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Diagnostic Accuracy of Intracellular uptake rates calculated using Dynamic Gd-EOB-DTPA–Enhanced MRI for hepatic fibrosis stage

Krishna Juluru, MD¹, Andrew H. Talal, MD, MPH², Rhonda K. Yantiss, MD³, Pascal Spincemille, PhD¹, Elizabeth K. Weidman, MD¹, Ashley E. Giambrone, PhD⁴, Sadaf Jalili, MS¹, Steven P. Sourbron, PhD⁵, and Jonathan P. Dyke, PhD¹

¹Department of Radiology, Weill Cornell Medical College, New York, NY

²Department of Medicine, State University of New York at Buffalo, Buffalo, NY

³Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY

⁴Department of Healthcare Policy and Research, Weill Cornell Medical College, New York, NY

⁵Division of Biomedical Imaging, University of Leeds, Leeds, UK

Abstract

Purpose—To assess the diagnostic accuracy of intracellular uptake rates (K_i), and other quantitative pharmacokinetic (PK) parameters, for hepatic fibrosis stage; to compare this accuracy with a previously published semi-quantitative metric, contrast enhancement index (CEI); and to assess variability of these parameters between liver regions.

Materials and Methods—Case-control study design. Dynamic Gd-EOB-DTPA-enhanced 1.5T MRI was performed prospectively in 22 subjects with varying known stages of hepatic fibrosis. PK parameters and CEI were derived from the whole livers and from three fixed regions of interest (ROI) in all subjects. Spearman rank correlation coefficients were computed to assess the relationship between fibrosis stages and each parameter. ROC curves were constructed to discriminate severe fibrosis (stages 3-4) from non-severe fibrosis (stages 0-2). Coefficient of variation (CV) was calculated to assess variability in parameters between ROIs.

Results— K_i and fibrosis stage were significantly correlated ($R=-0.55$, 95% CI [-0.79, -0.14], $p=0.01$). Area under ROC curve (AUC) in distinguishing severe from non-severe fibrosis for K_i was 0.84 (95% CI [0.65, 1.00]), and for CEI was 0.64 (95% CI [0.39, 0.89]) ($p=0.0248$). CV for K_i and CEI were 33.4 and 5.8, respectively. The only other parameter in the PK model having significant correlation with fibrosis stage was absolute arterial blood flow (F_a) ($R=-0.48$, 95% CI [-0.75, -0.05], $p=0.03$).

Conclusion—Hepatocyte intracellular uptake rate, K_i , derived from DCE-MRI, correlates with fibrosis stage and may contribute to a non-invasive biomarker of hepatic fibrosis.

Keywords

dynamic contrast enhanced MRI; DCE-MRI; perfusion; input; compartment; hepatic fibrosis; liver; Gd-EOB-DTPA

Introduction

Hepatic fibrosis results from repetitive or persistent hepatocellular injury and can develop due to various etiologies. In the western world, alcohol ingestion, hepatitis C virus infection, and non-alcoholic steatohepatitis are the most common causes (1). Hepatic fibrosis can progress to cirrhosis, hepatocellular carcinoma, and liver failure; as such, it was the 12th leading cause of death in the United States in 2014 (2). Liver biopsy remains the reference standard for quantifying fibrosis, despite its acknowledged sampling variability and potential adverse outcomes (3-6).

Non-invasive techniques to measure hepatic fibrosis have met with varying results (7). These techniques include serum markers (both indirect and direct) (8) as well as imaging techniques such as transient elastography via ultrasound (9), optical digital analysis of CT (10), and magnetic resonance (MR)-based techniques (11-14). A blood serum test known as “Fibrosure” in the US has shown some promise, but authors of a 2014 meta-analysis concluded that the test “has suboptimal accuracy in the detection of significant fibrosis and cirrhosis,” and highlighted the need to combine this test with other non-invasive modalities to improve accuracy (15).

