) that follow hemodialysis. There is a mixed link between HCV and ESRD. First of all, HCV may be directly related to ESRD by producing and storing circulating immune complexes (mixt cryoglobulinemia) within the renal matrix or by developing glomerulonephritis. On the other hand, ESRD itself is considered a risk factor for HCV infection through the fact that many patients receive transfusions or may be infected during the hemodialysis process or after renal transplant. Natural history surfaced the fact that HCV infection has a negative impact over hemodialysis patients with a rise in cirrhosis and CHC incidence, accelerating at the same time and ESRD progression and patient's mortality rate.

Thus, by using new direct antiviral agents (AAD), patients with advanced fibrosis or even a compensated form of cirrhosis benefit from hepatic function improvement and avoid liver transplant. Introduction of several therapeutic regimes allowed treatment individualization for each patient, offering other options for ESRD patients.

2. Chronic Viral Hepatitis C (HCV)

Along with evolution to cirrhosis, the risk of decompensation with specific signs of portal hypertension and liver insufficiency may appear. The most encountered clinical signs are jaundice (spontaneous bacterial peritonitis), variceal bleeding and hepatic encephalopathy. The patient's prognosis may be calculated by using the "Model of End Stage Liver Disease" (MELD) and "Child Turcotte Pugh" scores.

In a relatively low amount of time the new Interferon-free HCV regimes have reached a 90% response rate in patients with genotype type 1, have decreased the adverse events incidence, reduced the treatment duration rate and proved efficient in group cases that were previously

difficult to treat. The tolerability was improved by interferon removal and ribavirin limited use. Instead, AAD from different categories (NS3/4A inhibitors, NS5A inhibitors, and polymerase NS5B inhibitors) were combined so that may cover more steps within the virus cycle.

3. Liver fibrosis

Liver fibrosis is the results of liver tissue degradation. The main mechanism involved is the same as the one involved in wound healing process. Thus, a cascade of biological events that involve cells and soluble factors are aimed at the resolution of tissue injury. In general terms, these events are disposed in logic sequence activating step by step by resolving a recent process.

Though a regression was observed in animal cirrhotic experimental models, this process is not definitively sustainable in humans. Proof ore liver fibrosis or cirrhosis regression were reported in different liver disease types included viral and autoimmune hepatitis, non-alcoholic fatty liver disease, alcohol abuse. However, after examining these results by experienced liver pathologists, an agreement of a variable degree of fibrosis regression may occur but not a complete reversal when cirrhotic stage is instated. Over the years there have not been encountered compelling evidence of vascular abnormalities for a cirrhotic liver to regress to a normal state.

4. HCV and ESRD

Chronic Kidney Disease (CKD) is defined as a renal disease with a low glomerular filtration rate, high albumin urinary excretion and is considered a growing health problem. It affects a lot of people around the world and evolves to ESRD where renal substitution is required. The most common causes of CKD are diabetes mellitus and arterial hypertension. CKD is considered an independent factor for cardiovascular disease, cognitive disfunction, hospitalization and mortality.

Renal disease linked to HCV infection is mainly asymptomatic in dialysis long treated patients. HCV infection is high encountered in ESRD patients with hemodialysis and it remains the main cause of liver disease for this group of patients, although in recent years the prevalence has been reduced by almost a third. The prevalence varies between regions, with a high rate in developing countries.

PERSONAL CONTRIBUTUION

This current paper is focusing on patient's liver and renal disease characteristics in patients with ESRD that associate CHV chronic infection. By using different modern imagistic and biologic techniques in diagnosis assessment a proper monitorization for patients was made so that they may benefit from antiviral management, thus evaluating their prognostic and disease evolution.

Objectives:

- ESRD and HCV patient's characteristics assessment;
- Transient elastography as a main method to assess liver fibrosis;
- Use of biological markers for HCV assessment in ESRD patients;
- Antiviral therapeutic effect in HCV infected ESRD patients.

1. Materials and Methods

The study involved a multidisciplinary approach and it involved the Gastroenterology Clinic and Nephrology Clinic of Emergency County Hospital of Craiova as well as the Regional Nephrology Clinic of County Olt Hospital. 252 HCV diagnosed over a 4 year period of time (January 2014 – December 2018) patients were included from which 39 were diagnosed within the Nephrology Clinic, being known with ESRD and having dialysis.

