ROLE OF DIFFERENT CT TECHNIQUES IN GRADING OF LIVER FIBROSIS IN VIRAL HEPATITIS PATIENTS

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ABSTRACT

This study aims to evaluate the role of perfusion CT in grading of liver fibrosis, seeking for a simple tool that may be helpful in evaluation of a very common problem. Fifty one hepatitis C patients applying for Egyptian ministry of health antiviral therapy (Peginterferon alfa-2b 1.5 mcg/kg weekly + Ribavirin 800 mg daily) were included in the study. All the group were subjected to the routine clinical assessment, lab and imaging protocol. Lab assessment included CBC, AL, AST, liver and kidney function tests, HBsAg, HCV Ab, HCV PCR, abdominal ultrasound, and true cut liver biopsy. Perfusional CT scan was added and perfusional parameters was calculated. These results show that each of portal perfusion, total hepatic perfusion and transit time can be used to differentiate mild from moderate liver fibrosis. The best single factor was portal hepatic perfusion. Using portal hepatic perfusion value of 102 ml /min/100ml showed a sensitivity of and specificity of 83%. At this value efficiency of the test is about 80%.

Keywords: *liver fibrosis, CT, HCV, perfusion*

INTRODUCTION

The frequency of hepatic fibrosis is still continuously increasing in most countries mainly owing to an increase in hepatitis C viral (HCV) infection. Chronic HCV is one of the leading causes of chronic liver disease worldwide, with estimated worldwide prevalence of the disease between 64 and 103 million infected individuals (Wedemeyer et al, 2015). The increasing availability of treatments that halt or even reverse hepatic fibrosis calls for improvements in noninvasive measures of fibrosis to permit accurate monitoring of responses to therapy (Wedemeyer et al, 2015).

Liver biopsy is the most widely accepted reference standard in the assessment of hepatic fibrosis, but it is prone to interobserver variation and sampling error, and it is associated with pain in 40% of cases and with major complications in 0.5% (Materne et al, 2000).

Current serum biomarker panels and noninvasive imaging methods are well suited for use in the identification of advanced liver fibrosis, but they are

unreliable in distinguishing between early and intermediate stages of disease where therapy is most likely to be effective (Gülberg et al, 2002).

Transient elastography (widely known as fibroscan) is gaining more popularity and acceptance nowadays as a simple and non invasive tool however this tool is limited to diagnosis of cirrhosis and significant fibrosis. It couldn't be used to diagnose minimal fibrosis. As at this stage the fibrosis is limited to the portal tracts and no significant change in liver stiffness occurs (Friederich-Rust et al, 2008).

Perfusion imaging in liver fibrosis is based on the occurrence of substantial microcirculatory changes in this disease. These changes are caused by capillarization of the sinusoids, collagen deposits in the extracellular Disse space, and contraction of activated stellate cells (Gülberg V et al, 2002).

It has been previously shown that perfusion CT can be used to detect the microcirculatory changes that occur in cirrhosis (Ronot et al, 2010).

Moreover, perfusion CT may help to discriminate between minimal and intermediate stages of fibrosis. Imaging could play a role in the assessment of hemodynamic progression and response to treatment in liver disease, thereby lessening our dependence on more invasive and costly approaches (Ronot et al, 2010).

CT has several advantages, including accessibility for patients and medical teams, low cost, high speed, high spatial and temporal resolution, a linear relationship between attenuation and contrast agent concentration, and good reproducibility (Varenika et al, 2013).

BASIC PRINCIPLES OF CT PERFUSION IMAGING

Perfusion is the transport of blood to a unit volume of tissue per unit of time and usually refers to the blood transport at the capillary level. CT perfusion is based on the increase and subsequent decrease of contrast agent concentrations in tissues as a function of time. In contrast to MRI, tissue attenuation measured with CT and expressed in Hounsfield units is directly proportional to the local concentration of contrast agent in the tissues. This fact was first reported by (Axel 1980) and (Lee et al, 1990) makes CT assessment of tissue perfusion possible. (Kim et al, 2014).

This quantitative information cannot be obtained with conventional contrast material–enhanced CT where the degree of enhancement at certain time-points (ie, arterial or portal venous phase) is just a mixed result of entering and exiting of contrast agent and thus is usually assessed CT perfusion analysis is based on several fundamental requirements. One is sequential CT scanning of the same volume over time, performed before, during, and after intravenous administration of contrast agents to trace the temporal changes in CT attenuation in the tissue volume of interest (Varenika et al, 2013).

Another requirement for perfusion CT analysis is the selection of a vessel (usually an artery) supplying the tissue of interest to obtain a time-intensity curve (the arterial input function) by placing a region of interest (ROI) into the lumen of the vessel. Unlike in other organs, for which ROI is usually placed only onto the

artery, ROIs for hepatic CT perfusion should be placed on both artery and portal vein because the liver has a dual blood supply from the hepatic artery and the portal vein. This unique dual input makes perfusion imaging of the liver challenging. This issue will be discussed in detail below. The time-intensity curve is then compared with the time-intensity curve obtained from the tissue being analyzed (Varenika et al, 2013).

A third requirement of CT perfusion analysis is the application of kinetic models to calculate various perfusion parameters in the tissues being analyzed. For liver CT perfusion, two methods can be used, maximum slope method and compartment model-based method (Varenika et al, 2013).

CT Acquisition Protocol:

The typical CT perfusion protocol consists of a precontrast image acquisition followed by dynamic image acquisitions performed sequentially after intravenous injection of an iodinated CT contrast agent (Sahani,2012) And (Miles, 2003). The baseline precontrast CT scan can serve as a localizer to select the anatomic scan range for subsequent dynamic scanning. In the case of liver imaging, the scan range should ideally include the main portal vein to allow calculation of time-intensity curves of both the abdominal aorta and the portal vein. (Kambadakone and Sahani, 2009) and (Meijerink et al, 2008).

Contrast agents should be administered in small quantities at high flow rates to obtain a short and well-defined bolus. The iodine concentration of contrast materials should not be less than 300 mg iodine per milliliter and the total iodine dose injected should be approximately within the range of 12–18 g. A contrast bolus of 30–60 mL iodinated contrast agent followed by a 50-mL saline flush at an injection rate of 4 mL/sec or greater through an 18–20-gauge antecubital intravenous cannula is recommended. The amount of contrast material should be adjusted according to the concentration of the contrast agent (Miles et al, 2012). To obtain higher contrast-to-noise ratios, contrast agents with high iodine concentrations (≥ 350 mg iodine per milliliter) are usually recommended.

After CT data acquisition, various CT perfusion parameters can be calculated by using either a model-free or a model-based approach, with the former being easier to implement. Regardless of the algorithm used, several imaging processing steps should be performed for the calculation of CT perfusion parameters. The imaging processing includes selection of arterial and portal input functions, ROI definition. The perfusion analysis of the liver is calculated differently from other organs because the liver has a dual blood supply—the hepatic artery and the portal vein. The effective time-intensity curve obtained from liver tissue is therefore a result of an overlay of both the arterial and the portal venous components. (Varenika et al, 2013)

The normal liver is predominantly supplied by the low-pressure portal vein (75%) and supplemented by high-pressure hepatic artery (25%). However, several diseases such as liver cirrhosis leads to global changes toward increased hepatic arterial blood flow and decreased portal venous flow, although the underlying mechanism is different among the diseases. In liver cirrhosis, deposition of collagen in the space of Disse and subsequent increased resistance to incoming sinusoidal blood flow are known to be responsible for the decreased portal flow,

which is counteracted by an increase in hepatic arterial flow through the hepatic arterial buffer response (Pandharipande et al, 2005). Therefore, dedicated methods that allow a separation of the arterial and portal venous components are required for liver perfusion image analysis as well as for the diagnosis of various liver diseases.