New, non-invasive techniques for hepatic fibrosis measurement are needed. Dynamic contrast-enhanced MRI (DCE-MRI) with the application of pharmacokinetic (PK) models is one technique that has shown some promise. PK models first make assumptions about a tissue's vascular supply (single-input, dual-input) and then calculate exchange rates between tissue compartments (interstitial, intracellular) (Figure 1). Calculation of intracellular uptake rates through PK models has been limited in the past by the need for a contrast agent with an intracellular distribution. Unlike traditional contrast agents that are excreted only through the kidneys, gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) (Eovist, Bayer Schering Pharma AG, Berlin, Germany) is actively transported into hepatocytes and is partly excreted in bile (16). At least one study has utilized Gd-EOB-DTPA in DCE-MRI for diagnosing fibrosis, demonstrating correlations between fibrosis stage and parameters derived from a dual-input, single-compartment model (17); however, since the study used a single-compartment model, it could not assess intracellular uptake rate (K_i). Several other studies utilized static Gd-EOB-DTPA-enhanced MRI to demonstrate correlations between fibrosis stage and a parameter termed “contrast enhancement index” (CEI) (18-20), which was simpler to calculate. There are no known studies to date utilizing Gd-EOB-DTPA in DCE-MRI to assess how the intracellular uptake rate—as determined by application of a dual-input, two-compartment PK model—is affected by pathologically-determined fibrosis stage.

Our primary objective was to perform liver DCE-MRI to assess the diagnostic accuracy of PK parameters, K_i in particular, for hepatic fibrosis stage. We further sought to compare the

diagnostic accuracy of PK parameters with that of CEI. Finally, we sought to estimate the degree of variability in these parameters between different regions of the liver, as variability in liver function has been shown to impact the prediction of postoperative remnant liver function (21).

Materials and Methods

Patient Population

The study was approved by the Institutional Review Board. All patients provided written, informed consent. Volunteers with self-reported no past medical history, and particularly no history of liver or kidney disease, were recruited to serve as healthy subjects, and assigned to have a fibrosis stage of 0. Additional patients were recruited from the viral hepatitis clinic of a co-investigator (A.H.T.). Inclusion criteria were age greater than 18 years, chronic hepatitis C virus (HCV) infected patients who were scheduled to undergo a liver biopsy for assessment of liver histology or those who had already undergone a liver biopsy within 6 months of the scheduled MRI date. Patients with any contraindication to obtaining an MRI with intravenous contrast, including metal in the body, renal impairment ($\text{GFR} < 60 \text{ mL/min/1.73m}^2$), pregnancy, or breast feeding, were excluded from the study.

MRI

Patients were prospectively imaged supine and feet first using an eight-channel cardiac coil on a GE Excite TwinSpeed 1.5T scanner (GE Healthcare, Waukesha, WI). For each exam, pre-contrast, dynamic post-contrast, and delayed post-contrast images were obtained of the whole liver, in the axial plane, using a spiral liver acquisition with volume acquisition (LAVA) sequence (22). The imaging parameters for all spiral LAVA acquisitions were: flip angle 15° , 48 spiral leaves, variable density spiral (oversampling of 2 at the center of k-space gradually reduced to 0.7 at the edge of k-space), TR 5.7ms, TE 0.4ms, receiver bandwidth $\pm 125\text{kHz}$, spectrally selective inversion pulse for fat suppression, 70% partial slice encoding, acquisition matrix 256×256 , 32 slices, slice thickness 8mm, and field of view (FOV) 38 cm.

Acquisition of the dynamic post-contrast images began 10 seconds following intravenous power injection of 0.1 mL/kg Gd-EOB-DTPA at 1.5 cc/s followed by 20 cc saline flush. Up to 37 whole liver image sets (phases) were obtained over 80 seconds while the patient performed gentle, free-breathing (temporal resolution of approximately 2-4 seconds / phase). Delayed post-contrast images were then performed while the patient performed breathhold, and obtained by acquiring a single whole-liver phase every minute, on the minute, from minutes 2-20 post-contrast, therefore achieving a total of 19 additional phases. For all acquisitions, the receiver gain, the transmission gain, and all linear shimming parameters were kept constant.

