

**UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL**

DOCTORAL THESIS

**CURRENT METHODS FOR THE DIAGNOSIS AND
PROGNOSIS OF HEPATOCELLULAR CARCINOMA**

- ABSTRACT -

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GENERAL PART (STATE OF KNOWLEDGE)

CHAPTER I – HEPATOCELLULAR CARCINOMA EPIDEMIOLOGY

Hepatocellular carcinoma (HCC), a primitive malignant tumor of the liver, ranks as the fifth most frequent malignancy in men and the ninth in women, according to a 2012 estimate of the International Agency for Research on Cancer. HCC is the second most frequent cause of death caused by cancer worldwide, with over 700.000 annual cases. Currently, the highest incidence of HCC is found in East and South-East Asia, as well as in Central and West Africa, reaching values of approximately 15-20/100.000 in men and 10/100.000 in women, whereas in areas with a high human development index, such as North and South America, Australia, Northern and Western Europe, its incidence is lower than 7,5/100.000 in men and 2,5/100.000 in women. In Romania, Eastern and Southern Europe, incidence rates have intermediate values (approximately 10/100.000 in men and 3/100.000 in women).

CHAPTER II – MORPHOPATHOLOGICAL AND PATHOGENIC ASPECTS OF HEPATOCELLULAR CARCINOMA

Recent studies suggest that the initiation and progression of hepatocarcinogenesis might be influenced by various stressors belonging to the liver microenvironment. Thus, a series of common mechanism have been proven to alter the function and structure of hepatic cells. Chronic inflammation is one of the main factors which contribute to the initiation and progression of hepatocellular carcinoma. In almost all cases, chronic inflammation, most frequently produced by the hepatitis B or C virus and alcohol abuse, precedes the development of HCC. Over time, they generate fibrosis of the hepatic tissue and the formation of fibrous bands, which begin to incorporate the hepatic lobules, thus leading to cirrhosis. Hepatic cirrhosis represents the main etiological factor for the presence of hepatocellular carcinoma. The sequence of processes in the cirrhotic liver that lead to HCC includes the transformation of regeneration nodules into low-grade and afterwards high-grade dysplastic nodules, which initially evolve towards well-differentiated HCC and may in time become poorly differentiated HCC. At the moment, the accepted theory is that of multistage hepatocarcinogenesis, although perfect animal models for the cirrhotic liver are still unavailable.

Typically, hepatocellular carcinoma has a soft consistency with a heterogeneous macroscopic structure and is polychromatous due to hemorrhagic and necrotic areas. Regarding size, HCC can vary between 1 cm and 30 cm in diameter, usually having smaller

dimensions in the cirrhotic liver than in the absence of fibrosis. Classically, three macroscopic types are described: nodular, infiltrative and diffuse. Histologically, hepatocellular carcinoma is a well vascularized tumor with wide trabeculae, discrete cellular alteration, atypical cytology, increased mitotic activity, vascular invasion, absence of Kupffer cells and of any reticular networks. The most frequent cellular growth patterns are: the trabecular pattern, similar to normal hepatic tissue, with tumorous cell arranged in cellular bands of varying size, separated by sinusoid capillaries; the pseudoglandular pattern, a result of biliary canaliculi dilation or the central destruction of trabeculae; the compact pattern produced by trabeculae compacting.

CHAPTER III – DIAGNOSIS AND PROGNOSIS OF HEPATOCELLULAR CARCINOMA

The clinical presentation of a patient with HCC is highly variable depending on the stage of the disease. Typical symptoms of advanced hepatocellular carcinoma include those common to all malignancies, such as lack of appetite, weight loss and fatigue and are accompanied by abdominal pain and nonspecific digestive symptoms: diarrhea, constipation, meteorism. Pain usually appear in the context of tumorous hepatomegaly through the distention of Glisson's capsule and is frequently the first symptom that determines the patient to consult a doctor. Taking into consideration that most primitive hepatic malignant tumors occur in the cirrhotic liver, any deterioration of patient baseline health status may raise the suspicion of HCC. In advanced cases, liver palpation can reveal a painful enlargement of the liver, which can have an irregular, nodular surface, with increased consistency in the presence of liver cirrhosis.

