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Appendix E1: Materials and Methods

Patients

For patient enrollment, the exclusion criteria were as follows: *(a)* absolute contraindications to MR imaging, including pacemaker, automatic implantable cardioverter-defibrillator device, cochlear implant, ventriculoperitoneal shunt, aneurysm clip, deep brain stimulator, and severe claustrophobia; *(b)* absolute contraindications to liver biopsy, including coagulopathy and allergy to local anesthetic medication; *(c)* history of decompensated cirrhosis complicated by one or more of the following: esophageal variceal hemorrhage, ascites, hepatic encephalopathy, or spontaneous bacterial peritonitis; *(d)* women who are pregnant or breastfeeding; *(e)* history of liver transplantation or hepatic resection; *(f)* history of primary or secondary hepatic malignancy; *(g)* current or previous excessive alcohol consumption within 6 months of study enrollment, defined as more than 30 g/day for men and more than 20 g/day for women; *(h)* history of bariatric surgery more than 1 month prior to study enrollment; *(i)* current or previous history of therapy for an underlying liver disease including interferon-based medications, other antiviral agents, immunomodulatory therapy, biologic response modifier therapy, and complementary and/or alternative medications including (but not encompassing) milk thistle; and *(j)* any severe medical condition that, in the opinion of the principal investigator, would serve as grounds for exclusion from the study.

Liver Histologic Assessment

The METAVIR scoring system or Brunt classification (when appropriate) was used for histopathologic interpretations (29). For patients with chronic hepatitis C, the stage of fibrosis was assessed by using the METAVIR scoring system, where F0 represents no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. For patients with nonalcoholic and alcoholic liver disease, the Brunt classification was used, where stage F0 represents no fibrosis; F1, perisinusoidal or periportal fibrosis; F2, perisinusoidal and periportal fibrosis; F3, bridging fibrosis; and F4, cirrhosis.

MR Elastography and MR Imaging of Fat

The MR elastography parameters were as follows: flow-compensated gradient-echo MR elastography sequence; axial imaging plane; 32–44-cm field of view; 256 × 64 acquisition matrix; 30° flip angle; four 10-mm contiguous sections prescribed through the widest portion of the liver and imaged sequentially; repetition time msec/echo time msec = 50/20.2 msec; 60-Hz driver frequency; 16.7-msec through-plane motion sensitizing gradient (MSG); MSG sensitivity = 10.1 μm per radian; four time offset; bandwidth = ±31.25 kHz; and imaging time = 54 seconds split into four breath holds performed at the end of expiration.

The in-phase and out-of-phase hepatic fat imaging included the following parameters: fast gradient-echo sequence; axial images; 32–44-cm field of view; 256 × 128 acquisition matrix; 70° flip angle; 24 6-mm sections; repetition time msec/echo time 1 msec/echo time 2 msec = 100.0/2.13/4.94; receiver bandwidth = ±50 kHz; imaging time = 22 seconds split into two breath

holds performed at the end of expiration. Regions of interest were drawn by one of the authors (J.C.) in the four sections closest to the location of the MR elastographic sections, including only liver parenchyma and excluding blood vessels. Fat content was calculated as (IP – OP)/(2 × IP), where IP is the signal intensity of the in-phase images and OP is the signal intensity of the out-of-phase images (40,.41).

US VCTE

A VCTE examination consists of performing repeated measurements of the liver stiffness, with each measurement assessed as valid (a white wave front line shows up) or invalid (no white wave front line shows up) automatically by the VCTE system. When a VCTE operator started an examination, the goal was to have at least 10 valid measurements; if there were fewer than 10 valid measurements, more measurements were launched for approaching the goal. Three manufacture-certified operators (J.C., M.Y. and D.M.S., all with 3 years of experience) performed the VCTE examinations. At least two of the three operators were in the examination room for each patient. The first operator tried to get at least 10 valid measurements; if that failed, then the second operator started over with a new examination and tried to complete the examination until 10 valid measurements were achieved. After multiple trials, if both operators agreed that achieving 10 valid measurements (total attempted measurements could be more than 10) were not possible, then the examination was recorded as a technique failure (<10 valid measurements).

Statistical Analysis

Interobserver Agreement

The interobserver agreement was assessed by using the intraclass correlation coefficient (ICC) between the first two biopsy interpretations, as well as the MR elastographic measurements obtained by the two readers. No repeated measurements were made by different observers on the same patients for assessing the interobserver agreement of VCTE. An ICC of 1 indicates that the two observers are 100% equivalent.