Biopsy

All liver biopsy samples were obtained by the same hepatologist (A.H.T.) having over 15 years experience in performing liver biopsies. An area of the right lateral liver free of major blood vessels was first identified by ultrasound. The skin was cleansed with betadine and draped using standard sterile technique, and then locally anesthetized with 1% lidocaine. In

14 of the 22 patients, the biopsy was performed on a CT table, and the site of liver biopsy was localized by CT scan. A 22 gauge Klatskin needle was advanced into the liver approximately 5-6 cm from the skin surface to obtain the core biopsy. The specimen was placed in 10% neutral buffered formalin and transported to surgical pathology for analysis.

Histologic Analysis

Hematoxylin and eosin (H&E) stained slides were prepared from routinely processed, formalin fixed, paraffin-embedded liver biopsy specimens. All cases were reviewed by the same hepatic histopathologist (R.K.Y.) having more than 15 years of experience in liver biopsy interpretation. Cases were examined in a blinded fashion with no knowledge of radiological findings. Histology was assessed using the Sheuer system (23). Masson trichrome stain was used to determine the extent of fibrosis, which was staged as follows: Stage 0 was defined as no fibrosis; stage 1 showed fibrous expansion of portal tracts; stage 2 demonstrated fibrous expansion of portal tracts with periportal fibrosis; stage 3 displayed well-developed bridging fibrosis with architectural distortion; and stage 4 fibrosis was defined as the presence of cirrhosis (23). Stage was assigned to reflect the predominant pattern.

Image Analysis

All image analysis was performed by the same investigator (J.P.D.) having more than 15 years experience in DCE-MRI analysis, who was blinded to pathology results.

Image Registration—All MRI images were converted to Analyze format (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) and imported into the Statistical Parametric Mapping package (SPM 5.0; Wellcome Trust Centre for Neuroimaging, London, UK) for motion correction (24). SPM incorporates a sinc interpolation technique with a $9 \times 9 \times 9$ kernel and a Gauss-Newton optimization technique for a 6 parameter rigid body registration for realignment and reslicing of serially acquired images. In our MRI data, the greatest motion was due to respiration, and therefore the axis requiring the greatest amount of correction was the superior-inferior axis. Anterior-posterior and right-left motion were minimal.

Dual-input, two-compartment PK model (interstitial space and intracellular space) (Figure 1)—Following motion correction, MRI data were imported into the Platform for Medical Imaging (PMI 4.0, Leeds, UK) (25), a freely-available, previously validated software package for PK modeling (available at <https://github.com/plaresmedima/PMI-0.4>). Arterial and venous inputs were defined by regions of interest (ROIs) placed over the aorta and extra-hepatic portal vein, respectively. Three ROIs, each measuring 79 mm^2 (1 cm diameter), were then placed in different segments of the liver, in accordance with previously defined segmental hepatic anatomy (26), by the same investigator (E.K.W.) and confirmed by another investigator (K.J.) having over 7 years of experience in liver MRI interpretation. ROI-1 was placed in liver segment 8 (near the expected biopsy site, and confirmed by CT scan in 14 subjects), ROI-2 was placed in segment 4A, and ROI-3 was placed in segment 2 (Figure 2). Finally, a ‘whole liver’ ROI was defined by manual tracing of the entire liver on every slice of the MRI scans of every subject. Signal intensity changes

from each ROI and from the whole liver were fit by the PMI software to a dual-input, two-compartment PK model, previously defined and validated (27). A sample fit is shown in Figure 3.

Primarily derived parameters were: arterial flow (F_a , ml/min/100ml), portal venous flow (F_v , ml/min/100ml), extracellular volume (V_e , ml/100ml), and intracellular uptake rate (K_i , /100/min). Secondly calculated values were: total flow ($F_t = F_a + F_v$), arterial flow fraction (Art (%) = $F_a / (F_a + F_v)$), portal flow fraction ($Port$ (%) = $F_v / (F_a + F_v)$), extracellular mean transit time (MTT (s) = $V_e / (F_a + F_v + K_i)$), and hepatic uptake fraction (F_i (%) = $K_i / (F_a + F_v + K_i)$).