A biomarker with clinical value must fulfill several requirements, such as to differentiate between normal and malignant cells, to be organ specific, to correlate with tumor staging and to have prognostic value, high sensitivity (Se) and specificity (Sp), non-invasiveness and low costs. Currently, there are a number of molecules with diagnostic value, which are also used for treatment surveillance: alpha fetoprotein, alpha fetoprotein lens culinaris or des- γ -carboxyprothrombin.

HCC characteristics in multi-detector computed tomography (MDCT) are currently well-defined, tumors being hypervascular in the arterial phase with wash-out in the portal or late phases. Hepatocellular carcinoma can be surrounded by a hyperenhanced capsule, may present non-enhancing central necrosis or intratumoral hemorrhages. In advanced HCC,

MDCT may find images of portal invasion which presents itself as a filling defect in the portal veins, of capsular invasion or that of adjacent structures.

At this moment, gadoxetic disodium acid (hepatocyte specific contrast agent) enhanced magnetic resonance imaging (MRI), is considered the option of choice for hepatic lesions that are less than 2 cm due to its capacity to pass into the hepatobiliary tract. The use of this contrast agent allows a dual characterization of a hepatic lesions by adding a supplementary phase, the biliary phase, to the three standard vascular phases.

Contrast enhanced ultrasound (CEUS) provides characterization of focal liver lesions based on their behavior during the three phases of contrast enhancement: the vascularization pattern during the arterial phase and the presence or absence of wash-out during the portal or late phase. The distinctive appearance of HCC during CEUS is that of a hypervascular lesion in the arterial phase with wash-out either in the portal phase or in the late phase.

Quantitative contrast enhanced ultrasound consists of the analysis of time-intensity curves generated based on CEUS recordings and is a promising method of associating objective data to subjective image examination. Time-intensity curves are created for representative sequences, which contain clear frames of the hepatic tissue being studied. In order to describe these curves, a series of parameters have been established: the area under the curve (AUC), maximum intensity (IMAX), perfusion index (PI), rise time (RT), time to peak (TTP), rise slope (RS) and wash-out time.

The LI-RADS score (Liver imaging reporting and data system) was first introduced in 2011 with the purpose of standardizing CT and MRI image interpretation in patients with an increased risk of developing hepatocellular carcinoma. Piscalia et al. proposed to introduce a modified version for CEUS of the LI-RADS score in order to reduce diagnostic errors and interoperator variability. According to this classification the LR-1, LR-2, LR-M, LR-5V maintained their significance, but LR-3, LR-4, LR-5 were adapted to suit the specifics of contrast enhanced ultrasound.

Tumor resection represents the elective treatment for HCC in non-cirrhotic patients, with a 5-year survival rate of approximately 60-80%. In the case of cirrhotic patients, the prognosis is dependent on the degree of hepatic dysfunction, evaluated through the Child-Turcotte-Pugh (CTP) score, albeit with the risk of underestimating the severity of the subjacent hepatopathy. Presently, liver transplantation offers excellent results in patients with limited tumor extension according to the Milan criteria (HCC smaller than 5 cm or less than 3

nodules smaller than 3 cm). The 1-year survival rate surpasses 85%, while the 5-year survival rate is approximately 75%, with a recurrence rate of 10%. Until recently patients with viral hepatitis C associated HCC presented a lower survival rate than those with HCC of different etiology, due to a lack of efficient treatment for the underlying viral infection. However, new interferon free therapies have significantly improved these rates.

Trans-arterial chemoembolization (TACE) induces tumor necrosis with response in a percentage of patients that varies between 15% and 55%, thus slowing the tumor progression and preventing vascular invasion. Generally, the average survival of patients that benefit from TACE therapy for HCC is about 20 months, longer than those treated conservatively. As far as systemic anti-tumor therapy is concerned, at the moment, the only chemotherapy agent available for the treatment of HCC is Sorafenib, a tyrosine kinase inhibitor that prolongs lifespan with 4 months on average.