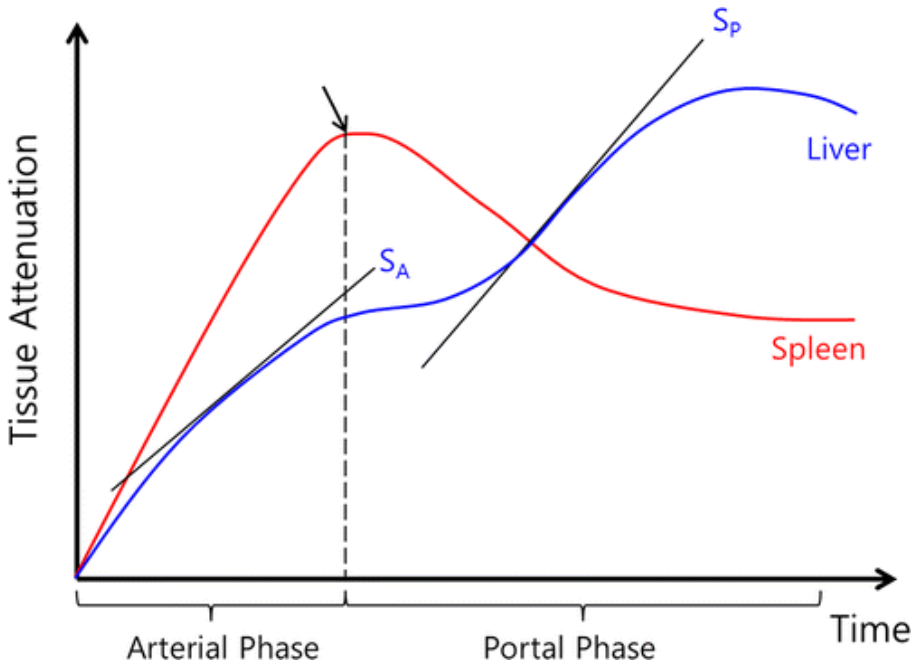

Figure 1. *Plot of time-intensity curve of spleen and liver from CT perfusion study using maximum slope method. Diagram shows how maximum slope for arterial perfusion (SA) and portal perfusion (SP) are derived. Time to peak splenic enhancement (arrow) indicates end of arterial phase and beginning of portal venous phase of liver perfusion, which is used for separating arterial and portal venous phases. Maximal slope (SA or SP) of liver time-intensity curve in each phase is divided by peak aortic and portal enhancement to calculate both hepatic arterial and portal perfusion, respectively. (Miles et al, 1993)*

In the model-free maximum slope method, time to peak splenic enhancement (the end of arterial phase and beginning of the portal venous phase of liver perfusion) is used for separating HAP and PVP. The maximal slope of the liver time-intensity curve in both the arterial and portal venous phase is divided by the peak aortic and portal enhancement to calculate arterial and portal liver perfusion (in mL/min/100 mL), respectively (Miles et al, 1998) (Fig 36). Furthermore, the HPI, which is the ratio of the arterial perfusion to the total hepatic perfusion [HPI = arterial perfusion/(arterial + portal perfusion)], can be calculated (Dugdale and Miles, 1999). However, this approach does not allow calculating other perfusion parameters, such as blood volume, or mean transit time (MTT). A major limitation of this method is that to satisfy the assumption of no venous outflow, a relatively high injection rate (15–20 mL/sec) must be used, which is not technically feasible in routine clinical practice (Dugdale and Miles, 1999).

To obtain additional perfusion parameters, several kinetic model-based approaches have been developed (Brix et al, 2010) and (Sourbron and Buckly, 2012). Table E1 (online) summarizes different kinetic models used for liver CT perfusion imaging in previous studies. Kinetic models applied to the liver vary according to the physiologic and hemodynamic assumptions made, including the following (Thng et al, 2010):

Unlike other organs, the unique dual vascular input to the liver from both the hepatic artery and portal vein makes data analysis of CT perfusion liver imaging challenging. To circumvent this problem, a single-input model for perfusion imaging of hepatic metastases has been proposed with the assumption that the vascular supply of liver metastases is predominantly arterial. However, this assumption may not hold true for all histologic types of metastases as some liver metastases may have a mixed vascular supply (Liu and Matsi, 2007) and (Koh et al, 28). It was reported that a dual vascular input model (arterial and portal venous) for analysis of CT perfusion data sets improves test-retest reproducibility (Ng et al, 2012). Indeed, the separation of hepatic arterial and portal venous blood supply in normal liver tissue and liver lesions is important for characterization and treatment response evaluation of liver nodules (Cuenod et al, 2001). For instance, when hepatocellular dysplastic nodules evolve to HCC, the intranodular portal supply decreases while the intranodular arterial supply increases in parallel with formation of unpaired arteries. Therefore, separating tumor perfusion into arterial and portal components can potentially help detect early cancerous changes in hepatocellular nodules (Varenika et al, 2013).

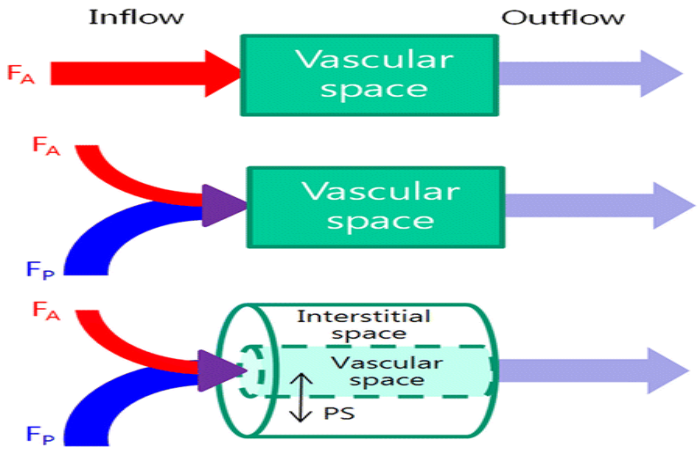

Figure 2. *Schematic diagrams show key features of single-input, dual-input, single-compartment, and dual-compartment models. Single-input model (top) assumes vascular supply to hepatic lesions is mainly from hepatic artery, although normal liver is supplied from both hepatic artery and portal vein. Dual-input model (middle) adopts physiologic status of liver which is supplied by low-pressure portal vein (75%) and supplemented by high-pressure hepatic artery (25%). Using single-compartment model (top and middle), only vascular compartment is considered. Dual-compartment model (bottom) assumes dynamic distribution of contrast agent between two compartments. Using a dual-compartment model, kinetic properties such as permeability surface area product (PS) can be quantified. (Kim et al, 2014).*

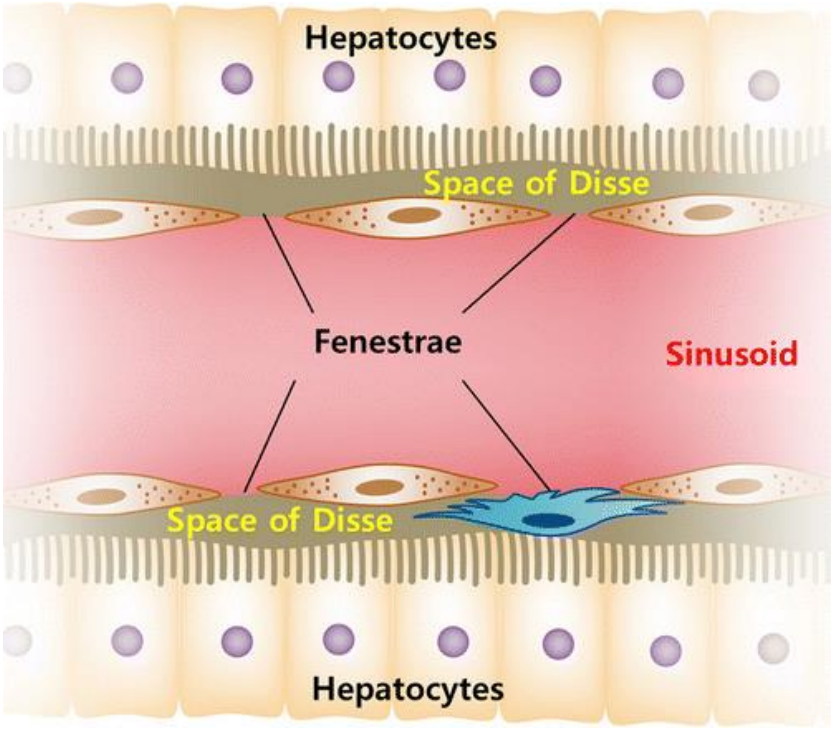

Figure 3. *Behavior of normal liver can be approximated by a single-compartment model because the space of Disse (equivalent to interstitial space of other organs) communicates freely with sinusoids through fenestrae. However, in disease states such as liver cirrhosis, deposition of collagen impedes free exchange of contrast material between the two spaces, requiring use of dual-compartment model. (Kim et al, 2014)*