CEI—CEI was calculated using signal intensities from the same ROIs and whole liver as generated above. CEI has been previously defined (20) as follows:

$$\text{Contrast enhancement index} = \frac{\frac{\text{Liver}_{\text{post}}}{\text{Muscle}_{\text{post}}}}{\frac{\text{Liver}_{\text{pre}}}{\text{Muscle}_{\text{pre}}}} = \frac{\text{Liver}_{\text{post}} \times \text{Muscle}_{\text{pre}}}{\text{Liver}_{\text{pre}} \times \text{Muscle}_{\text{post}}} \quad (1)$$

where the *pre* and *post* subscripts describe the average signal intensities pre-contrast and 20-minute post-contrast, respectively.

Statistical Analysis

Categorical variables are presented as N (%) and continuous variables as mean $\pm$ standard deviation. Fibrosis stage was classified into three groups: none (stage 0), mild (stages 1-2), and severe (stages 3-4). Differences between patient characteristics and hepatic fibrosis stage were evaluated with the non-parametric Kruskal-Wallis test or Fisher's test, where appropriate. Spearman rank correlation coefficients and 95% confidence intervals were computed to assess the relationship between continuous fibrosis stages and derived parameters for whole liver and single ROI measurements. To compare different fibrotic groups across derived parameters, the Kruskal-Wallis test was used with bootstrap adjusted p-values for post-hoc multiple comparisons. Receiver operating characteristic (ROC) curves were constructed and area under the curve (AUC) values were computed for K_i and CEI in discriminating severe fibrosis (stages 3 - 4) from non-severe fibrosis (stages 0 - 2). Fisher's z transformation was used to statistically compare the correlation of fibrosis stage and derived parameters between whole liver and single ROI measurements. To assess differences in mean parameter values between whole liver and single ROI measurements, a Wilcoxon signed-rank sum test for paired data was used. Variation in derived parameters across all fibrosis stage groups was expressed in terms of the coefficient of variation (CV) derived from ROI measurements in three liver locations and estimates were compared with the Kruskal-Wallis test. All p-values were two-sided with statistical significance evaluated at $\alpha = 0.05$. All analyses were performed, and ROC curves generated, using SAS 9.3 (SAS Institute, Cary, NC). Box plots were generated using BoxPlotR (28).

Results

Patient Population

Twenty-three patients met the inclusion / exclusion criteria. MRI data from one patient was excluded entirely because of severe artifact that prevented liver evaluation. The remaining 22 patients (Table 1) were more commonly male gender (13 male, 9 female). The average age of patients was 48 ± 13.5 years, and average time between biopsy and MRI was 23.1 ± 50.7 days. There were 4 healthy volunteers who did not obtain a biopsy, assigned fibrosis stage 0. The remaining 18 patients had biopsy results available. There were no significant differences in age or biopsy-MRI time interval between the three groups. In the time interval between biopsy and MRI, two patients received treatment for Hepatitis C. The remainder received no treatment in the biopsy-MRI interval.

Primary Objective: Assess and compare diagnostic accuracies of PK parameters and CEI for hepatic fibrosis stage

Whole liver PK parameter correlations with, and averages between, fibrosis stages are shown in Table 2a. A statistically significant negative correlation with fibrosis stage was demonstrated for intracellular uptake rate K_i ($R=-0.55$, 95% CI $[-0.79, -0.14]$, $p=0.01$).

Average K_i values demonstrated statistically significant differences between livers with no fibrosis (average $K_i=8.09/100/\text{min}$) and mild fibrosis (average $K_i=3.94/100/\text{min}$) ($p=0.05$), and between livers with no fibrosis (average $K_i=8.09/100/\text{min}$) and severe fibrosis (average $K_i=2.24/100/\text{min}$) ($p<0.001$) (Table 2a, Figure 4). Average arterial flow F_a demonstrated statistically significant differences between livers with no fibrosis (average $F_a=78.56$ ml/min/100g) and livers with severe fibrosis (average $F_a=33.17$ ml/min/100g) ($p=0.01$).

Two patients (referenced in Patient Population section) received treatment for hepatitis virus infection in the biopsy-MRI interval. When these two patients were removed from the analysis, a statistically significant negative correlation was again demonstrated between fibrosis stage and K_i ($R=-0.58$, 95% CI $[-0.81, -0.16]$, $p=0.01$).

Whole liver CEI correlations with, and averages between, fibrosis stages are also shown in Table 2a. CEI demonstrated no significant correlation with fibrosis stage ($R=-0.30$, 95% CI $[-0.63, 0.15]$, $p=0.18$) and no significant differences in means between fibrosis groups (Table 2a).

