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Noninvasive methods for the detection and diagnosis of hepatic diseases compared to the previous standard of care

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SCHOOL OF MEDICINE

Thesis

NONINVASIVE METHODS FOR THE DETECTION AND DIAGNOSIS OF
HEPATIC DISEASES COMPARED TO THE PREVIOUS STANDARD OF CARE

by

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B.A., Boston University, 2009

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2016
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DEDICATION

I would like to dedicate this thesis to my father, Stanley L. Sotnik. He endured hepatitis C then cirrhosis with its numerous complications quietly and bravely for more than twenty years and ultimately succumbed on May 9, 2014. He was my inspiration, my strength, and my motivation in this endeavor. While some of the medical procedures and interventions discussed in the following may have benefited him had they been available at an earlier time, he was at peace with his illness. It was with pride that I completed this work for him.
ACKNOWLEDGMENTS

I want to thank Gwynneth D. Offner, Ph.D. for her guidance, patience, and support throughout my time in the MAMS program. She is an example that great teaching goes beyond what is presented in a lecture hall and I will always be grateful for her compassion.
NONINVASIVE METHODS FOR THE DETECTION AND DIAGNOSIS OF
HEPATIC DISEASES COMPARED TO THE PREVIOUS STANDARD OF CARE

ELIZABETH IVY SOTNIK

ABSTRACT

As the global burden of liver disease evolves, a need for noninvasive detection and diagnosis has emerged. For over fifty years biopsy has been the standard to which all other disease detection and confirmation methods have been compared. With the development of several noninvasive methods in the detection of liver disease, biopsy has come under scrutiny for its cost-effectiveness, reliability and safety. Serologic testing has proven useful but not specific overall for the determination of disease stages. Liver stiffness is a relatively new parameter used in the diagnosis and monitoring of hepatic disease. It is a quantifiable through the use of an ultrasound-based method of transient elastography using a tool Fibroscan. The implementation of transient elastography has changed the paradigm of liver disease diagnostics with a more cost effective, reproducible, reliable, and well-tolerated option.
While the hepatitis C virus is the primary cause of fibrosis and cirrhosis, numerous studies of the application of liver stiffness measurement to varying disease etiologies have broadened the scope of the method. Optimizing reliability criteria has been the focus of many studies to find a standard by which cirrhosis may be ruled out and fibrosis staging may be accomplished. Novel non-interferon based HCV therapies are altering the course of disease progression and may affect the need for continued development of noninvasive monitoring procedures. However, globally the impact of advanced liver disease is rising with an increase in mortality by fifty million cases per year from 1990-2010 indicating the continued relevance and need for widely applicable noninvasive procedures for both diagnosing hepatic disease and informing treatment options.
# TABLE OF CONTENTS

Title ........................................................................................................................................................................i
Copyright Page ..................................................................................................................................................ii
Approval Page ..................................................................................................................................................iii
Dedication ..........................................................................................................................................................iv
Acknowledgements .........................................................................................................................................v
Abstract ...........................................................................................................................................................vi
Tables ............................................................................................................................................................ix
Figures ..............................................................................................................................................................x
Abbreviations ..................................................................................................................................................xi
Introduction ......................................................................................................................................................1
Body ..................................................................................................................................................................13
Conclusion .......................................................................................................................................................29
References .........................................................................................................................................................33
Vita ....................................................................................................................................................................38
LIST OF TABLES

Table 1: META VIR Fibrosis Stages.................................................................4
Table 2: Necrosis Stages..............................................................................5
Table 3: Hepatitis Stages.............................................................................5
Table 4: APRI Parameters...........................................................................9
Table 5: Liver Stiffness Range.................................................................12
Table 6: Correlation between META VIR and FS Values.....................18
Table 7: Specificity and Sensitivity of Fibroscan.....................................19
Table 8: LSE reliability categories.............................................................22
Table 9: Independent predictors of poorly reliable Fibroscan examinations....25
Table 10: Disease Type and TE Characteristics.......................................26
LIST OF FIGURES

Figure 1: Invasive and noninvasive methods to determine liver fibrosis………6

Figure 2: Pressure Associated Conditions………………………………………13

Figure 3: The Fibroscan®.................................................................14

Figure 4: Schematic of a transient elastography system.............................16

Figure 5: Elastic wave propagation in liver with different fibrotic stages……16
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALD</td>
<td>alcoholic liver disease</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>APRI</td>
<td>aspartate aminotransferase to platelet ratio</td>
</tr>
<tr>
<td>ARFI</td>
<td>acoustic radiation force imaging</td>
</tr>
<tr>
<td>ASH</td>
<td>alcoholic steatohepatitis</td>
</tr>
<tr>
<td>CAP</td>
<td>controlled attenuation parameter</td>
</tr>
<tr>
<td>CEMRI</td>
<td>contrast enhanced MRI</td>
</tr>
<tr>
<td>CEUS</td>
<td>contrast-enhanced ultrasound</td>
</tr>
<tr>
<td>CHC</td>
<td>chronic hepatitis C</td>
</tr>
<tr>
<td>CLD</td>
<td>chronic liver disease</td>
</tr>
<tr>
<td>CT</td>
<td>computer tomography</td>
</tr>
<tr>
<td>DAA</td>
<td>direct-acting antiviral</td>
</tr>
<tr>
<td>DWMRI</td>
<td>diffusion-weighted magnetic resonance imaging</td>
</tr>
<tr>
<td>FLD</td>
<td>fatty liver disease</td>
</tr>
<tr>
<td>HA</td>
<td>haluronic acid</td>
</tr>
<tr>
<td>HCC</td>
<td>hepatocellular carcinoma</td>
</tr>
<tr>
<td>HVPG</td>
<td>hepatic venous pressure gradient</td>
</tr>
<tr>
<td>kPa</td>
<td>kilopascals</td>
</tr>
<tr>
<td>LSM</td>
<td>liver stiffness measurement</td>
</tr>
<tr>
<td>MRE</td>
<td>magnetic resonance elastography</td>
</tr>
<tr>
<td>NAFLD</td>
<td>nonalcoholic fatty liver disease</td>
</tr>
<tr>
<td>NALD</td>
<td>nonalcoholic liver disease</td>
</tr>
<tr>
<td>NASH</td>
<td>nonalcoholic steatohepatitis</td>
</tr>
<tr>
<td>NPV</td>
<td>negative predictive value</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>OLT</td>
<td>orthotopic liver transplantation</td>
</tr>
<tr>
<td>PBC</td>
<td>primary biliary cirrhosis</td>
</tr>
<tr>
<td>PHT</td>
<td>portal hypertension</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SWE</td>
<td>shear wave elastography</td>
</tr>
<tr>
<td>TE</td>
<td>transient elastography</td>
</tr>
<tr>
<td>US</td>
<td>ultrasound</td>
</tr>
<tr>
<td>WHO</td>
<td>world health organization</td>
</tr>
</tbody>
</table>
INTRODUCTION