Single-Compartment versus Dual-Compartment Model: Single-compartment models assume that the intravenously administered contrast agent is confined to only one compartment (ie, the vascular space), whereas dual-compartment models assume that there is dynamic distribution of contrast agent between two compartments (ie, the vascular space and the interstitial space). This assumption can be made because the space of Disse (equivalent to extravascular-extracellular space in other organs) communicates freely with the sinusoids through relatively large fenestrae (Fig 24) (Materne et al, 2000). However at advanced stages of cirrhosis this approximation may not be true owing to increased resistance to incoming sinusoidal blood flow caused by deposition of collagen in the space of Disse and altered sinusoidal architecture through the loss of fenestrae between sinusoidal endothelial cells (Koh et al, 2008).

CT Perfusion Parameters

Single-compartment models allow for estimates of blood flow, blood volume, and MTT. Blood flow refers to the volume flow rate of blood through the vasculature (expressed as mL/min/100 mL). Blood volume is the volume of blood within the vasculature that is actually flowing (expressed in units of mL/100 mL). MTT is average time it takes for blood to traverse between the arterial inflow and the venous outflow, measured in seconds (Varenika et al, 2013).

MR Versus CT Perfusion Imaging

Generally, the same principles which apply to CT perfusion are also applicable at MR perfusion. The previously described dual input single compartment model is the method utilized by most authors.

In 2003 Annet et al used 1.5 tesla scanner with a bolus of gadolinium chelate. They obtained images in axial plane using T1-weighted fast spoiled gradient-echo sequence. They combined cardiac and respiratory gating to obtain an image every cardiac beat. They calibrated the obtained enhancement levels against tubes containing different gadolinium concentrations. They calculated hepatic arterial, portal and total flow, transit time and arterial fraction in a manner comparable to CT. They reported substantial correlation between different flow parameters and severity of cirrhosis

In 2007 Hagiwara et al used 1.5 tesla scanner combined with 3D gradient aquistion protocol. They injected a bolus of gadolinium chelate and acquired images in a coronal plane using a breath hold protocol which required holding breath for 60 seconds. To facilitate this they used pure oxygen nasal prongs. They overcome the problem of non linear relationship between signal intensity and gadolinium concentration by using previously prepared tables based on in vitro experiments. They reached a temporal resolution of approximately an image every four seconds.

The problem of in vitro calibration originates from the fact that calibration needs to be repeated after any change in examination parameters. For example changes in the flip angle cause dramatic change in the signal intensity (Fig 25). More over, changes in the chemistry of contrast agent or the containing solution cause alteration of signal intensity. (Fig 26)

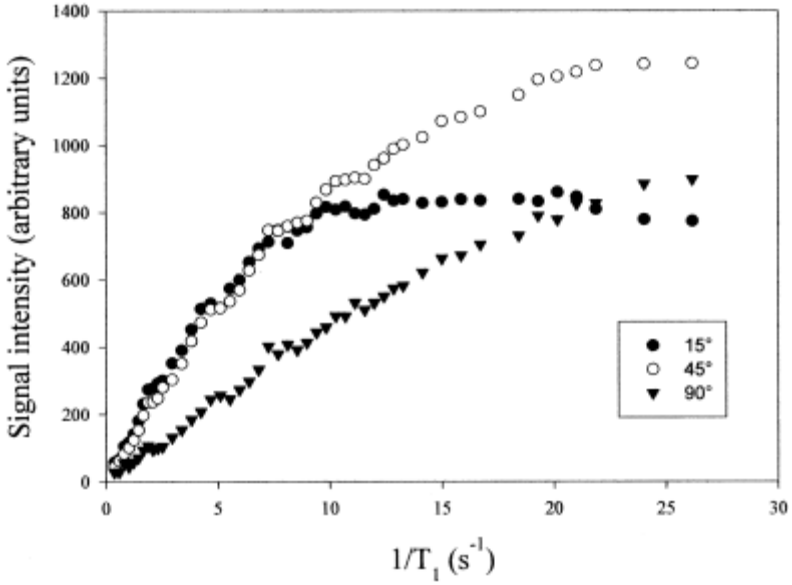

Figure 4. *Effect of flip angles (15°, 45°, 90°) on the relationship between the signal intensity of the fast T1-weighted sequence (TR/TE 6.8/2 ms, 20 cm field of view with 60% rectangular field of view, 256 × 128 matrix, preparation time 290 ms) and the 1/T1 of the tubes filled with different concentration of Gd-DTPA in saline. (Materne et al 2001)*

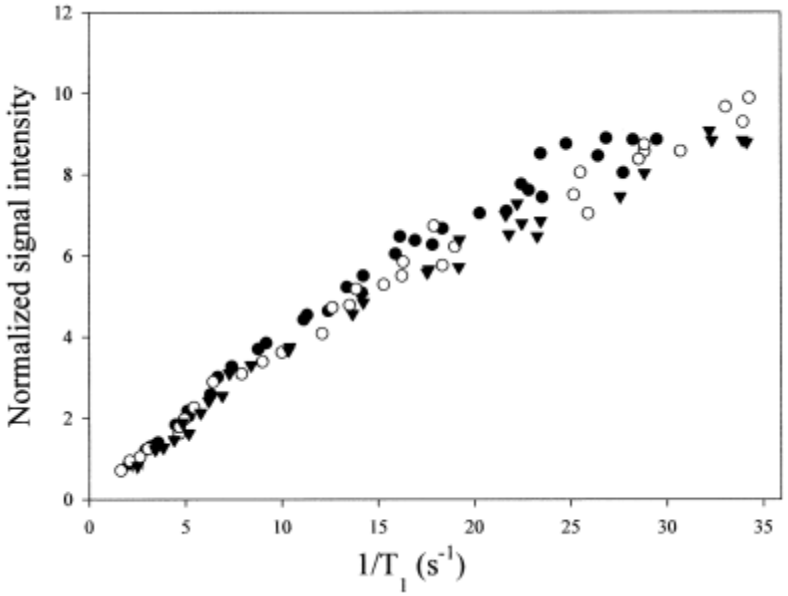

Figure 5. *Scatterplot of the relationship between signal intensity of the perfusion sequence (45°) and 1/T1 of the tubes filled with Gd-DTPA in saline (black dots), Gd-DOTA in saline (white dots), and Gd-DOTA in blood (triangles). The signal intensity of the tubes is normalized to that of the reference tube to take differences of receiver gain into account (Materne et al 2001).*

PATIENTS AND METHODS

Fifty one hepatitis C patients applying for Egyptian ministry of health antiviral therapy (Peginterferon alfa-2b 1.5 mcg/kg weekly + Ribavirin 800 mg daily) were included in the study. All the group were subjected to the routine clinical assessment, lab and imaging protocol. Assessment included CBC, ALT, AST, liver and kidney function tests, HBsAg, HCV Ab,HCV PCR, abdominal ultrasound, and true cut liver biopsy. Perfusional CT scan was the only added investigation to the routine investigation protocol. A written consent was included.