In a ROC analysis as a performance measure in discriminating severe fibrosis (stages 3-4) from non-severe fibrosis (stages 0-2), the area under the curve (AUC) of K_i was 0.84 (95% CI $[0.65, 1.00]$), and the AUC of CEI was 0.64 (95% CI $[0.39, 0.89]$) ($p=0.0248$) (Figure 5). A K_i threshold of 2.7/100/min achieved an optimal sensitivity of 66.7% (95% CI $[29.9\%, 92.5\%]$) and specificity of 75% (95% CI $[42.8\%, 94.5\%]$).

Secondary Objective: Estimate the degree of variability in derived parameters between different regions of the liver

Significant differences were observed in average K_i values between those obtained from whole liver signal intensities (average $K_i=4.19/100/\text{min}$) and those obtained from ROI-1

only (average $K_i=3.50/100/\text{min}$) ($p=0.002$) (Table 2b). F_a and F_i demonstrated no statistically significant differences between values derived from whole liver and ROI-1 alone.

Significant differences were also observed in average CEI values between those obtained from whole liver signal intensities (average $\text{CEI}=1.61\pm0.59$) and those obtained from ROI-1 only (average $\text{CEI}=1.73\pm0.59$) ($p=0.004$) (Table 2b).

Large variations in a majority of PK parameters were demonstrated when derived from different ROI signal intensities in a given subject (Table 3). Coefficients of variation (CV) for PK parameters ranged from 25% to 87%. CV for CEI was lower, 5.8%.

Discussion

In this hypothesis-generating study, we demonstrate that intracellular uptake rate K_i , obtained by the application of a dual-input, two-compartment PK model to Gd-EOB-DTPA-enhanced MRI, is negatively correlated with hepatic fibrosis stage, with values that significantly differ between livers without fibrosis and livers with mild or severe fibrosis. In an ROC analysis designed to distinguish severe fibrosis from non-severe fibrosis, the AUC for K_i was 0.84. The AUC for CEI, a previously published measure of hepatic fibrosis, was 0.64.

Application of a dual-input, two-compartment PK model is the logical choice when imaging the liver with Gd-EOB-DTPA, a contrast agent having both interstitial and intracellular distributions. Prior studies have utilized Gd-EOB-DTPA-enhanced MRI in the identification of liver disease, including fibrosis, primary biliary cirrhosis, and primary sclerosing cholangitis (29-32), using PK model-independent parameters.

Prior studies have noted deficiencies in serum biomarkers in the assessment of hepatic fibrosis, and the need to combine these with other noninvasive biomarkers to improve accuracy (15). K_i may serve this purpose, and additional hypothesis-testing studies are needed for validation. The reasons for diminished intracellular uptake with progressive liver disease have yet to be elucidated. The interstitial changes of hepatic fibrosis, particularly in advanced stages, could obstruct the delivery of Gd-EOB-DTPA to cell-surface transporters. These interstitial changes may be chronic (fibrous tissue) or acute (inflammatory cells and substances). Alternatively, the cell-surface transporters themselves may be affected. It has been shown that intracellular transport mechanisms of Gd-EOB-DTPA are mediated by organic anion transporting polypeptides (OATPs) and Na^+ /taurocholate cotransporting polypeptides (NTCPs) (33). These polypeptides also mediate the transport of a broad range of organic solutes, including drugs and toxins (34). Hepatic fibrosis could lead to qualitative (malfunction) or quantitative (decreased count) changes in these transporters. The quantitative changes may be secondary to a decreased number of hepatocytes per unit liver tissue in the setting of hepatic fibrosis.

Regardless of the underlying reasons, the ability to quantify hepatocyte intracellular uptake rate has some important implications. Because the transporters involved also mediate the transport of a variety of drugs, a novel (but yet to be validated) possibility is to incorporate

intracellular uptake rate in the dosing of certain medications, delivering a dose optimized to achieve desired intracellular drug concentrations within the individual patient. Likewise, some drugs, including antiretrovirals, have been shown to be inhibitors of hepatic OATPs (35). Measurement of intracellular uptake rate in patients receiving these drugs may help to better quantify the degree of drug toxicity in the individual patient.