Confounding Effects on Liver Stiffness

In the patients who had successful MR elastographic and reliable VCTE examinations, Spearman correlation coefficients (ρ) among the liver stiffness (VCTE and MR elastography), liver fibrosis stage, and inflammation grade were calculated by using a multivariate comparison analysis.

In addition, 10 fixed-effect factors (fibrosis, inflammation, inflammation × fibrosis [interaction], liver fat content, etiology, sex, BMI, biopsy type, biopsy sample portal tract number, and age) were analyzed for their effects (*P* values) on liver stiffness. A mixed-effect model (restricted maximum likelihood method) was used, where patient ID was the random effect. To show the interaction effect of inflammation and fibrosis on liver stiffness, univariate linear regression of liver stiffness versus inflammation was analyzed for patients in each fibrosis stage separately.

Appendix E2: Results

Interobserver Agreement

In the 105 patients who had successful MR elastographic examinations, the interobserver agreement ICC between the two MR elastography readers was 0.95. In the 111 patients who underwent liver biopsy, the interobserver agreement ICC between the two biopsy interpretations was 0.89. The two biopsy interpretations had a difference of at least one fibrosis grade in 30% of patients (33 of 111 patients).

Confounding Effects on Liver Stiffness

In the patients who had successful VCTE (excluding unreliable examinations) and successful MR elastographic examinations (n = 77), Table E1 shows the Spearman correlation coefficient (ρ) between liver stiffness, liver fibrosis, and inflammation. Liver fibrosis was correlated with liver stiffness measured by using VCTE ($\rho = 0.68$, $P < .0001$) and MR elastography ($\rho = 0.73$, $P < .0001$); $P = .54$. Liver inflammation was correlated with liver fibrosis ($\rho = 0.72$, $P < .0001$); it was also strongly correlated with liver stiffness measured by using VCTE ($\rho = 0.49$, $P < .0001$) and MR elastography ($\rho = 0.58$, $P < .0001$); $P = .44$. Among the 10 fixed-effect factors, for both VCTE and MR elastography, two factors had statistically significant effect on liver stiffness: *(a)* fibrosis ($P \leq .009$) and *(b)* the fibrosis and inflammation interaction ($P \leq .006$).

The inflammation effect on liver stiffness depended on the fibrosis stage. Figure E1 shows univariate linear regressions of liver stiffness versus inflammation in each fibrosis stage (F0, F1, F2, F3, and F4 fibrosis), separately. For MR elastography, liver stiffness (Y) increased with inflammation (X) in F0 fibrosis ($Y = 2.42 + 0.49 \times X$, $R^2 = 0.105$) and in F4 fibrosis with a higher slope ($Y = 5.05 + 1.41 \times X$, $R^2 = 0.286$). For VCTE, liver stiffness (Y) decreased with inflammation (X) in F0 fibrosis ($Y = 6.15–0.43 \times X$, $R^2 = 0.004$) and increased with inflammation in F4 fibrosis ($Y = 16.21 + 12.54 \times X$, $R^2 = 0.213$).

Table E2 shows the effects of 10 factors on liver stiffness. Among the 10 factors, for both VCTE and MR elastography, fibrosis had the strongest effect on liver stiffness ($P \leq .009$). The fibrosis and inflammation interaction had the second strongest effect ($P \leq .006$), whereas inflammation alone did not have a statistically significant effect ($P \geq .052$) on liver stiffness. None of the other seven factors (liver fat, etiology, sex, BMI, age, biopsy type, and portal tract number) was found to have a statistically significant effect on liver stiffness ($P \geq .057$ for both MR elastography and VCTE).

Appendix E3: Discussion

Examination Success Rate

Previous VCTE studies have shown that obesity had a great effect on VCTE technique failures. In the study of 141 patients (mean BMI = 25.9 kg/m^2 ± 4.0) with only the traditional M probe, VCTE failed in 10 of the 141 patients who had relatively higher BMI (32.8 kg/m^2 ± 1.8) and 13 of the 141 patients who had ascites (16). Even with the XL probe later designed specifically for obese patients, reliable liver stiffness measurements could only be obtained in 57%–65% of patients with BMI over 30 kg/m^2 and 75%–80% of the overall population (42,43). A previous study with an obese patient population similar to ours (mean BMI = 40.5 kg/m^2, range = 30–64 kg/m^2) had a technical failure (<10 valid measurements) in 54.5% of patients (54 of 99 patients) with use of the M probe alone and a combined technical failure of 23.2% (23 of 99 patients) with use of both the M and XL probes (44).