Inclusion criteria were HCV antibody positive, HCV PCR positive and age range between 17 and 50 years.

Exclusion criteria were ascites, serum creatininen level > 1.2, known allergy to contrast media, non-characterized hepatic focal lesions, bleeding tendencies and portal vein thrombosis

All patients were examined in the clinic of tropical medicine at El-Fayoum university hospital to choose those fitting the inclusion criteria and exclude patients not fit for therapy, biopsy or CT examination

The biopsies were performed at the tropical medicine departments using 16 and 18 gauge needles under the sonographic guide. Interpretation was done at external labs. All specimen were given a score of 0 to 4 according to the METAVIR score and 0 to 18 according to ISHAK score the stage of fibrosis was given a stage of 0 to 6.

Perfusional CT scan was performed at radiology department of el-fayoum university hospital using Asterion 4 slice Toshiba machine with automatic injector. The protocol of examination included.

Overnight fasting

A precontrast study of the abdomen and pelvis is performed. The study is used to assess liver morphology, and to choose the suitable section for perfusion study which typically includes the portal vein or one of its main branches. The scanning parameters was The perfusion CT protocol parameters were as follows: 80 kVp, 100 mAs, matrix, 512 X 512, slice thickness = 6 mm.

Canulation of the antecubital vein with 14 G canula. 40 ml of water soluble non ionic contrast media 350 mg /ml concentration is injected at a rate of 4 ml /min using an automated injector.

The patient is asked to breathe slowly and superficially and was informed about flushing sensation during contrast injection. Continuous imaging of the selected section is then performed at a rate of one image per second for 75 second.

The images are then transferred to a desktop computer, analyzed using a special software generated specially for this purpose. The software is based on an open source DICOM viewer published on web by Mccausland center for brain imaging, south carolina.

Three regions of interest are located at the aorta, portal vein and peripheral liver tissues. The average housefield value of the three regions is calculated and plotted against time after substraction of the basal attenuation value before contrast administration.

The curves are then fitted according to "A single compartment dual input" model proposed by Materne et al,2000 which could be summarized as following

The liver, including sinusoids, interstitium, and cells, was considered to be a single compartment; and two inflow rate constants, k1a and k1p (aorta and portal vein, respectively) were used because the liver receives its blood supply from both vessels. One outflow rate constant, k2, was also included in the model, resulting in the following equation:

$$\frac{dC_L(t)}{dt} = k_{1a}C_a(t) + k_{1p}C_p(t) - k_2C_L(t), \quad (1)$$

where Ca(t), Cp(t), and CL(t) represent the concentration versus time curves from the aorta, portal vein, and liver compartments. Because the contrast agent does not enter the RBCs, the time series Ca(t) and Cp(t) were divided by one minus the hematocrit. Solving for CL(t) and adding two delay parameters, τa and τp, which represent the transit time from the aorta and the portal vein to the liver ROI, we obtain:

$$C_L(t) = \int_0^t [k_{1a}C_a(t' - \tau_a) + k_{1p}C_p(t' - \tau_p)]e^{-k_2(t-t')}dt', \quad (2)$$

where t′ is a dummy integration value. An unweighted least squares fit was performed for the parameters k1a, k1p, and k2. The measurement of k1a + k1p reflects liver perfusion as:

$$k_{1a} + k_{1p} = F \cdot E, \qquad (3)$$

where F (mL · min-1 · 100 mL-1) is liver perfusion and E is the extraction fraction. The extraction fraction was assumed to be 1.0 in the liver. In addition, the arterial fraction of liver perfusion (%) was calculated as 100 · k1a / (k1a + k1p). The distribution volume (%) of the contrast agent was calculated as 100 · (k1a + k1p) / k2. The mean transit time (seconds) was calculated as 1 / k2.

The Matlab software is then used to perform the curve fitting process.

RESULTS

The studies were performed between feb 2014 and feb 2015. After reaching the targeted number of patients, the study was ended. Fortunately this was before the introduction of sovaldi and other oral antiviral therapies as the liver biopsy was removed from the governmental guidelines for initiation of antiviral therapy.

During this period 51 patients was included in the study. Thirty male and twenty one female, although the HCV has no sex predilection it looks that males seek therapy more than females.

The age range was between eighteen and fifty one. Patients with METAVIR score 0 or 1 have mean age of 30.6 and SD of about 7.1. Patients with score 2 have mean age of 33.7 with SD about 10.1. Patients with metavir score of 3 or 4 have mean age of 38.4 and SD of about 10.7. A result which may reflect the progressive nature of the disease.

Table 1. *Mean and standard deviation of age of different fibrotic stages*

Metavir score	Mean age (years)	SD (years)
0 or 1	30.6	7.1
2	33.7	10.1
3 or 4	38.4	10.7

The group shows ALT level of average 2.1,SD = 0.83. Eight patients have within normal ALT levels. The group shows AST level of average 1.4, SD = 0.48. Twelve patients have normal levels. The average Bilirubin level was about 2.0 with SD 0.81. seven patients have normal levels. Four patients showed completely normal liver enzymes and total bilirubin levels. The grade of fibrosis according to Metavir score shows no correlation with any of these individual tests.

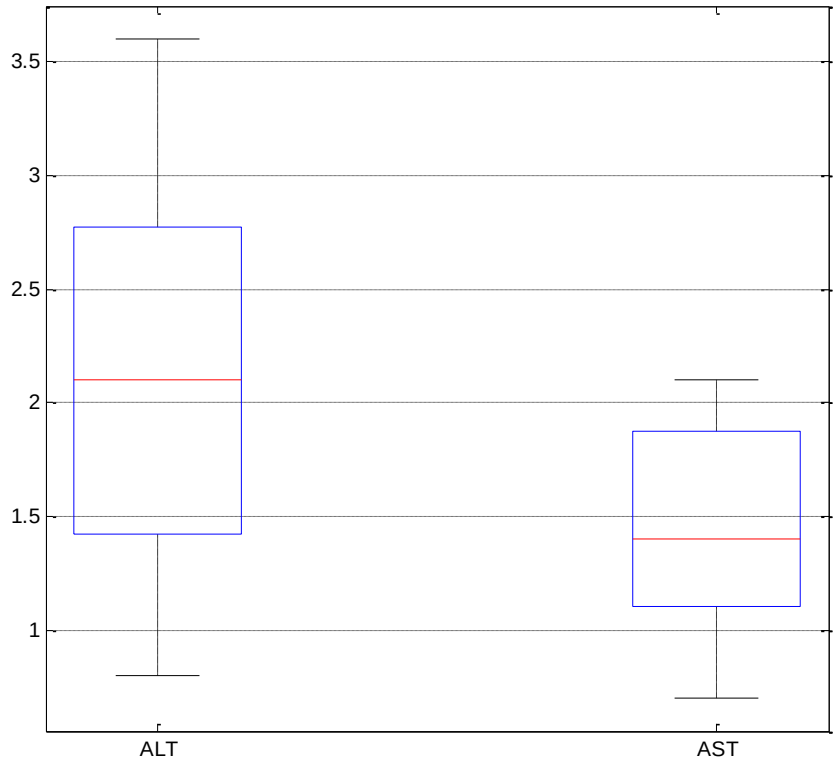

Figure 6. *Box plot of ALT and AST as times of normal of the entire group*

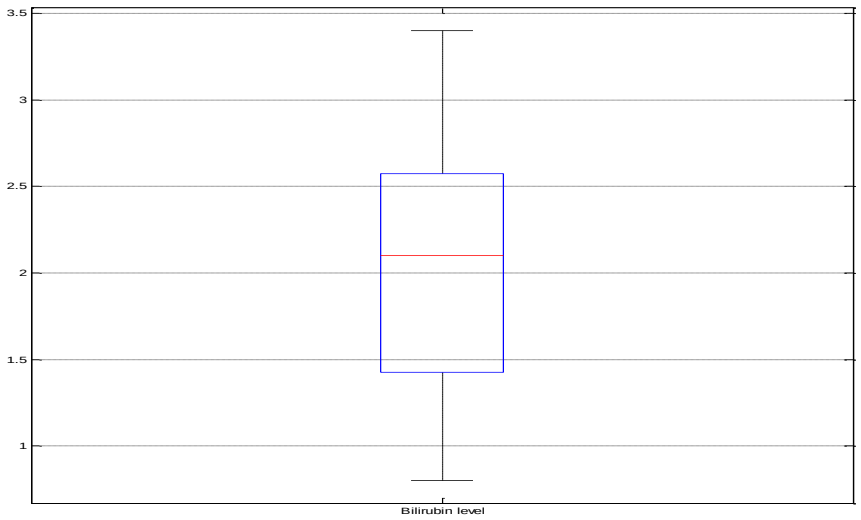

Figure 7. *Box plot of bilirubin level o the entire group*

All patient underwent ultrasound guided liver biopsy. The number of portal tracts was between 3 and 17 with average 11.6 and SD = 5.1.