Liver fibrosis assessment

Liver fibrosis was measured using Fibroscan (Echosens, Paris, France) according to the national guidelines related to liver elastography in 2014. The procedures were performed by a single experienced operator from Gastroenterology Clinic of University of Medicine and Pharmacy of Craiova Romania on a group of patients on which was used the standard M transductor with a frequency of 3,5 MHz. Tissue stiffness was expressed in kilopascals (kPa) and patients were grouped according to fibrosis stages. Measurements were made for each patient by positioning them on their back with right arm above their head to enlarge the intercostal spaces and allowing

the transductor to access the right liver lobe. Results were taken into considerations after 10 measurements were made with an IQR with a rate of success of over 60%.

A first step in liver fibrosis assessment was based in performing the procedure on 39 patients with ESRD before and after dialysis to evaluate the effect of hemofiltration process over fibrosis. Two measurements were made, one just before the dialysis process and the next just after dialysis. The results were statistically analised using SPSS Statistics 20.0 (Chicago IL, America).

The quantity and length of fluid that was taken from each patient was measured during the dialysis process.

The next step in assessing liver fibrosis by transient elastography consisted in comparing the ESRD HCV patients and a separate group of HCV patients without any renal disorders. Biologic parameters where compared and several scored of hepatic fibrosis where calculated by using as a reference method Fibroscan.

We also used hyaluronic acid as a biomarker for liver fibrosis assessment with normal values < 50 ng/ml, which were compared with transient elastography results. To identify hyaluronic acid value a quantitative analysis was performed based on antibody linked to an enzyme (ELISA – hyaluronic acid) which is detected by a colorimetric agent with special kit.

We determined ARN-VHC for every patient and treatment response was considered as "undetectable" three months after finishing therapy.

Antiviral therapy

According to the national guidelines for HCV infection, we followed treatment efficacy in all patients with ESRD, regardless of fibrotic stage and ARN-VHC value and we also assessed the liver fibrosis by transient elastography and hyaluronic acid.

Therapy was based on the combination of ombitasvirum + paritaprevirum + ritonavirum with 2 pills taken in the morning with food and 1 pill of dasabuvirum in the morning and 1 during the evening with food. Treatment therapy lasted for twelve weeks, time when the patient was monitored. We considered response to therapy the result of ARN-VHC after three months after therapy, moment where patients had another fibrosis assessment procedure by Fibroscan and hyaluronic acid.

2. Results and Discussions

HCV infection is associated with a high morbidity and mortality rate, consecutive to liver disease. Public involvement is on a growing trend, because HCV may evolve to a cirrhotic stage and also to CHC but is also well known that may be associated with extraintestinal manifestations such as autoimmune, metabolic, renal, cardiovascular neurologic or even limfoproliferative situations. Not only that HCV is associated with CKD but also it accelerates renal deterioration by rapidly evolving to ESRD with dialysis. Moreover, morbidity and mortality will rise and patients will require organ transplant

One of the major objectives of response to therapy is considered the severity assessment of liver disease by determining the fibrotic stage, which will allow treatment individualization by choosing the correct treatment and its length. However, when the subjects are patients with CKD this might be challenging.

Our study was a prospective one in which we focused on ESRD HCV patients, by highlighting individual characteristics and disease particularities by assessing liver disease and necessary therapy so that prognostic may be improved.

Transient elastography was the reference method and permitted noninvasive assessment of liver disease. Thus, we have included a larger number of patients by eliminating the reticence of liver biopsy, technique which may associated with adverse events. By using it we could easily assess the liver disease before and after ESRD as well as after antiviral therapy.

We observed that the highest modifications were recorded within patients with a low level of fibrosis when compared with the ones with an advanced stage. We identified the fact that some patients did not present a liver disease but only chronic HCV infection and immediately after dialysis the Fibroscan values were decreased. This may be happening due to the fact that CKD in the dialysis stage after retrieval of 2,5 l of fluid, a decrease of EI was observed. When comparing F3 and F4 stages, no significant decrease was observed.

Another relevant step was after measuring the hyaluronic acid. This was supposed to come as an easy method to express liver disease in patients with HCV in order to enlarge the noninvasive horizon of available tests. The particularity in this situation represents the fact that plasmatic values of hyaluronic acid may be influenced by CKD. This may be encountered due a permanent inflammatory reaction caused by the dialysis process. Testing hyaluronic acid on the

ESRD and HCV patients showed high values especially in high stage fibrosis, thus validating the test.

The use of hyaluronic acid in combination with other noninvasive techniques with the purpose to assess liver fibrosis may represent an important option pe disease evolution prognostic. Thus, the fact that hyaluronic acid is synthetized within the stellate liver cells and degraded by the endothelial sinusoidal cells will reflect a new perspective for liver fibrosis differentiation.

ESRD and HCV therapeutic management is rather similar with the ones with a normal renal function, therefore these patients do not require important changes in their medication. Traditionally, patients are treated with DAA which involve sofosbuvir and NS5A inhibitor with a treatment duration of 12 weeks regardless of fibrosis stage.