The accurate detection, diagnosis, and treatment of hepatic disease is becoming increasingly important globally. Total mortality worldwide from cirrhosis and hepatocellular carcinomas rose by fifty million per year over the span of two decades according to the first-ever World Health Organization (WHO) study of liver disease mortality conducted from 1990 to 2010 [36]. Advanced liver disease is now considered a substantial contributor to global mortality with the incidence of both cirrhosis and liver cancer on the rise. In the United States alone chronic hepatitis C (CHC) infection is a major etiology of hepatic disease and as such is a growing public health concern making detection essential for prognoses and treatment decisions. According the WHO report published in 2012, Hepatitis C was the primary cause of both liver cancer and cirrhosis in 2010, accounting for 41% and 40% of cases respectively. Comorbidities and coinfections with hepatic disease are also becoming more prevalent. One such example is coinfection with human immunodeficiency virus (HIV). Since the first decade of the widespread use of highly active antiretroviral (HAART) therapies the course of HIV infection has been dramatically altered and mortality reduced. As a result of this shift, liver disease has emerged as a leading cause of morbidity and mortality among HIV patients who are dually infected with the hepatitis C virus (HCV) [37]. What develops out of these statistics and information is the clear need for a method of early detection and diagnosis which fits within the restrictions of the current
insurance system as well as the parameters of clinical settings here in the United States but with the potential for global applications.

All chronic liver diseases whether of toxic, genetic, autoimmune, or viral origin cause the organ to undergo typical histological changes that ultimately lead to fibrosis, cirrhosis, and the excess deposition of extracellular matrix \[21\]. Liver fibrosis is the result of the healing of parenchymal injury in chronic liver diseases and, left uninterrupted, this process will result in liver cirrhosis and its various complications. The extent of the scarring and hardening of hepatic tissue due to the wound response process is a good prognostic measure for individuals with liver disease. Assessing the stages of fibrosis accurately is essential for informing the course of treatment as well as monitoring disease progression. Many techniques have been explored and used over more than fifty years to allow for early and reliable detection of fibrosis but these previous care standards are limited in their utility and practicality for ongoing assessment. Invasive procedures lead to greater risk of infection and complications as well as longer patient recovery times, increased costs, and decreased likelihood of patient compliance with serial monitoring.
**Biopsy**

For approximately fifty years, biopsy has been the gold standard of evaluation and histological diagnosis for liver fibrosis and cirrhosis. The clinical value of a biopsy is procuring a live sample from which disease gradation and staging can be histologically determined. Grading reflects necroinflammatory activity and integrates lesion severity while staging reflects the extent of fibrosis (Fig.1, 2). Information about liver disease can be assessed and determined through biopsy, leading to its primary role among diagnostic methods. The METAVIR scoring scale for fibrosis and cirrhosis allows pathologists to grade fibrotic activity as: F0-F4, no fibrosis and cirrhosis respectively with F1, 2, 3 representing intermediate levels of fibrosis. Despite universal use, the biopsy procedure has significant drawbacks and risks. On a practical level, biopsy is expensive, requiring hospital admission and the use of a specialized room, instruments, and a team of medical staff. It is, therefore, far from an ideal procedure to repeat often for continuous disease monitoring. Interobserver variation in histological interpretation makes a diagnosis based on biopsy less objectively reliable when there is disagreement between pathologists. While this is a rare occurrence, it is still a consideration, particularly when diagnosing intermediate stages when there are fewer histological distinctions in the organ tissue. As with any invasive procedure, a risk exists, Sharma et al. found a mortality rate of 0.01% and rate of complications of 0.3% in their 2014 review of noninvasive diagnostic methods [31]. Another factor that limits the utility of biopsy is acquiring the specimen itself, or
sampling error. A satisfactory tissue sample is typically 10-15 mm in length and 1.0-1.4 mm in diameter containing four to six portal tracks. A sample this size represents approximately 1/50,000 of the entire liver and is often not representative of the disease state of the organ as a whole. Accurate determination of intermediate stages is a particular limitation which cannot always be overcome by optimal sample size. Diagnoses of F0 and F4 are typically more reliable due to more representative and less ambiguous histological patterns. Even with the chance of an accurate diagnosis, patients are often unwilling to undergo repeated biopsies due to invasiveness and recovery time. These limitations have led to the need to develop noninvasive and cost-effective procedures which either maintain or improve upon the diagnostic accuracy of biopsy.