Variability in PK parameters was assessed by comparing parameters obtained from whole liver signal intensity with parameters obtained from three small ROIs in the liver. We found a high degree of variability (high CV) in PK parameters between different regions of the liver. Additionally, statistically significant differences were observed in K_j values between those obtained using whole liver signal intensity and those obtained using ROI-1 signal intensity alone. This may be indicative of the variability in the number of infected hepatocytes in HCV, estimated to be between 20-40% (36). It may otherwise be due to the small size of the regions of interest drawn, and consequent statistical noise. Whether arising from technical considerations or real differences, the variability in PK parameters between liver regions raises important concerns about sampling bias, due to either small ROIs or small biopsy specimens. This bias has implications in a variety of assessments, including prediction of postoperative remnant liver function (21). Techniques employing whole liver segmentation, as we have utilized in our study, may help to better characterize liver disease burden and lead to more reproducible data. Further studies investigating the reproducibility of DCE-MRI data are needed.

The study has several limitations. The case-control design may contribute to an overestimation of diagnostic accuracy (37). Also, the sample size was small. The MRI acquisition was performed using a sliding window reconstruction technique; although each phase in the dynamic portion is spaced 2 seconds apart, each phase nonetheless required approximately 8 seconds of data to reconstruct. Therefore, there was a component of “temporal averaging” in our data which may blunt the peak signal intensity in the dynamic images.

A large number of approximations has been made in the model to analyze these data, including: (a) fast exchange of tracer within the extra- and intracellular space - ie. well mixed spaces, (b) negligible leakage of tracer out of the intracellular space within the acquisition time, (c) linear relationship between signal and concentration (ie. assume small enough concentrations), (d) small differences in signal scaling factor between input and tissue ROI's (ie. no major coil sensitivity changes, or B1-effects), (e) fast exchange of water between the various tissue compartments, (f) constant relaxivities of contrast agent, (g) negligible T2*-effects of the tracer, (h) no partial volume errors in the portal vein. All these approximations may be subject to criticisms. Further refining the modeling, however, may introduce additional parameters that cannot be determined from the data, necessitating additional calibration measurements (ie. more measurements and processing), or inclusion of pre-determined values from the literature (which will also be inaccurate). Also, refining a model in this way may increase the accuracy, but the added complexity will likely also reduce the precision due to propagation of errors. More detailed analyses of the sources of variance and respective solutions in DCE analysis are needed to determine the optimal trade-off points between the conflicting requirements of accuracy, precision, practicality and cost.

The last and perhaps most important limitations are the demands on acquisition and the extent of post-processing required to perform DCE-MRI analysis in the liver. There are few, if any, turn-key solutions for post processing of liver DCE-MRI data. High levels of experience and comfort are needed with image registration, ROI placement, and curve fitting due to the complexities of underlying imaging data.

Nonetheless, there are no comparable studies utilizing Gd-EOB-DTPA and DCE-MRI with a two-compartment PK model that includes data up to 20 minutes post injection. A study by Chen et al in 2012 utilized DCE-MRI and Gd-EOB-DTPA, but used a single compartment PK model, and therefore intracellular uptake rate was not assessed (17). A study by Goshima et al in 2012 also utilized Gd-EOB-DTPA, but with a three-phase-only (non-dynamic) MR technique, and therefore no PK modeling; instead, the study assessed CEI, amongst other static parameters (18). That study demonstrated a significant correlation between CEI and hepatic fibrosis stage, which our study was unable to confirm. One explanation may be that the CEI in that study was calculated from average signal intensities of multiple ROIs placed in the liver, whereas in our study, the CEI was calculated from a single whole liver signal intensity.

In conclusion, we find that intracellular uptake rate (K_i), derived from the application of a dual-input, two-compartment PK model to DCE-MRI with a liver-specific contrast agent, is correlated with hepatic fibrosis stage. K_i may contribute to a non-invasive biomarker of hepatic fibrosis. Clinical applicability of this technique would be enhanced by developing standards for imaging acquisition in addition to software tools that streamline the post processing.