SPECIAL PART (PERSONAL CONTRIBUTION)

OBJECTIVES

The main objectives of this doctoral thesis are focused on evaluating the diagnostic performance of contrast enhanced ultrasound in hepatocellular carcinoma, as well as developing a prognostic model for patients with cirrhosis and hepatocellular carcinoma. Thus, regarding CEUS, the present study is meant to estimate the diagnostic capacity of this imaging technique, which was recently introduced in the characterization and diagnosis of HCC. The novelty of study I of this thesis is the introduction of the 2016 LI-RADS CEUS score, which was initially developed for CT and MRI. Moreover, the question was raised if a quantitative analysis of contrast enhanced ultrasound could positively influence HCC diagnosis. Consequently, there is a preference for a multimodal approach based both on current imaging methods, as well as clinic and laboratory findings. The second study aims to discover markers or prognostic scores for patients with HCC with emphasis on the already existing classifications, such as Child-Turcotte-Pugh for cirrhosis and Barcelona-Clinic Liver Cancer for HCC. Taking into consideration that prognosis depends on indicating the correct therapy and on its curative potential, an important objective of this research was to evaluate the survival of patient in relation to the therapy that was chosen.

MATERIAL AND METHODS

The doctoral thesis included two groups of patients, in a mixt, retrospective and prospective study, who presented to the Gastroenterology Clinic of Craiova Clinical Emergency County Hospital, or to the Gastroenterology and Hepatology Research Center of

Craiova, and were followed during a 5-year period between 01.07.2010 – 01.07.2016. The first group comprised of 132 patients with or without underlying hepatic disease, that were diagnosed with a focal liver lesion either during a routine check-up or as part of a screening program for those with liver cirrhosis. Patients that presented only biliary cysts were excluded, as this lesion is rarely misdiagnosed, together with those that presented multiple hepatic metastases in the setting of an already documented malignant neoplasia. The second group comprised of 78 patients, that presented to the Gastroenterology and Hepatology Clinic of “Antoine Béclère” Hospital in France and who were included in the study with the its permission as part of a international exchange program sponsored by the POSDRU/159/1.5/136893 project. All the patients of group II were followed over a period of 5 years while monitoring clinical, laboratory and imaging parameters in order to determine their prognosis and 5-year survival.

Regarding data processing, the IBM - Statistical Analysis Software Package was used to provide a descriptive analysis of the study groups. Furthermore, the program was also used to calculate the diagnostic performance of CEUS through the following parameters: sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Kaplan-Meier survival curves were generated taking into consideration the presence of ascites, hepatocellular insufficiency, for every CTP and BCLC class and for every type of treatment underwent.

The quantitative analysis of CEUS recordings was made using Image Pro-Plus and contrast agent arterial phase uptake curves were drawn for three types of lesions: HCC, hepatic hemangioma and focal nodular hyperplasia (FNH). The curves were created with reference to normal hepatic tissue, at the same depth of liver parenchyma, obtaining, in fact, a representation of the surplus in contrast agent uptake of the lesions compared to the surrounding liver.

Study I – Diagnostic performance of CEUS in hepatocellular carcinoma

The HCC patient subgroup included 15 women and 38 men with ages between 43 and 78 years, with an average of $63,65 \pm 8,68$ years.

Regarding CEUS sensitivity, the highest values were calculated for focal liver steatosis (Se = 97,9%), focal fatty sparing (Se = 91,6%) and focal nodular hyperplasia (Se = 97,9%). In the case of malignant lesions, for HCC the sensitivity was 83,3%, whereas for liver metastasis it was 81,7%. A similar value (Se = 83,1%) was obtained for hepatic

hemangioma, while CEUS sensitivity for regeneration nodules was only 68,6%. Together with increased sensitivity, CEUS also presented high diagnostic specificity for focal fatty sparing (Sp = 96,3%) and for focal liver steatosis (Sp = 92,4%). In a hierarchy of focal liver lesions based on CEUS specificity, intermediate positions are taken by hepatic hemangioma (Sp = 91,2%), HCC (Sp = 90,3%) and hepatic metastasis (Sp = 89%), while regeneration nodules (Sp = 88,4%), liver abscess (Sp = 88,4%) and FNH (Sp = 86,8%) occupy the lowest positions. The results support a national multicentric study that established a series of reference values for CEUS sensitivity, specificity, positive predictive value and negative predictive value. Despite these values being over 85% for the majority of focal liver lesions included in the study, there are still challenges in differentiating between regeneration nodules and hepatocellular carcinoma lesions.