Table 1: METAVIR Fibrosis Stages

<table>
<thead>
<tr>
<th>METAVIR Score</th>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>none</td>
<td>no fibrosis</td>
</tr>
<tr>
<td>F1</td>
<td>moderate</td>
<td>portal fibrosis without septa</td>
</tr>
<tr>
<td>F2</td>
<td>significant</td>
<td>portal fibrosis with few septa</td>
</tr>
<tr>
<td>F3</td>
<td>severe</td>
<td>numerous septa without cirrhosis</td>
</tr>
<tr>
<td>F4</td>
<td>cirrhosis</td>
<td>cirrhosis</td>
</tr>
</tbody>
</table>
### Table 2: Necrosis Stages

<table>
<thead>
<tr>
<th>Necrosis Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no inflammation</td>
</tr>
<tr>
<td>1</td>
<td>peri-portal inflammation no hepatocellular necrosis</td>
</tr>
<tr>
<td>2</td>
<td>inflammation with mild hepatitis</td>
</tr>
<tr>
<td>3</td>
<td>severe portal inflammation with moderate hepatitis</td>
</tr>
<tr>
<td>4</td>
<td>marked inflammation with severe hepatitis</td>
</tr>
</tbody>
</table>

### Table 3: Hepatitis Stages

<table>
<thead>
<tr>
<th>Hepatitis Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>compensated</td>
</tr>
<tr>
<td>B</td>
<td>beginning to decompensate</td>
</tr>
<tr>
<td>C</td>
<td>decompensated</td>
</tr>
</tbody>
</table>

### Disease Indicators & Diagnostic Methods

As with any pathology, liver disease has numerous clinically relevant markers and indicators. The development of tools which assess the presence or indeed absence of these markers has been vital in the evolution of accurate diagnostics and appropriate treatment regimens. Hepatic disease diagnostic methods can be broken down into categories and subcategories (Fig. 1) [21]. Invasive methods include laparoscopy, endoscopy, and biopsy which until recently has been the standard for liver disease detection and diagnosis. Laparoscopic and endoscopic procedures are not particularly sensitive in their ability to identify disease indicators in vivo. Noninvasive methods can be radiologic or serologic in nature. Radiologic and imaging methods include magnetic resonance imaging (MRI), ultrasound (US), computer tomography (CT), and elastography. Conventional
imaging via US, MRI, and CT while noninvasive, is not sensitive to the explicit signs of cirrhosis such as the genesis of new blood vessels called collaterals and the nodular surface of the organ ultimately limiting the utility of these three methods. Serum-based disease biomarkers can be broadly classified as either direct or indirect. Direct methods assess extracellular matrix turnover and hepatic fibrogenesis. Liver function decline is indirectly assessed via the presence of various enzymes in blood and serum samples. Radiologic and serum-based markers of fibrosis correlate well with biopsy scores, especially when used to exclude the presence of fibrosis and cirrhosis, a clinically useful feature which can often allow for biopsy to be avoided [30].

![Figure 1: Invasive and noninvasive methods to determine liver fibrosis, adapted from Meuller & Sandrin, 2010](image)

Figure 1: Invasive and noninvasive methods to determine liver fibrosis, adapted from Meuller & Sandrin, 2010 [21]
Fibrotest®, APRI, and Other Serologic Assessments

An ideal serologic diagnostic test would be specific for hepatic tissue, easy to execute, and perform independently of concurrent inflammatory and fibrotic processes. The biomarkers assessed with these ideal tests would be freely available within the serum, independent of an inflammatory response, not influenced by secretion, and correlated with the process of fibrogenesis [31]. This list of requirements breaks down into identifying a factor that can: 1. be assessed independently of others present in the serum, i.e. one that is not bound to or influenced by another molecule, 2. one whose circulating levels do not increase when acute inflammation occurs, 3. a factor which is not a component of ongoing metabolic processes and eliminated by the liver, 4. a biomarker which is involved in the wound healing process that results in the deposition of new liver tissue.

Serum markers categorized as indirect reflect changes in hepatic function, i.e. platelet count, coagulation factors, and serum transaminases. Direct markers of fibrosis indicate matrix turnover and consist of synthesis and degradation enzymes, collagen deposition factors, and several proteinases [31]. There are three major second generation panel tests which provide diagnostic information regarding fibrosis stage; these are FibroTest, Hapascore, and FibrometerA [7]. These panel tests have the specificity to discriminate between mild and clinically significant fibrosis as well as advanced fibrosis and cirrhosis. Out of the three second generation serologic tests, Fibrotest is the most used and widely studied. Chrostek et al. and Shaheen et al. discuss the utility and accuracy of Fibrotest
and its application to various disease etiologies\cite{7,29}. The Fibrotest is a composite of five serum biomarkers: α-2-macroglobulin, apolipoprotein A1, haptoglobin, γ-glutamyltranspeptidase, and bilirubin. Chrosteck et al. indicated in their 2014 study that Fibrotest had a 100% negative predictive value (NPV) for the absence of significant fibrosis and a greater than 90% positive predictive value (PPV) for significant fibrosis\cite{7}. These findings indicate that Fibrotest is highly sensitive because the test ruled out fibrosis in 100% of the cases in which patients did not have significant fibrosis and resulted in a positive reading in 90% of cases in which patients did have significant fibrosis\cite{7}. In the Shaheen et al. review of nine studies assessing Fibrotest, they compared the sensitivities and specificities of the test’s diagnostic thresholds and determined that studies with an increased prevalence of advanced fibrosis reported improved test performance\cite{2}. Therefore, while it is sensitive, Fibrotest is not adequately specific and limited in its ability to distinguish lower and intermediate grades of fibrosis.