HCV infection has become curable, which was also confirmed in our study which pointed out no ARN VHC levels after finishing the treatment. Choice of treatment was made according to European guidelines of HCV therapy and according economic considerations taking into account the fact that was fully reimbursed by state authorities. All patients followed a three months therapy, and worth mentioning is that none of them required the use of ribavirin.

We measured the fibrosis before and after the therapeutic regime. Several studies have suggested fibrosis regression after therapy by using noninvasive methods. In our study we observed that a decrease of values of methods involved were encountered in patients with liver fibrosis and also cirrhosis. However, this does not directly mean that a fibrosis regression occurred but an inflammation decreased especially in the first month after therapy. Also, the use of hyaluronic acid with lower levels encountered after therapy might suggest regression, although for validation a larger number of patient should be used.

3. Conclusions

HCV is a major health problem and may evolve to chronic liver disease, cirrhosis and even HCC;

Liver cirrhosis evolution involves as a first step the presence of fibrosis and its assessment is of major importance for HCV management;

Our study focused on the management of ESRD HCV infected patients by monitoring patients and assessing their evolution before and after therapy;

ESRD and HCV relationship highlights the necessity of multidisciplinary approach between nephrologist and gastroenterologist so that HCV may be eradicated in this type of patients and thus offering a long monitoring period. The advantages have appeared along with DAA drugs;

The study points out the need of introducing this treatment in patients with ESRD to prevent future complications as well as obtain a better quality of life for patients that associate hepaticand extrahepatic manifestations.

HCV eradications is considered an important challenge and will require a more implication and a multidisciplinary approach so that both patient and clinician have direct access to therapy and thus prevent infection.

Along with every DAA drug, management options extend. Patients that suffered from previous interferon management failure will benefit of new combination drugs. Although HCV antibodies are present in chronic infection and considered nonfunctional, the immune process allows a large number of patients to obtain remission after an acute infection may be used to create a HCV vaccine.

With this study we tried to focus on a specific group of patients with HCV infection that by associating ESRD have different characteristics. Thus, we suggested a new approach for this type o patients starting from diagnosis monitorization and therapeutic approach.

References

1. Gao, B., W.I. Jeong, and Z. Tian, *Liver: An organ with predominant innate immunity.* Hepatology, 2008. **47**(2): p. 729-36.
2. Szabo, G., P. Mandrekar, and A. Dolganiuc, *Innate immune response and hepatic inflammation.* Semin Liver Dis, 2007. **27**(4): p. 339-50.
3. Paik, Y.H., R.F. Schwabe, R. Bataller, M.P. Russo, C. Jobin, and D.A. Brenner, *Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells.* Hepatology, 2003. **37**(5): p. 1043-55.
4. Seki, E., S. De Minicis, C.H. Osterreicher, J. Kluwe, Y. Osawa, D.A. Brenner, and R.F. Schwabe, *TLR4 enhances TGF-beta signaling and hepatic fibrosis.* Nat Med, 2007. **13**(11): p. 1324-32.
5. Guo, J., J. Loke, F. Zheng, F. Hong, S. Yea, M. Fukata, M. Tarocchi, O.T. Abar, H. Huang, J.J. Sninsky, and S.L. Friedman, *Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of Toll-like receptor 4 to hepatic stellate cell responses.* Hepatology, 2009. **49**(3): p. 960-8.
6. Huang, H., M.L. Shiffman, S. Friedman, R. Venkatesh, N. Bzowej, O.T. Abar, C.M. Rowland, J.J. Catanese, D.U. Leong, J.J. Sninsky, T.J. Layden, T.L. Wright, T. White, and R.C. Cheung, *A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C.* Hepatology, 2007. **46**(2): p. 297-306.
7. Watanabe, A., A. Hashmi, D.A. Gomes, T. Town, A. Badou, R.A. Flavell, and W.Z. Mehal, *Apoptotic hepatocyte DNA inhibits hepatic stellate cell chemotaxis via toll-like receptor 9.* Hepatology, 2007. **46**(5): p. 1509-18.
8. Gabele, E., M. Muhlbauer, C. Dorn, T.S. Weiss, M. Froh, B. Schnabl, R. Wiest, J. Scholmerich, F. Obermeier, and C. Hellerbrand, *Role of TLR9 in hepatic stellate cells and experimental liver fibrosis.* Biochem Biophys Res Commun, 2008. **376**(2): p. 271-6.
9. Marra, F., *Chemokines in liver inflammation and fibrosis.* Front Biosci, 2002. **7**: p. d1899-914.