The specimen showed variable degree of steatosis 9 showed no steatosis, 12 showed mild steatosis 20 showed moderate and 10 showed severe degree.

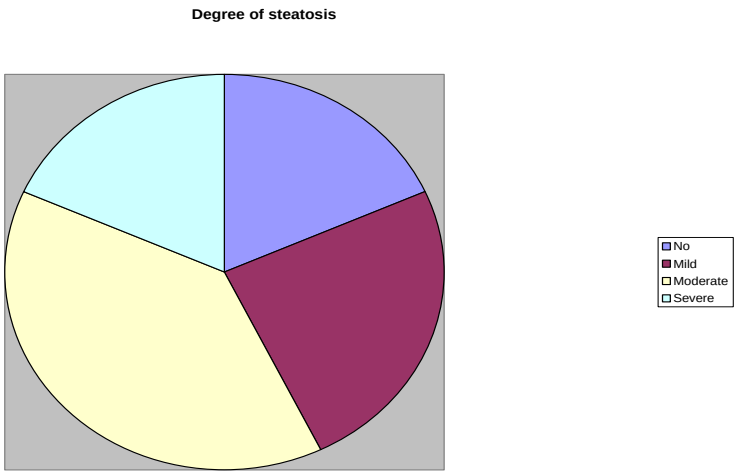

Figure 8. *Distribution of the degree of steatosis in the study group*

The degree of steatosis shows good correlation with the mean Hounsefield unit of the selected hepatic region of interest which is a well understood phenomenon. Moreover the degree of steatosis shows good correlation the precontrast standard deviation of value selected hepatic region of interest. A parameter which probably compare the density of hepatic parenchyma with the internal minute portal tracts. If the density of the hepatic parenchyma is similar to the portal tracts the CT numbers of the region will be more homogenous with smaller SD value. On the opposite hand in case of severe steatosis, the CT numbers of the hepatic parenchema is much less than the minute non avoidable portal tracts which results in larger SD value. On the other hand the Standard deviation value doesn't show correlation with the degree of fibrosis in this study.

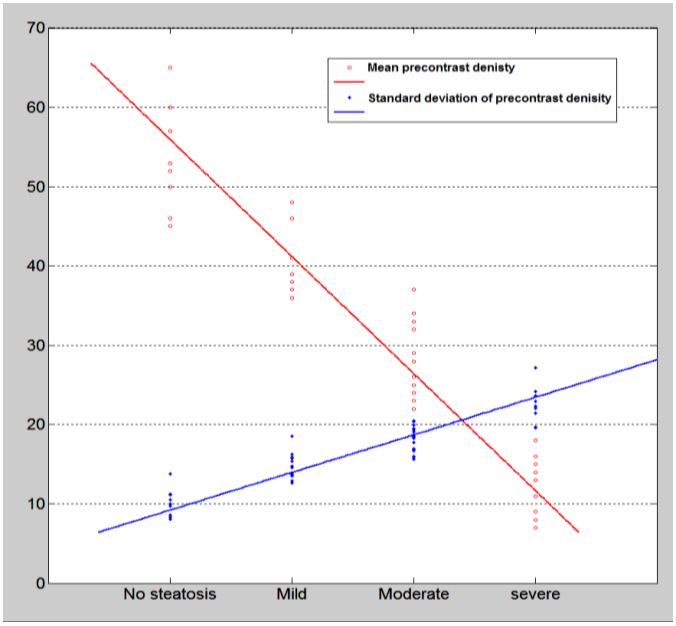

Figure 9. *Retrograde fitting of the degree of steatosis against mean precontrast density and standard deviation*

Table 2. *Fitting parameters of the degree of steatosis against mean precontrast density and standard deviation.*

Degree of steatosis versus mean precontrast density	Degree of steatosis versus standard deviation precontrast density
Linear model f(x) = p1*x + p2 Coefficients (with 95% confidence bounds): p1 = -0.06775 (-0.07411, -0.06138) p2 = 3.79 (3.567, 4.014) Goodness of fit: SSE: 4.857 R-square: 0.9032 Adjusted R-square: 0.9012 RMSE: 0.3148	Linear model f(x) = p1*x + p2 Coefficients (with 95% confidence bounds): p1 = 0.2109 (0.1853, 0.2365) p2 = -1.946 (-2.391, -1.5) Goodness of fit: SSE: 7.607 R-square: 0.8483 Adjusted R-square: 0.8452 RMSE: 0.394

According to metavir score patients were divided into two groups ; group1 with no or mild fibrosis and METAVIR score 0 or 1, group 2 with moderate or dvanced fibrosis and METAVIR score 2 or more.

The first group contained 19 patients, the second group contained 32 patients. All 51 patients underwent perfusional CT scan. In four cases the study was canceled, one patient got terrified as the contrast flowed in his arm and sit down and leaving the CT machine. Two patients has excessive respiratory movements getting the portal vein and its main branches out of the field. One patient was very obese, its images showed poor resolution. In rest of the patients, the fitting process was performed and the perfusional parameters were calculated. The results are shown in the following table.

Table 3. Different perfusional parameters in the two study groups

	Group 1	Group 2
Hepatic arterial perfusion	24.7 ± 8.6	26.9 ± 14.0
Hepatic portal perfusion	157.9 ± 64.8	86.0 ± 16.0
Transit time	12.6 ± 3.0	15.9 ±3.2
Arterial fraction	14.9 ± 8.1	23.0 ± 9.4
Total Hepatic perfusion	182.6 ±70.9	113.0 ±22.5

The perfusional parameters show a significant degree of overlap yet the portal perfusion and total hepatic perfusion were significantly higher in the first group. The portal perfusion was 157.9 ± 64.8 ml/min/100 ml in the first group compared to 86.0 ± 16.0 ml/min/100 ml. The total hepatic perfusion was 182.6 ±70.9 ml/min/100 ml in the first group compared to 113.0 ±22.5 ml/min/100 ml in the second group. The arterial perfusion was slightly higher in the second group but not to a significant extent.