Aspartate aminotransferase to platelet ratio index (APRI) is another noninvasive and readily available tool and has been widely studied due to its universal applicability and simplicity. Compared to other serum panel tests, APRI is a single measure which evaluates the level of the circulating blood enzyme aspartate aminotransferase (AST). AST is an enzyme associated with the
parenchymal or functional cells of the liver and is typically measured as part of a liver function test. When measured as a ratio with platelet cells, it is an indicator of cellular turnover and breakdown. In a 2013 meta-analysis by Chou et al. which Sharma et al. referenced in their 2014 review, APRI was shown to have 55% specificity and 81% sensitivity for the diagnosis of fibrosis in hepatitis C patients across the 28 studies included in the analysis. Toniutto et al. aimed to determine the usefulness of this test in their 2007 study to detect fibrosis in transplant patients with recurrent HCV. They found an overall sensitivity of 0.76, specificity of 0.77, PPV of 46%, and a NPV of 93% among their study population indicating that APRI is a good method for ruling out the presence of fibrosis (Table 4). While useful for its NPV and lower cost, APRI accuracy has not been established for a broader range of etiologies and its diagnostic value is lower than some other serum biomarkers.

### Table 4: APRI Parameters, data from Toniutto et al, 2007

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients (n=102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.76 (0.58-0.89)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.77 (0.72-0.80)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.46 (0.35-0.53)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.93 (0.87-0.97)</td>
</tr>
</tbody>
</table>

A newer development is a panel which detects serum microRNAs as potential biomarkers of nonalcoholic fatty liver disease (NAFLD). MicroRNAs are a class of non-coding, highly conserved RNAs that control post-transcriptional gene
expression. As such they are linked to cellular differentiation, development, metabolism, proliferation, and apoptosis. They have more recently been found to regulate a spectrum of liver functions \[^{[34]}\]. A pilot study using a serum microRNA panel to diagnose NAFLD by Tan et al. indicated an increased sensitivity of diagnostic accuracy compared to other biomarkers for NAFLD \[^{[34]}\]. While more research on a larger scale is needed, this method of diagnosis may prove promising specifically for NAFLD diagnosis. However, it is not universally applicable to all etiologies of hepatic disease thus limiting its utility.

Although serologic markers reflect profibrogenic activity, they do not always correlate directly with the absolute amount of matrix deposited, limiting their diagnostic utility \[^{[21]}\]. Serum-based testing is a valuable tool in a clinical setting but may be insufficient to make a complete diagnostic determination due to specificity limitations. Used as an initial and less expensive starting point, serologic testing has the ability to determine the presence or absence of fibrosis but not the specific stage. Overall, serum markers for fibrosis correlate well with the two extremes of the METAVIR scale and clinically these types of assessments may eliminate some need for more invasive procedures but they do not represent a replacement for biopsy. Continued research into the pathophysiology of fibrosis and cirrhosis may identify new markers and encourage the development of diverse serum testing.
Liver Stiffness

Liver stiffness (LS) is a relatively new parameter used in the diagnosis and monitoring of hepatic disease. It is quantifiable through the use of an ultrasound-based method of transient elastography using a tool called Fibroscan. The stiffness of the liver changes over time with the progression of fibrosis and is therefore an observable and qualifiable measure of disease state and functions as a particularly reliable surrogate marker for advanced fibrosis and cirrhosis. LS is expressed in kilopascals (kPa) and is a measurement that is dependent on many factors: the extracellular matrix of the liver, the constraints applied on the organ, the internal pressure of the organ, and the viscous effects over the time constant that the liver is subjected to during testing \[21\]. Liver stiffness measurement (LSM) is ideal for serial monitoring of disease progression over time and carries fewer of the risks and a fraction of the cost of the previous clinical standard. Mueller and Sandrin presented the range of kPA values which correlate to fibrotic stage in their 2010 review of liver stiffness as a novel parameter in the ongoing search for noninvasive methods of diagnosing hepatic diseases (Table 5, Figure 2) \[20\]. Considerations in accurately assessing liver stiffness include whether the patient is undergoing alcohol detoxification causing hepatocellular swelling, experiencing an acute inflammation flare related to liver disease, or undergoing tumor infiltration as all these scenarios can lead to overestimation of LS and result in an inaccurate categorization of fibrosis stage. Figure 2 also indicates other conditions which affect liver stiffness which alter
LSM but do not affect fibrosis stage. Controlling for these circumstances, LSM is a more global representation of the condition of the organ than biopsy. The latter method only assesses a small sample of tissue which may not give a full diagnostic picture. The goal of novel diagnostic techniques is to improve early detection of and thus inform appropriate treatment. Additional applications of LSM would be earlier recognition of fibrosis and cirrhosis-related complications such as portal hypertension, esophageal varices, liver cancers, as well as monitoring response to treatment.

Table 5: Liver Stiffness Range, adapted from Mueller & Sandrin, 2010 [21]

<table>
<thead>
<tr>
<th>Stiffness</th>
<th>kPa</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>soft</td>
<td>&lt;6</td>
<td>normal, F0</td>
</tr>
<tr>
<td>intermediate</td>
<td>6-8</td>
<td>intermediate F1/2</td>
</tr>
<tr>
<td>moderate</td>
<td>8-12.5</td>
<td>fibrosis, F3</td>
</tr>
<tr>
<td>stiff</td>
<td>&gt;12.5</td>
<td>cirrhosis, F4</td>
</tr>
</tbody>
</table>
TRANSIENT ELASTOGRAPHY

Elastography and Fibroscan®

The development of elastographic techniques yielded four major categories: static elastography, dynamic elastography, transient elastography, and remote elastography [25]. Some of these methods are limited by high sensitivity, boundary conditions of the tissue tested (i.e. external forces that exert pressure), acquisition times, compatibility with specific organs, and prohibitive costs. Transient elastography (TE) differs from other ultrasound-based techniques by
the kind of mechanical stimulation it relies upon. The use of transient vibration offers advantages over other methods: 1. the transmitted elastic wave can be temporally separated from the reflected waves thereby making the method less sensitive to eternal forces that affect the stiffness of the tissue tested; 2. acquisition time is short enabling measurements to be conducted on moving organs making the technique well-adapted to the study of the liver. Fibroscan (Figure 3) [25], which uses transient elastography technology (TE), is a tool for liver stiffness evaluation (LSE) capable of assessing soft biological tissue stiffness in vivo.