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List of Abbreviations

DCE-MRI

Dynamic contrast-enhanced MRI

PK	pharmacokinetic model
Gd-EOB-DTPA	gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid
OATP	organic anion-transporting polypeptide
NTCP	Na+/taurocholate cotransporting polypeptide
CEI	contrast enhancement index
FOV	field of view
LAVA	Liver acquisition with volume acquisition
ROI	region of interest
F_a	arterial flow (ml/min/100ml)
F_v	portal venous flow (ml/min/100ml)
V_e	extracellular volume (ml/100ml)
K_i	intracellular uptake rate (/100/min)
F_t	total flow (ml/min/100ml)
F_i	hepatic uptake fraction (%)
<i>Art</i>	arterial flow fraction (%)
<i>Port</i>	portal flow fraction (%)
MTT	extracellular mean transit time (s)
ROC	receiver operator characteristic
AUC	area under the curve
CV	coefficient of variation

Figure 1.

Dual-input, two-compartment pharmacokinetic perfusion model parameters. F_a : arterial flow (ml/min/100ml); F_v : portal venous flow (ml/min/100ml); K_i : intracellular uptake rate (/100/min); V_e : extracellular volume (ml/100ml); OATP: organic anion-transporting polypeptides; NTCP: Na⁺/taurocholate cotransporting polypeptide.

Figure 2.

MRI liver acquisition with volume acquisition (LAVA) images demonstrating locations of regions of interest (ROIs) placed in three different segments of liver for the purpose of assessing potential sampling bias in pharmacokinetic parameters. In all subjects ROI-1 was placed in segment 8, near expected site of liver biopsy; ROI-2 was placed in segment 4A; ROI-3 was placed in segment 2.

Figure 3. Whole liver signal intensity vs. time after injection in two subjects, one with fibrosis stage 0 (subject 27, diamond), and one with fibrosis stage 4 (subject 17, circle). Curves represent fits to a dual-input, two-compartment PK model.

Figure 4. Boxplot of whole liver intracellular uptake rates (K_i), categorized by fibrosis stage. Mild fibrosis is stages 1 and 2. Severe fibrosis is stages 3 and 4. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means.

Figure 5. Receiver operating characteristic (ROC) curves of whole liver K_i and CEI in discriminating severe fibrosis (stages 3 - 4) from non-severe fibrosis (stages 0 - 2). Values listed next to each parameter are areas under the curves (AUC). K_i : intracellular uptake rate (/100/min); CEI: contrast enhancement index.

Table 1

Subject Demographics

	Overall (n=22)	Fibrosis Stage			P-value
		None (Stage=0) (n=6)	Mild (Stage=1-2) (n=7)	Severe (Stage=3-4) (n=9)	
Gender [n (%)]					0.0007
Female	9 (41%)	6 (100%)	0 (0%)	3 (33%)	
Male	13 (59%)	0 (0%)	7 (100.0)	6 (67%)	
Age (years)	48.0 +/- 13.5	35.3 +/- 15.3	53.4 +/- 11.3	52.1 +/- 8.5	0.1523
Hepatitis C infected [n (%)]	18 (82%)	2 (33%)	7 (100%)	9 (100%)	
Biopsy performed [n (%)]	18 (82%)	2 (33%)	7 (100%)	9 (100%)	
Biopsy to MR (days)	23.1 +/- 50.7	3.0 +/- 2.8	23.6 +/- 47.9	27.2 +/- 60.1	0.6296