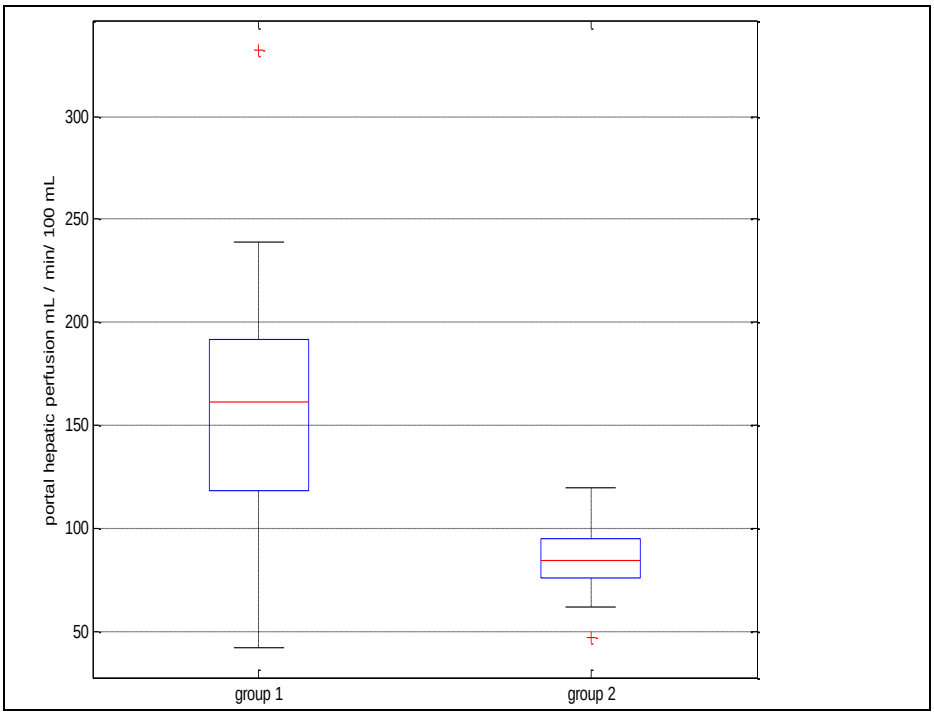

Figure 10. *Box plot the portal hepatic perfusion of the two study groups*

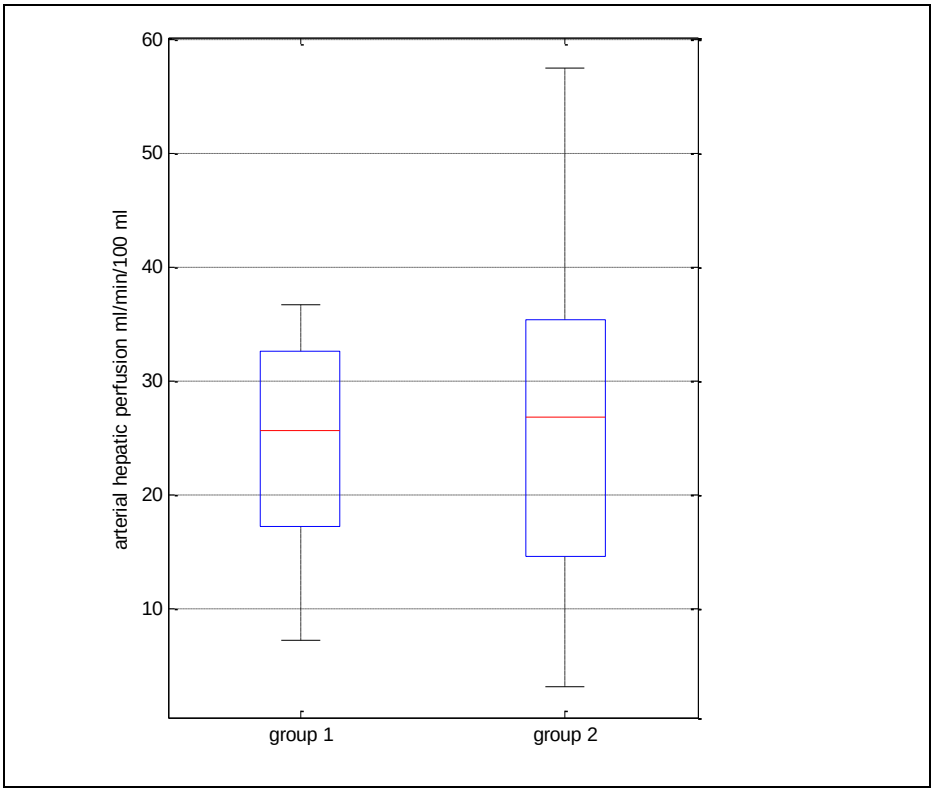

Figure 11. *Box plot the arterial hepatic perfusion of the two study groups*

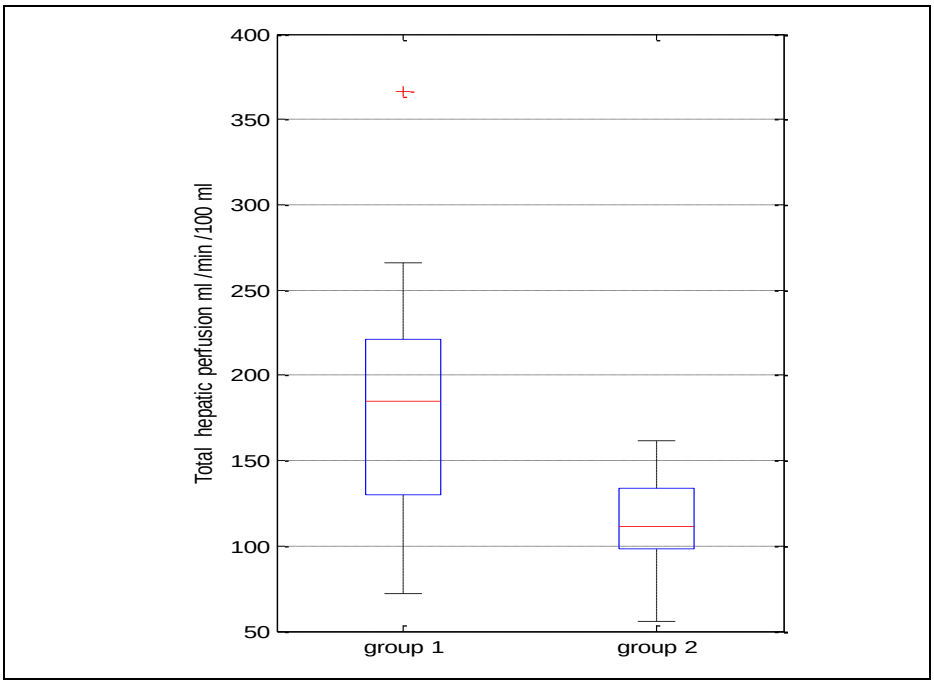

Figure 12. *Box plot the total hepatic perfusion of the two study groups.*

The transit time was significantly less. It was about 12.6 seconds ± 3.0 in the first group and 15.9 seconds ± 3.2 in the second group. The arterial fraction was significantly higher in the second group. Arterial fraction was 14.9 ± 8.1% in the first group and 23.0 ± 9.4% in the second group.

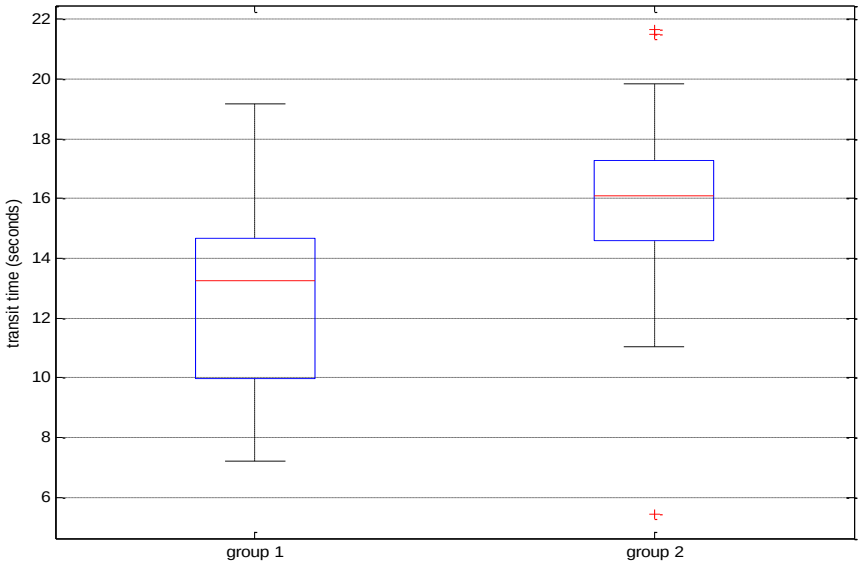

Figure 13. *Box plot the transit time of the two study groups*

These results show that each of portal perfusion, total hepatic perfusion and transit time can be used to differentiate mild from moderate liver fibrosis. The best single factor was portal hepatic perfusion. Using portal hepatic perfusion value of