Figure 3: The Fibroscan®, taken from Sandrin et al, 2003 [25]
To perform a liver stiffness measurement as shown in Figure 4 [9], the Fibroscan probe is positioned at the intercostal space on the right side of a patient who is laying in the dorsal decubitus position with their right arm in maximal abduction (i.e. lying on the back with the right arm behind the head) to access the right lobe of the liver which contains the region of interest (ROI). A typical ROI in a LSE is often about 100 times larger than a biopsy sample. An additional advantage of TE is that a single ultrasonic transducer can be used as a low frequency vibration generator, emitter, and receiver. The transducer probe generates vibrations of a low frequency, typically 50Hz, which are transmitted through liver tissue resulting in a shear wave propagating through the ROI at a particular velocity. The probe uses pulse-echo ultrasound (US) to then follow the propagation of the initial wave to measure its velocity which determines liver stiffness (LS). This is possible to achieve with one probe because of the aforementioned temporal separation of transmitted and reflected waves. Liver elasticity is determined from the velocity of the low frequency shear wave which propagates through the ROI tissue. The slope of the shear wave pattern depicted in Figure 5 increases with advanced fibrosis grade [25]. As fibrosis spreads through the liver in worsening disease states, tissue elasticity decreases resulting in a faster propagation velocity of shear waves through the organ which corresponds to a steeper slope. Therefore, more advanced disease means a stiffer liver and an elevated LSM calculated in kPa [25].
Figure 4: Schematic of a transient elastography system, taken from Cournane, Browne, and Fagan, 2012

Figure 5: Elastic wave propagation in liver with different fibrotic stages, adapted from Sandrin et al, 2003
**Fibroscan Studies**

The METAVIR fibrosis scale of F0-F4, a staging scale used to categorize biopsy results, remains the standard to which noninvasive method measurements are compared and correlates to Fibroscan cutoff values (Table 6) [30]. Liver stiffness measured by TE is indicative of the level of fibrosis and disease state and is measured in kilopascals (kPa). Numerous studies have developed cut off values which correspond to each stage and those numbers varied depending on disease etiology. In 2014, Sharma et al. published a prospective study to determine the usefulness of Fibroscan in the evaluation of liver fibrosis [30]. Their objective was to find a correlation between liver stiffness measurement (LSM) and the fibrosis stage assessed by liver biopsy (LB) and determine predictors of discordance between the two methods. A secondary goal was to find similar correlation between aspartate transaminase to platelet ratio index (APRI) and LB outcomes. Both the METAVIR classification system and AUROC values were used to evaluate TE and APRI accuracy in 185 patients who underwent both LB and TE. Fibrosis stages were graded by two blinded and independent pathologists. After eliminating some participants due to unreliable TE readings or inadequate biopsy results, the sample was reduced to 175 patients. A significant difference was noted in the LSM values for patients with F0 compared to mild fibrosis of F1-2 and advanced fibrosis of F3-4. The AUROC for significant fibrosis, i.e. F>2, was 0.98 with an optimal TE cutoff value of ≥10.0 pKa. The sensitivity and specificity at this cutoff were 98% and 89% respectively. The
optimized cutoff value to predict cirrhosis was determined at ≥14.7 pKa with a sensitivity of 97% and a specificity of 96%. Elevated bilirubin of >10.5 mg/dL was an independent predictor of a false TE reading. A median APRI value for patients without fibrosis was 0.47, with a significant difference between no fibrosis and mild fibrosis. The difference in the APRI value between mild and advanced fibrosis was also statistically significant. In comparing the two noninvasive methods the AUROC as well as sensitivity and specificity for TE are greater than those same parameters calculated for APRI. Thus the researchers determined that LSM is a reliable predictor of hepatic fibrosis and is, in fact, superior to APRI for noninvasive diagnosis of fibrosis and cirrhosis [30].

Table 6: Correlation between METAVIR and FS Values, data from Sharma et al, 2014 [30]

<table>
<thead>
<tr>
<th>METAVIR score</th>
<th>FS Values (kPa)</th>
<th>Fibrosis Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>≤4.5</td>
<td>no fibrosis</td>
</tr>
<tr>
<td>F1</td>
<td>7.5</td>
<td>fibrosis without septa</td>
</tr>
<tr>
<td>F2</td>
<td>≥10</td>
<td>fibrosis with few septa</td>
</tr>
<tr>
<td>F3</td>
<td>≥12.5</td>
<td>numerous septa without cirrhosis</td>
</tr>
<tr>
<td>F4</td>
<td>≥14.7</td>
<td>cirrhosis</td>
</tr>
</tbody>
</table>

In their systematic review of diagnostic test accuracy for hepatitis C-related fibrosis published in 2007, Shaheen, Wan, and Meyers discussed how accurate diagnosis is crucial for determining disease prognosis and making treatment decisions. As hepatitis C is the primary cause of advanced liver disease worldwide and affected 3.2 million Americans at the time of their study, the
researchers functioned within the framework that standard interferon-ribavirin therapies only resulted in sustained virologic response in 50-60% of patients with only a minority of HCV patients having access or eligibility for treatment [29]. A priority for clinical research is the development of accurate staging methods in order to estimate prognosis and inform treatment. In this study the three researchers compared Fibroscan to biopsy as well as other noninvasive diagnostic methods with their primary outcome as the differentiation of mild from moderate fibrosis, i.e. F0-1 from F2-3. This outcome was chosen because the point of antiviral therapy indication is usually when a patient transitions into the moderate stage. Specifically for cutoffs of 7.1-8.8 kPa, Fibroscan had summary sensitivity and specificity of 64% and 87% respectively. The secondary outcome was the identification of cirrhosis, F4. The researchers found Fibroscan has improved accuracy for this outcome compared to biopsy, with a sensitivity of 86% specificity of 93% for LS threshold of 12.5-14.8 kPa [29].