Table 2a
Correlation and Average Perfusion Parameters using whole liver values

			Fibrosis stage			K-W Test	Multiple comparison adjusted p-value		
Model / Parameter	Correlation with fibrosis stage (95% CI)	p value	None (Stage = 0)	Mild (Stage = 1-2)	Severe (Stage = 3-4)	p value	No vs. Mild	No vs. Severe	Mild vs. Severe
Dual-input, dual-compartment (n=21)									
F _a (ml/min/100g)	-0.48 (-0.75,-0.05)	0.03	78.56 +/- 35.42	55.48 +/- 33.59	33.17 +/- 11.14	0.07	0.33	0.01	0.25
F _v (ml/min/100g)	0.20 (-0.26,0.57)	0.41	24.63 +/- 21.59	11.23 +/- 29.71	37.62 +/- 48.32	0.09	0.84	0.84	0.38
F _i (ml/min/100g)	-0.19 (-0.57,0.27)	0.20	103.18 +/- 29.00	61.29 +/- 31.31	70.80 +/- 51.48	0.16	0.35	0.40	0.98
Art (%)	-0.30 (-0.64,0.16)	0.19	73.33 +/- 24.69	90.26 +/- 25.76	61.29 +/- 31.31	0.08	0.57	0.72	0.13
Port (%)	0.30 (-0.16,0.64)	0.19	26.67 +/- 24.69	9.74 +/- 25.76	38.71 +/- 31.31	0.08	0.57	0.72	0.13
V _e (ml/100ml)	0.01 (-0.42,0.44)	0.95	43.67 +/- 12.77	41.03 +/- 10.17	45.31 +/- 15.96	0.89	0.94	0.98	0.81
MTT (s)	0.33 (-0.13,0.66)	0.15	25.56 +/- 10.75	41.45 +/- 17.97	54.98 +/- 46.19	0.20	0.73	0.26	0.74
F _i (%)	-0.33 (-0.66,0.13)	0.15	6.95 +/- 3.60	7.15 +/- 4.23	4.63 +/- 5.19	0.26	1.00	0.64	0.53
K _i (/100/min)	-0.55 (-0.79,-0.14)	0.01	8.09 +/- 5.24	3.94 +/- 1.18	2.24 +/- 1.70	0.02	0.05	<0.001	0.47
CEI (n=22)	-0.30 (-0.63,0.15)	0.18	1.83 +/- 0.49	1.54 +/- 0.27	1.51 +/- 0.29	0.47	0.33	0.24	0.99

A: Signal amplitude; Kel: elimination rate constant; Kep: interstitial to plasma rate constant; F_a: absolute arterial blood flow; F_v: absolute portal venous blood flow; F_i: absolute total blood flow; Art: arterial flow fraction; Port: portal flow fraction; V_e: volume of extracellular space; MTT: extracellular mean transit time; F_i: hepatic uptake fraction; K_i: hepatic uptake rate; CEI: contrast enhancement index

Table 2b
Comparison of correlation and average perfusion parameters, CEI between whole liver and ROI-1

	Correlation with fibrosis stage (95% CI), whole liver	Correlation with fibrosis stage (95% CI), ROI-1	p value	Average, whole liver	Average, ROI-1	p value
F _a (ml/min/100g)	-0.48 (-0.75,-0.05)	-0.45 (-0.73,0.01)	0.90	51.41 +/- 31.28	52.39 +/- 40.73	0.66
F _i (%)	-0.33 (-0.66,0.13)	-0.43 (-0.72,0.01)	0.73	6.02 +/- 4.50	4.43 +/- 3.34	0.19
K _i (/100/min)	-0.55 (-0.79,-0.14)	-0.58 (-0.80,-0.18)	0.90	4.19 +/- 3.53	3.50 +/- 3.29	0.002
CEI	-0.30 (-0.63,0.15)	-0.30 (-0.64,0.15)	0.99	1.61 +/- 0.59	1.73 +/-0.59	0.004

K_{el}: elimination rate constant; F_a: absolute arterial blood flow; F_i: hepatic uptake fraction; K_i: hepatic uptake rate; CEI: contrast enhancement index

Table 3
Coefficient of variation between three ROIs

Model / Parameter	Coefficient of variation (%)
Dual-input, dual-compartment (n=21)	
F_a (ml/min/100g)	42.3
F_v (ml/min/100g)	87.4
F_t (ml/min/100g)	32.6
Art (%)	48.2
Port (%)	83.6
V_e (ml/100ml)	24.7
MTT (s)	27.6
F_i (%)	41.7
K_i (/100/min)	33.4
CEI (n=22)	5.8

F_a : absolute arterial blood flow; F_v : absolute portal venous blood flow; F_t : absolute total blood flow; Art: arterial flow fraction; Port: portal flow fraction; V_e : volume of extracellular space; MTT: extracellular mean transit time; F_i : hepatic uptake fraction; K_i : hepatic uptake rate; CEI: contrast enhancement index