For the CEUS LI-RADS score, sensitivity and specificity were calculated in three situations: all lesions with a LI-RADS score higher than LR-3 were considered HCC, lesions with a score higher than LR-4 was considered HCC or only LR-5 was equivalent to HCC. This approach allows the setting of a cut-off value, over which the diagnosis of HCC can be placed based on the LI-RADS score. To this purpose a ROC curve was generated in order to establish a relationship between the sensibility and sensitivity depending on the value of the LI-RADS score. An area under the curve of 0,653 ($> 0,5$) confirms the fact that the results of the analysis is statistically significant. The LR-5 score taken separately has a low sensitivity (53,4%), but a high specificity (85,3%), whereas, when considering all three scores LR-3, LR-4, LR-5 diagnostic for HCC, the values are reversed: high sensitivity (95,7%) and low specificity (20,3%). An optimum equilibrium between Se and Sp is obtained by associating LR-4 with LR-5, meaning that o lesions that receives these scores is a HCC with a sensitivity of 80,7% and a specificity of 78,7%. This study uses for the first time the complete CEUS LI-RADS score on a group of 131 patients with 178 lesions. Taking into consideration this score was only recently introduced, studies that evaluate its diagnostic capacity are important in further developing and improving its performance.

In quantitative CEUS, FNH presents the fastest (TTP = $9,6 \pm 3,9$ s) and the most intense enhancement (IMAX = $129 \pm 35,4\%$ more intense by comparison to normal liver parenchyma at the same depth). The rise slope, which represents the ratio between IMAX and TTP, illustrates the abrupt increase from 0 to IMAX and a high value ($13,4 \pm 4,1$ pentru FNH) if another indicator of intense arterial hyperenhancement. From this point of view HCC ranks second, also presenting fast contrast agent uptake (TTP = $14,5 \pm 4,2$ s) and

intense hyperenhancement ($IMAX = 109 \pm 19,6\%$) with a RS of $7,5 \pm 3,6$. The slowest uptake is seen in hepatic hemangioma due to its characteristic enhancement pattern with initial peripheral hyperenhancement which extends centrifugally until the entire lesion becomes isoenhanced. This particular behavior explains the lower IMAX values in quantitative CEUS ($48 \pm 21,9\%$), as well the long time period necessary to obtain IMAX ((TTP = $24,3 \pm 6,1$), with a very low rise slope (RS = $1,9 \pm 1,5$). In the field of quantitative CEUS, there are only a few studies regarding its applications in distinguishing between hepatocellular carcinoma and focal nodular hyperplasia based on contrast agent uptake parameters, probably due to limited standardization, which makes comparative analysis of recordings difficult. Presently, to our knowledge, a quantitative CEUS characterization of hepatic hemangioma has not been published and therefore the current data can improve the diagnostic accuracy of CEUS.

Study II – Evaluating hepatocellular carcinoma prognosis

Study II included 78 patients, 16 women and 60 men, aged between 42 and 86 years, with an average of $69,12 \pm 9,86$ years.

Kaplan-Maier curves were generated for patients with HCC, that presented hepatic insufficiency at the moment of diagnosis and for those without hepatic insufficiency (HI). According to the resulting chart, the percentage of patients without liver insufficiency that were alive at any given time was higher than those with liver insufficiency over the course of the entire 5 year period. Regarding the survival period of each subgroup, subjects with HI died on average after 2,17 years after HCC diagnosis with a median value of 1,41 (meaning after 1,41 years the percentage of patients that were still alive was 50%), whereas those without HI lived on average 3,07 years with a median of 3,43 years. Survival surpassed 5 years in 38,4% of patients without HI, while in the other subgroup only 17,9% lived over 5 years from the moment of HCC diagnosis.

The same statistical method was applied for patients with or without ascites (clinically or through imaging methods) at the moment of HCC diagnosis. Thus, according to the Kaplan-Meier curves, over the course of almost the entire 5 year period, the percentage of patients without ascites that were alive at any given moment was higher than of those that presented with ascites. The average survival of subject with ascites at the initial moment of diagnosis was 1,06 years with a median value of 1,08 years, while in the case of those without ascites the average was 2,93 years with a median of 3,26 years. Of

the patients that presented ascites at the moment of HCC diagnosis only 11,1% survived more than 5 years, while in the other subgroup the percentage reached 33,3%.