Table 7: Specificity and Sensitivity of Fibroscan, data from Shaheen, Wan, & Meyers, 2007 [29]

<table>
<thead>
<tr>
<th>Fibrosis Stage</th>
<th>pKa Threshold</th>
<th>Sensitivity AUC (95% CI)</th>
<th>Specificity AUC(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>7.1-8.8</td>
<td>0.64 (0.5-0.76)</td>
<td>0.87 (0.8-0.91)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>12.5-14.8</td>
<td>0.86 (0.78-0.91)</td>
<td>0.93 (0.9-0.95)</td>
</tr>
</tbody>
</table>
LSM Reliability Criteria & Cutoff Value Optimization

Over time investigators have continued to determine appropriate and optimized cut off values for the measurements that correlate both with LSM reliability and accuracy of determining fibrotic stages. Accurate positive and negative predictive values are an important aspect of this work. Positive predictive values apply mainly to fibrosis and indicate that a patient with a LSM which indicates fibrosis truly does have that level of fibrotic activity. Negative predictive values are more often applied to ruling out cirrhosis. This is a value range for LSM which accurately indicates that cirrhosis is not present. Another key parameter is LSM reliability which correlates to diagnostic accuracy and the predictive values just discussed. A liver stiffness evaluation (LSE) is usually considered reliable when it fulfills the following criteria: a minimum of ten usable measurements obtained with a minimum of a 60% success rate and an interquartile range/median ratio (IQR/MR) of less than or equal to 0.30. The last parameter ensures the elimination of any measurement which is more than 30% greater or less than the median value determined from the ten successful measurements thereby excluding extreme outliers which could affect test results.

Boursier et al. published a study in 2013 involving 1,165 patient participants with varying disease etiologies in which their aim was to determine whether an unreliable LSE according to these standards was any less accurate than a
reliable LSE and if there was a need for revision of reliability parameters[^3]. This was the first study to demonstrate the relevance of the reliability definition. Previous studies did indicate that LSE meeting the three established criteria did not provide tests with better diagnostic accuracy that those LSE which did not meet criteria. In fact, at least 15% of the LSE in clinical settings were excluded from analyses after being deemed unreliable when their data may have resulted in accurate diagnoses. Researchers evaluated the relevance of the standard reliability criteria in order to improve upon them by using diagnostic accuracy as the desired outcome. Each patient underwent biopsy and histological diagnosis as well as LSE. Out of the 1,165 LSE, 75.7% fulfilled standard reliability criteria. Area under receiver operating characteristic curve (AUROC) values for significant fibrosis, severe fibrosis, and cirrhosis were not significantly different between reliable and unreliable LSE. Researchers found that the number of valid measurements and the success rate for obtaining them were not independent predictors of discrepancies between histologically-determined fibrotic stage and LSE median. However, the parameter which did independently predict discrepancies between LSE median and biopsy result was IQR/M, causing researchers to conclude that LSE accuracy is a function of increasing intervals of IQR/M. LSE medians of 7.1 kPa and 12.5 kPa were used as the diagnostic cut-offs for significant fibrosis and cirrhosis respectively, or F2 and F4 on the METAVIR scale, referencing the Castéra et al study from 2005[^4]. The ultimate conclusions drawn by Boursier and colleagues were: 1. the usual definition for
LSE reliability was not relevant, 2. reliability depends upon the IQR/M parameter and 3. three distinct reliability subgroups are a better way to categorize LSE: very reliable, reliable, and poorly reliable (Table 7) [3].

Table 8: LSE reliability categories, adapted from Boursier et al, 2013 [3]

<table>
<thead>
<tr>
<th>Reliability</th>
<th>IQR/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>very reliable</td>
<td>≤0.10</td>
</tr>
<tr>
<td>reliable</td>
<td>&gt;0.10 and ≤0.30</td>
</tr>
<tr>
<td>poorly reliable</td>
<td>&gt;0.30</td>
</tr>
</tbody>
</table>

In their investigation of the reliability of TE, published a year after the Boursier study, Pang et al. indicated that the outdated reliability definition for LSE may have led to the elimination of 10% of results which could have had a potentially significant impact on clinical practice as well as research studies. Using revised reliability definitions, approximately two thirds of these unused results would have been classified as reliable or very reliable [22]. The researchers conducted a large study which took place between July 2008 and June 2011, determining the feasibility and reliability of TE with a participant pool of 2,335 patients. Patient and operator characteristics were selected to represent a routine clinical practice in an effort to determine which properties of both were predictive of poorly reliable results. In this study cirrhosis was presumed at a cut off of ≥12.5 kPa and the reliability of LSM was divided into three categories based on the Boursier
study: very reliable, reliable, and poorly reliable. In the study 29% of measurements were very reliable, 66% were reliable, and 4.9% were poorly reliable. This was based on the reliability standard of a minimum of ten valid measurements with a success rate of ≥60% and an IQR/M≤30%. Poorly reliable measurements were defined as having IQR/M≥30% with median liver stiffness ≥ 7.1 kPa. By standardizing what defined a poorly reliable outcome, researchers were able to isolate patient and procedural characteristics which negatively impacted LSM reliability, they were as follows: older age, male sex, comorbid medical conditions, alcohol abuse, elevated BMI, and higher mean liver stiffness. Despite what researchers hypothesized, operator experience was not found to be a predictor of poor reliability in LSM [22).