102 ml /min/100ml showed a sensitivity of and specificity of 83%. At this value efficiency of the test is about 80%

Using transit time value of 14.5 seconds showed a sensitivity of and specificity of 73% with efficiency 63.8%

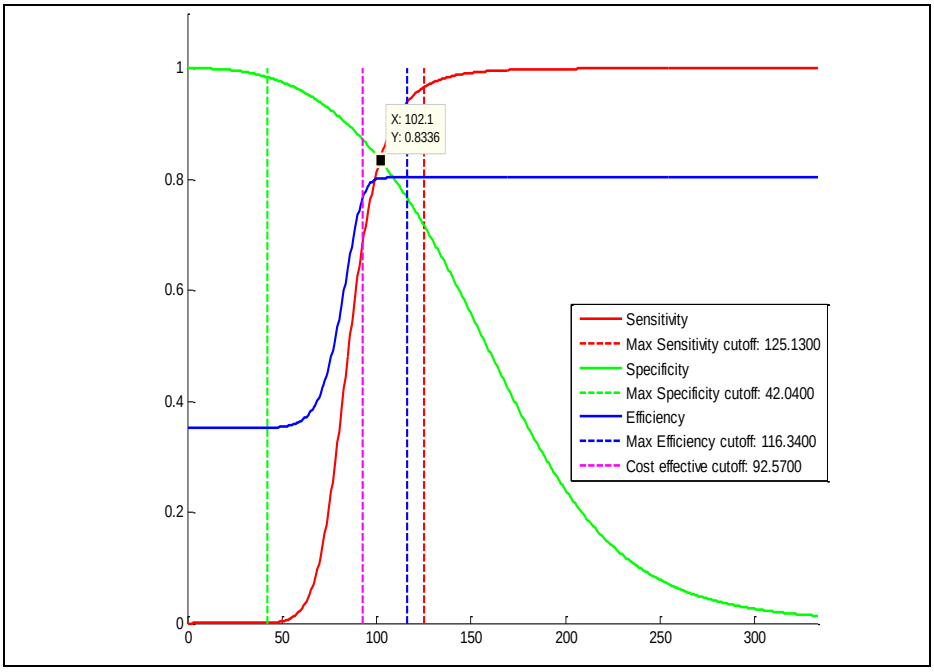

Figure 14. *Sensitivity and specificity of portal hepatic perfusion as a single classifier of mild and moderate fibrosis*

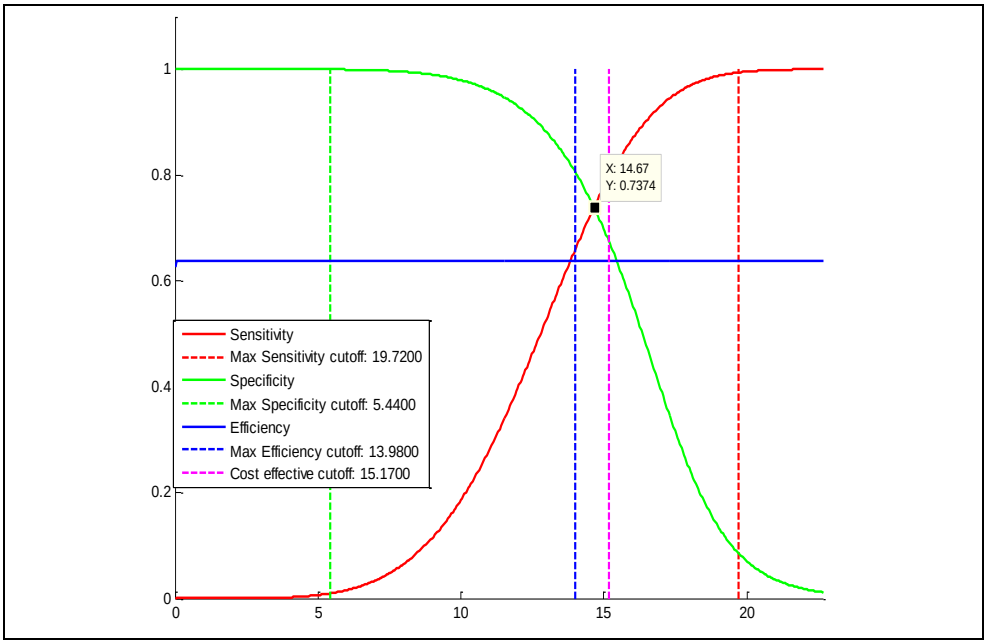

Figure 15. *Sensitivity and specificity of transit time as a classifier of mild and moderate fibrosis*

DISCUSSION

Nowadays, after the introduction of several therapeutic lines for the treatment of hepatitis C virus, the need for a non invasive diagnostic tool for the early detection and follow up of hepatic fibrosis is progressively increasing.

Maternal et al (2000) described the combination of continuous CT imaging with the application of the dual input single compartment model to obtain the various perfusional parameters. They validated this method by the simultaneous assessment of hepatic perfusion using radiolabelled microspheres in animal model. This method showed better reproducibility than the formerly described maximum slope method with less affection with the extrahepatic systemic factors. This method has simple computational requirement and is easily applicable. We depended on their equations to calculate the different perfusional parameters.

The use of CT rather than MR for quantification of hepatic perfusion has the advantage of higher spatial resolution as well as the linear relation between contrast concentration and CT density which is not achievable in MR.

This method still has its drawbacks and limitations. It needs correction of the respiratory movement errors. The procedure necessitate exposure to radiation yet the exposure dose is less than the ordinary triphasic CT scan. The procedure can't be conducted in cases of portal vein thrombosis, extensive arteriovenous shunts, excessive body movements as well as other contraindication of CT and iodinated contrast media

In 2001 van beer et al compared the perfusional parameters of cirrhotic patients compared to non cirrhotic group, they noticed a significant decrease in total liver perfusion and portal perfusion with increased arterial perfusion and transit time. They reported the increase in arterial perfusion to be insufficient to compensate the decrease in portal perfusion and hence the reduction in total hepatic perfusion. They reported no significant change in volume of distribution between the two groups. Their work showed no the difference between perfusional parameters in patients with chronic liver disease and non diseased healthy control group to be insignificant. This finding is not strong enough to contradict our results as the term chronic parenchymal liver disease doesn't necessitate occurrence of fibrosis. Moreover the small number of the group of chronic parenchymal liver disease they studied added to absence of a factor which determine the degree of fibrosis further weaken this result

In 2005 Guan et al were the first to focus on the use of CT perfusional changes in the early stage of fibrosis in animal model. Their work showed that perfusional changes occur early in the disease progress.

In 2006 Hashimoto et al studied patients with chronic liver disease. They classified patients clinically according to Child Pugh classification. They reported that Child B group has hepatic arterial flow significantly higher than Child B group. They reported hepatic arterial fraction in Child A, B and C groups to be 18.6±8.3, 29.8±11.2 and 40.2±11.1%. When we compare this finding to ours we find that the mean arterial fraction in our group is 20.0 ±11.2 which matches with the fact that most of our patient are child group A

In 2009 Chen et al showed the main transit time to be higher in patients with decompensated liver cirrhosis than those with compensated liver cirrhosis. Our results on the other hand focus on the earlier stages of the pathology.

In 2010 Ronot et al studied the perfusional parameters in a group of hepatitis C patient. They correlated the perfusional changes with histopathological grade of fibrosis. They showed F1 patients to have significantly higher portal perfusion compared to F2 and F3 patients. Also they noticed significant prolongation in transit time in F2 and F3 group compared to F1.