Concerning the evaluation of prognosis based on CTP score, Kaplan-Meier curves were generated for each class (A, B and C). The best survival was obtained by patients with CTP A, followed by those with CTP B, whereas the worst prognosis was observed in patients CTP C. The average survival from the moment of HCC diagnosis for class CTP A was 3,12 years with a median of 3,53 years, for class CTP B the average was 2,93 years with a median of 3,26 years, whereas for class CTP C the figures were significantly lower with an average of 0,38 years and a median of 1,42 years. The percentage of class CTP A patients that survived over 5 years was approximately 21,7% compared to only 12,8% of those class CTP B, whereas all class CTP C patients died during the first 6 months.

The evaluation of patient prognosis in HCC is based on the BCLC classification. Average survival of patients with BCLC 0 after diagnosis was 4,39 years with a median of 5 years, with the majority of them (64,1%) having a lifespan of over 5 years. BCLC A patients lived on average 3,12 years with a median value of 3,62 years, for BLCL B patients the average was 1,92 years with a median of 2,67 years, whereas for those with BCLC C the average survival rate was 1,92 years with a median of 1,09. The percentage of patients with BLCL 0 that surpassed the 5 years survival mark was 64,1%, for BCLC A patients the percentage was 39,7% and for BCLC B 10,2%. Patients with a BCLC score of C or D died before the end of the 5 year surveillance period.

The average survival period of patients that underwent surgical treatment was 3,16 years, with a median value of 3,45 years (44,9% were still alive 5 years after diagnosis), while for those that did not benefit from this type treatment it was 1,59 years with a median of 0,89 years (13,3% were still alive 5 years after diagnosis).

In order to determine the impact of trans-arterial chemoembolization on prognosis, the statistical analysis was performed only on the patient subgroup that did not benefit from surgery. In the absence of palliative treatment by TACE, the longest survival rate was 3 years and 7 months with an average of 1,25 years, in contrast with those that were treated, who lived on average 3,07 years with a median of 3,26 years. The 5 year survival rate in the TACE subgroup was 27,2%, whereas the patients treated with sorafenib or only with symptomatic treatment did not surpass the 4 year survival mark.

In each of the above mentioned situations, the value of p as resulted by applying the chi-square Mantel-Cox test was lower than 0,001, which confirms that the difference between the studied subgroups was highly significant.

CONCLUSIONS

Statistical analysis of CEUS diagnostic performance revealed a sensitivity of over 90% for focal hepatic steatosis, focal fatty sparing and FNH. In the case of malignant lesions, HCC and hepatic metastasis, sensitivity was approximately 80%. CEUS presented a high specificity in the diagnosis of focal hepatic steatosis and focal fatty sparing and only a slightly lower specificity for HCC and hepatic hemangioma, while for hepatic metastases, regeneration nodules, hepatic abscess and FNH, it was under the 90% threshold.

Optimal sensitivity and specificity values for CEUS LI-RADS score in HCC diagnosis were obtained when both LR-4 și LR-5 were considered diagnostic for HCC.

CEUS quantitative analysis revealed that FNH presents the fastest and most intense enhancement, followed by hepatocellular carcinoma, while the slowest contrast substance intake was seen in the case of hepatic hemangioma.

Survival surpassed 5 years in 38,4% of patients without hepatic insufficiency, while in the other subgroup only 17,9% lived over 5 years from the moment of HCC diagnosis. Of the patients that presented ascites at the moment of HCC diagnosis only 11,1% survived more than 5 years, while in the other subgroup the percentage reached 33,3%.

The best survival was obtained by patients with CTP A, followed by those with CTP B, whereas the worst prognosis was observed in patients CTP C.

The percentage of patients with BCLC 0 that surpassed the 5 years survival mark was 64,1%, for BCLC A patients the percentage was 39,7% and for BCLC B 10,2%. Patients with a BCLC score of C or D died before the end of the 5 year surveillance period.

The average survival period of patients that underwent surgical treatment was 3,16 years and the 5-year survival rate was 44,9%, while for those that did not benefit from this type treatment it was 1,59 years with survival rate of 13,3%.

The 5 year survival rate in the TACE subgroup was 27,2%, whereas the patients treated with sorafenib or only with symptomatic treatment did not surpass the 4 year survival mark.