**Fibroscan® Limitations**

According to a June 2015 editorial; by Ioan Sporea about the state of liver elastography research there are still drawbacks to the method [33]. Limitations are related to techniques and equipment as well as patient and technician characteristics. The process, which is a nonecho-guided blind method, cannot be performed on patients with ascites; it is also influenced by several confounding factors such as elevated aminotransferase levels, biliary obstruction, and heart failure. The TE machine is also very expensive and must be biannually calibrated. Numerous studies have indicated limitations based on patient weight
and some probe modifications have been made to counteract these, i.e. the XL and S probes.

Necroinflammatory activity flairs, causing increased ALT levels are the main confounding factor for an accurate LSM using TE. These acute hepatic flares which are common in chronic liver disease result in a higher LSM due to the tissue’s response to elevated enzymes. The increased stiffness is not related to fibrosis therefore the TE result gives an inaccurate diagnostic picture. Ascites are a complication at the end stages of liver disease which result from scarring and pressure inside the hepatic vessels causing the accumulation of fluid in the peritoneal cavity leading to abdominal swelling. This condition alters LSM because the shear wave generated by TE does not propagate through a fluid medium. The buildup of fluid in the abdominal cavity of a patient with ascites results in a barrier between the probe and the liver. Heart failure results in a similar effect on LSM as ascites. The pooling of fluid which is the result of congestive heart failure makes obtaining an accurate LSM difficult due to the same limitations on shear wave propagation through a liquid medium. Finally, obesity is a predictor of a poorly reliable LSM. Among the 434 patients in the Pang et al. study who had available BMI data, poorly reliable scans occurred in 10% of patients with a BMI≥30kg/m² compared to 3.1% among non-obese patients [22]. Pang et al. determined numerous patient characteristics which lead
to decreased reliability of TE in their 2014 study (Table 9) \[^{22}\]. The most prominent effects on LSM reliability were observed for obese patients and those with presumed cirrhosis according to the revised reliability standards discussed earlier. Disease etiology and comorbidity clearly affect the results of Fibroscan. In their 2014 review of the clinical utility of Fibroscan as a diagnostic tool, Wilder and Patel included a chart indicating disease type and characteristics of TE for identifying cirrhosis among those diseases (Table 10) \[^{39}\]. The predictive values of the LSE varied among the conditions with the most marked reduction in PPV among patients with HBV. The test involving patients with HCV-HIV coinfection displayed higher PPV and NPV, however, the pKa cut off value for cirrhosis was much higher compared to that used for HCV alone. The authors concluded that different TE cutoff values should be employed depending on the etiology of liver disease and LSM must be cautiously interpreted in the setting of inflammation \[^{39}\].

Table 9: Independent predictors of poorly reliable Fibroscan examinations, Adapted from Pang et al, 2014 \[^{22}\]

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03</td>
<td>1.01-1.05</td>
</tr>
<tr>
<td>Male Sex</td>
<td>1.32</td>
<td>0.87-1.99</td>
</tr>
<tr>
<td>BMI ≥30kg/m(^2)</td>
<td>2.93</td>
<td>0.95-9.05</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>1.58</td>
<td>1.05-2.37</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>2.22</td>
<td>1.31-3.76</td>
</tr>
<tr>
<td>Liver Stiffness</td>
<td>1.03</td>
<td>1.02-1.05</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>5.24</td>
<td>3.49-7.89</td>
</tr>
<tr>
<td>M Probe vs XL probe</td>
<td>0.64</td>
<td>0.38-1.06</td>
</tr>
<tr>
<td>Operator Experience</td>
<td>0.86</td>
<td>0.67-1.10</td>
</tr>
</tbody>
</table>
Table 10: Disease Type and TE Characteristics, taken from Wilder and Patel, 2014 [39]

<table>
<thead>
<tr>
<th>Liver disease</th>
<th>Study</th>
<th># patients</th>
<th>% METAVIR F=4</th>
<th>Cutoff (kPa)</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
<th>AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV</td>
<td>Cardoso et al.</td>
<td>363</td>
<td>9%</td>
<td>≥12.5</td>
<td>0.84</td>
<td>0.94</td>
<td>0.58</td>
<td>0.98</td>
<td>0.93</td>
</tr>
<tr>
<td>HBV</td>
<td>Cardoso et al.</td>
<td>202</td>
<td>8%</td>
<td>≥11</td>
<td>0.75</td>
<td>0.90</td>
<td>0.39</td>
<td>0.98</td>
<td>0.94</td>
</tr>
<tr>
<td>PBC</td>
<td>Corpechot et al.</td>
<td>103</td>
<td>14.5%</td>
<td>16.9</td>
<td>0.93</td>
<td>0.99</td>
<td>0.93</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>PSC</td>
<td>Corpechot et al.</td>
<td>66</td>
<td>14%</td>
<td>14.3</td>
<td>1.00</td>
<td>0.88</td>
<td>0.56</td>
<td>1.00</td>
<td>0.95</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Gaia et al.</td>
<td>72</td>
<td>12.5%</td>
<td>10.5</td>
<td>0.78</td>
<td>0.96</td>
<td>0.70</td>
<td>0.97</td>
<td>0.94</td>
</tr>
<tr>
<td>HCV + HIV</td>
<td>Vergara et al.</td>
<td>169</td>
<td>15%</td>
<td>14.6</td>
<td>0.91</td>
<td>0.88</td>
<td>0.83</td>
<td>0.94</td>
<td>0.94</td>
</tr>
</tbody>
</table>

**Additional Applications**

While transient elastography using Fibroscan is not without limitations, there are still broad applications for the method, particularly since it is noninvasive. There have been numerous studies addressing the utility of TE for the diagnosis and monitoring of liver diseases of many etiologies. Although HCV is the primary etiology which leads to the development of fibrosis and cirrhosis, there are many others such as alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), chronic hepatitis B infection (HBV), etc. Another critical aspect of caring for patients with advanced liver disease is detecting signs of decompensation such as biliary atresia or portal hypertension, fibrosis with steatosis, and many more. Comorbidities are becoming increasingly relevant clinically as well, most prominently with HIV and HCV coinfection. Many studies have indicated that LSM is useful, reliable, and accurate in the detection of
fibrosis and cirrhosis due to etiologies other than HCV. The method is also suited to determine the presence of other complications and characteristics of end stage liver disease.