In 2015 Wang et al induced fibrosis in a group of rats using CCl4.They classified the animal into five groups according to the histological degree of fibrosis. They studied the CT perfusional parameters in the five groups. They noticed a significant change in portal perfusion between F0 and F1 groups. Comparing F1 to F2 groups; They noticed a significant change in portal perfusion, arterial perfusion and total perfusion. This work represented a trial to predict the stage of fibrosis using perfusional CT parameters. We tried to do the same but we focused on transition from F1 to F2 derived by our group characteristics.

Our results as well as the different studies show the perfusional parameters to be altered with fibrosis. The perfusional parameters changes occur at an early stage of fibrosis. The progress of the pathological process is associated with more pronounced perfusional changes.

According the METAVIR scoring system the degree of fibrosis is classified into five grades. Grade 0 is the grade of no fibrosis. In grade 1 the fibrosis is limited to the portal tracts. The published work up to now shows that the only parameter that changes in grade 1 fibrosis patients is the reduction in the portal venous perfusion (2015 Liuhong et) which actually make sense as it matches with the pathological change. In grade 2 patients fibrosis extends to affect the whole hepatic lobule with few septa. The published papers shows that portal perfusion decreases, the arterial perfusion increases and the transit time increase. The whole hepatic perfusion also decreases. Our results emphasize these data. Our results shows transit time to be the best single factor to differentiate patient with grade I fibrosis from patients with grade 2 or 3 fibrosis. In grade 3 and grade 4 fibrosis the pathological changes are profound and all the perfusional parameters change.

On the other hand the perfusional parameters are affected by non hepatic systemic factors and there's wide individual variability in the perfusional parameters. Both factors limit the clinical use of this tool in grading fibrosis. Our results show that transit time and portal venous perfusion are the earliest parameters to change. The low specificity of these tests limit the use of this tool to screening. The liver biopsy up to now remains the gold standard for diagnosis and grading of hepatic fibrosis.

Paucity of published work in this topic as well as the heterogeneity of the study design between different publishers makes it difficult to compare our results to each published experiment. For example our results can't be compared to the results obtained from animal model although both experiments compare the perfusional parameters to histological fibrotic changes but the perfusional parameters are of course different between different mammalian species. On the other hand published work focusing on human patients but comparing the

perfusional changes to clinical data as compensated versus decompensated liver disease or different stages of Child Paugh classification can't be compared to our results. As these articles focus on the advanced fibrotic stages in which the clinical manifestations start to appear. Our experiment on contrary focused on the early stages of the disease which is asymptomatic.

Our experiment is similar to that published by Ronot el al in 2010. The results of both experiments are generally similar. However they reported the arterial hepatic perfusion to decrease in the advanced stages of fibrosis and they reported the arterial fraction to be almost constant between the different fibrotic stages. These results can't be explained on physiopthological bases. The increase in hepatic arterial perfusion is a well reported issue in the advanced stages of fibrosis. Our results show a weak increase in hepatic arterial perfusion with a significant increase in hepatic arterial fraction.

A second look to the arterial perfusion as estimated by Ronot et al shows a very high degree of variability in the early fibrotic group represented by the increased standard deviation value reported as (28 ml/min/100ml) to be close to the value of the mean (31 ml/min/100ml). These result shows that the arterial perfusion is much more susceptible to extra-hepatic systemic factors compared to the portal perfusion, making its assessment of little value.

During our experiment, the procedure showed a failure rate of about 8%. 4% resulting from excessive respiratory movement and 2% resulting from marked obesity resulting in low signal/noise ratio. 2% represented a patient that got terrified from the flushing sensation of the contrast into his arm. This rate can be minimized by leaving the patient to rest on the CT table for a while before staring the examination actually this may have an effect on perfusional parameters. Every patient should be informed about the flushing sensation before the start of the injection. The signal/noise ratio in Obese patients may be improved by increasing the tube voltage. The dorsal veins of the hand should never be used for perfusional studies, because even if they are usually unable to tolerate the high rate of contrast injection. Their rupture is unpleasant event that results in a large subcutaneous collection causing pain and agony to the patient. Yet it doesn't interfere with completion of the study after canulation of a suitable antecubital vein. The condition is self limited and the body absorbs the collection in few days.

Our results show that each of portal perfusion, total hepatic perfusion and transit time can be used to differentiate mild from moderate liver fibrosis. The best single factor was portal hepatic perfusion. Using portal hepatic perfusion value of 102 ml /min/100ml showed a sensitivity of and specificity of 83%.

Using transit time value of 14.5 seconds showed a sensitivity of and specificity of 73%.

To increase the accuracy of this tool researchers tried to neutralize the extra-hepatic systemic factors modifying hepatic perfusion by comparing the hepatic perfusion to the perfusion of other organs. In 2012 Motosigi et al compared the hepatic to splenic perfusion. This should be an easy job as a part of the spleen is usually included in the same section containing the portal vein which is needed for studying hepatic perfusion. Their results are promising yet unfortunately most of the pathological processes inducing fibrosis affect the spleen as well probably

altering the splenic circulation to a some extent. Further research may suggest other organs to compare the hepatic perfusion with. The pancreas and kidney may be a suggestion.

Another method for increasing the accuracy of this tool would be combination of different perfusional parameters into scoring system. Our results show that the transit time and portal perfusion can be combined for exclusion of significant fibrosis. If the transit time below 9 seconds or portal perfusion is above 109 ml /sec/100 ml the fibrosis grade is F1 or less. This criteria has a specificity of 87% and a sensitivity of 85%. More perfusional parameters could be combined to increase the accuracy.

The modern CT scanner may be able to integrate these perfusional tests into routine triphasic CT protocols to increase the diagnostic the value of these protocols. This may be the single possible clinical application of this procedure nowadays with the current limitation of theses tests.

The accidentally noticed relation between the degree of steatosis and the degree of variability of Hounsfield density of a certain area of the liver estimated through measurement of the standard deviation was not reported before. It needs further investigation.

The pain, agony and potential complications of liver biopsy represented the rational for our study and for similar work. Unfortunately they represent a major limitation to the broad studying of perfusional parameters in healthy subjects. Up to now no study compared the perfusional parameters in grade 0 fibrosis patients to other grades. Such work remains limited to animal models. This is because it's difficult to convince healthy subject to undergo liver biopsy only for the sake of research. In the group of patients we studied only two out of fifty one patients tuned out to be F0, probably because of the insidious onset of hepatitis C which results in a delay of its diagnosis.

Another important limitation to the local future research in this subject is that liver biopsy is no more obligatory requirement for initiation of governmental antiviral therapy.

LIMITATIONS AND RECOMMENDATIONS

The main limitation of this study was the small number of F 0 patients in this group (only two). A study with larger number of F 0 patients would add better idea about the change in the perfusional parameters in the earlier stages of the disease.

Another limitation was the utilization of basic software to calculate the perfusional parameters. More dedicated software would conclude more perfusional criteria.

Further research is recommended to stabilize the cut off values and to find ways to neutralize the inter-personal variation in hepatic perfusion parameters. We recommend studying hepatic perfusion parameters in completely healthy subjects to find out the effect of height, body weight, liver and spleen size, heart rate and cardiac output on hepatic perfusion. We recommend comparing the perfusion of the diseased liver to other healthy organs located at the upper abdomen.

LIMITATIONS AND RECOMMENDATIONS

Each of portal perfusion, total hepatic perfusion and transit time could be used to differentiate mild from moderate liver fibrosis. The best single factor was portal hepatic perfusion. Using portal hepatic perfusion value of 102 ml /min/100ml showed a sensitivity of and specificity of 83%. At this value, efficiency of the test is about 80%.

Further research is still recommended to stabilize the cut off values and to find ways to neutralize the inter-personal variation in hepatic perfusion parameters.

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