Interobserver Agreement

Our study showed that the interobserver agreement between the two MR elastography readers was very high (ICC = 0.95). For the interobserver agreement between the two histopathologic interpretations, the ICC was 0.89. The number of cases with a difference of at least one fibrosis stage between the two interpretations was 30% (33 of 111 patients). Our findings were consistent with reports in the literature about the interreader variability of liver biopsy (4) and MR elastography (45). For VCTE, although we did not collect data for an interobserver agreement analysis, a recent published study has shown that VCTE had very high interobserver (ICC = 0.98, n = 26) and intraobserver (ICC = 0.95, n = 34) agreements (46).

Confounding Effects on Liver Stiffness

Inflammation was shown in our study to be a confounding factor that affects liver stiffness, but there is no consensus yet about how it influences the stiffness in the literature. For example, in a study of 129 patients with MR elastography and US shear-wave elastography (SWE), there were no significant differences in mean liver stiffness among patients with different levels of necroinflammatory activity: For stiffness measured with MR elastography, P = .06; with SWE, P = .49 (35). In a study of 131 patients with use of transient elastography with acoustic radiation force impulse and VCTE, inflammation was found to increase the liver stiffness in patients with stage F0–F1 fibrosis but not in patients with severe fibrosis (47). In a study of 72 patients with nonalcoholic steatohepatitis using VCTE, the correlation of lobular inflammation and liver stiffness was found to be significant at univariate analysis (r = 0.364, P = .002), but not at multivariate analysis (r = 0.481, P = .187) (48). In a study of 58 patients with nonalcoholic fatty liver disease using MR elastography, inflammation alone was found to increase liver stiffness in patients before the onset of liver fibrosis (49). In a study of 113 patients with chronic hepatitis B using MR elastography, necroinflammation grades 2–3 yielded higher liver stiffness than grade 0–1 in F0 fibrosis (P < .001) and F1 fibrosis (P = .045), but not in F2 fibrosis (P = .056) and F3–F4 fibrosis (P = .069) (50). Finally, in an MR elastographic study of 239 patients, liver stiffness was significantly greater in patients with inflammation grade 3 than in those with grade 1 or 2 (P = .03), regardless of fibrosis (12).

In our study, with both MR elastography and VCTE, we did not find a statistically significant effect of inflammation alone on liver stiffness ($P \geq .052$); though, given the relatively low number of patients with severe inflammation, this could also be interpreted as a trend. On the other hand, the interaction of inflammation and fibrosis had a statistically significant effect on liver stiffness (P < .006). This interaction effect indicated that the effect of inflammation on liver stiffness depended on the fibrosis stage, which has not been studied before. It is equally correct that the fibrosis stage effect on liver stiffness depends on the inflammation grade. For MR elastography, liver stiffness increased with inflammation for each fibrosis stage except F3, and the slope was much greater in stage F4 fibrosis than in stage F0–F2 fibrosis (Fig E1). This acceleration could be explained by the biomechanical nonlinearity of tissue (the stiffness increases with increasing tissue strain) (51). We hypothesize that when a patient has end-stage fibrosis, the liver parenchyma is in a stiff mechanoenvironment due to the stiffening extracellular matrix; therefore, necroinflammation-related inflamed or edematous tissue could generate more tension or strain in this stiffening mechanoenvironment, resulting in even higher liver stiffness due to nonlinearity. This needs further investigation.

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Table E1. Spearman Correlation Coefficient and Comparison

Correlation Between	Spearman ρ	Prob > |ρ|	Comparison
Fibrosis and MR elastography	0.73	<0.0001	*P* = .54
Fibrosis and VCTE	0.68	<0.0001	
Inflammation and MR elastography	0.58	<0.0001	*P* = .44
Inflammation and VCTE	0.49	<0.0001	

Inflammation and fibrosis	0.72	<0.0001	NA
VCTE and MR elastography	0.73	<0.0001	NA

Note.—MR elastography and VCTE included patients who had both successful MR elastography and successful (reliable) VCTE examinations. NA = not applicable.

Table E2. Confounding Effects on Liver Stiffness Measurements

Method	F	A	F × A	Liver Fat	Etiology	Sex	BMI	Biopsy Type	Portal Tract Number	Age
VCTE	0.009*	0.052	0.006*	0.684	0.643	0.506	0.057	0.717	0.797	0.456
MR elastography	0.0001*	0.056	0.005*	0.391	0.378	0.427	0.057	0.689	0.575	0.454

Note.—MR elastography and VCTE included patients who had both successful MR elastography and successful (reliable) VCTE examinations. F = fibrosis, A = inflammation activity, F × A = interaction of fibrosis and inflammation.

* Indicates a statistically significant effect ($P \leq .05$).