Until recently noninvasive approaches have proven inaccurate in the early prediction of clinical decompensation in cirrhotic patients [23]. In their 2011 study Robic et al. assessed the accuracy of liver stiffness as a predictor of portal hypertension and other related complications in patients with chronic liver disease. Portal hypertension (PHT) determines, to a great extent, the prognosis of patients with chronic liver disease. The prognostic index for PHT is the hepatic venous pressure gradient (HVPG), the determination of which requires an invasive hepatic vein catheterization. Additionally, this procedure is not offered in all medical settings and is not cost-effective. Several reports which indicated a correlation between liver stiffness and HVPG prompted the researchers to determine if TE had applications in assessing PHT. One hundred patients underwent both invasive HVPG measurement procedures as well as LSM via TE. In previous studies the researchers had determined a pKa threshold value of 21.1 was useful in discriminating between patients with and without significant PHT. Using this previously established criteria there was significant correlation between LSM values and traditional HVPG which indicated elevated PHT. Researchers concluded that LSM is a reliable noninvasive option for both
diagnosing clinically significant PHT and informing the treatment course for
patients with decompensating liver disease[^23].

The comorbidity of HIV and HCV is becoming increasingly prevalent due to
progress in highly active anti-retroviral therapies (HAART) which has dramatically
decreased AIDS-related mortality. Individuals with the HIV infection are living
longer and liver disease is emerging as a leading cause of morbidity. An
alternative to biopsy is particularly important for immune-compromised patients
who are more susceptible to infection and other complications from an invasive
procedure. Vergara et al. published a study in 2007 discussing the use of TE for
assessing liver fibrosis in patients with HIV-HCV coinfection to determine if it was
as reliable as when used to diagnose HCV mono-infected patients[^37]. This was a
cross-sectional study of six tertiary care hospitals and a population169 patients
was included. The diagnostic performance of previously established LS cut off
values was evaluated, determining that 20% of patients had been misclassified
after the comparison of biopsy and LSM data. Diagnostic accuracy of LSM was
very high for detecting cirrhosis but less so for discriminating between mild and
moderate fibrosis. According to Vergara et al., their study results were within the
range of previous reports involving HCV mono-infection. However, TE
performance was less reliable among HIV-HCV infected patients than HIV-
uninfected patients which fulfilled expectations[^37]. Further cutoff value validation
is needed for TE to be equally reliable in this patient population.
CONTINUING RELEVENCE OF LSM

For over fifty years, biopsy has been the standard to which all other disease detection and confirmation methods have been compared. With the advancement of several noninvasive methods, particularly in the detection of liver disease, biopsy has come under scrutiny for its cost-effectiveness, reliability, and safety. Biopsy will never be eliminated as a valuable clinical tool, however, there is a case being made for noninvasive procedures to become the primary method for both detection and monitoring of hepatic diseases. As the number of companies involved in the development of ultrasound-based (US) elastography increases, it is likely that liver elastography modules will be included on all or most US models in the future which will minimize cost[^32]. Additionally, other shear wave elastography (SWE) methods are in the process of validation. Substantial evidence supports the transition from biopsy to only noninvasive methods but it takes time to fully implement new medical standards. Most recently, however, a surge in the development of HCV treatments brings the continued need for noninvasive disease monitoring into question with treatment efficacy at 90% in some cases.

HCV Treatment and Implications

The hepatitis C virus (HCV) infection remains a significant public health burden as the leading cause of liver cirrhosis and hepatocellular cancers with more than
170 million individuals infected globally. As this population ages, the risk of additional complications is increasing and viral eradication is associated with better outcomes. For the last decade, standard HCV treatment based on the combination of pegylated-interferon and ribavirin (PR) was limited by sub-optimal response rates (54-56%) and significant toxicity\textsuperscript{[14]}. Now that the hepatitis C viral lifecycle is well characterized, new treatments which directly interact with replication mechanisms have advanced rapidly. Over the last five years, trials and clinical implementation of these non-interferon based combination therapies called direct-acting antivirals (DAA) are leading HCV treatment in a new direction of shorter duration and improved outcomes.

The evolution of DAA therapies radically changes the previous HCV treatment paradigm by eliminating the use of interferon as well as creating more universally tolerated, highly effective options. An ideal DAA has a high barrier resistance, few drug-drug interactions, minimal toxicity, high potency, and a pharmacokinetic profile which allows for once daily dosing\textsuperscript{[14]}. Among the growing DAA classes are protease inhibitors, polymerase inhibitors, nucleos(t)ide inhibitors, structural protein inhibitors, and host-targeting agents all of which have shown varying degrees of efficacy. Drugs of this kind have greater specificity and are less likely to affect a patient’s immune mechanisms. Patient characteristics which affect therapeutic viability are whether the individual is treatment-naïve, the viral load,
advanced liver disease, and comorbid conditions. The variables noted lead researchers to construct trials which manipulated treatment intervals in an effort to study relapse rates and minimum effective durations. While many of these new treatments remain in various stages of clinical development, approved DAA’s have dramatically improved twelve week success rates up to >90% for most HCV genotypes. With continued advancement of DAA’s, reduction in treatment duration for therapeutic effect and prohibitive drug costs will need to be addressed.

Approved by the FDA in 2014, HARVONI® is a combination of two drugs which inhibit viral replication, Ledipasvir and Sofosbuvir. The former is a phosphoprotein inhibitor which prevents the assembly and secretion of HCV from the host cell, while Sofosbuvir mimics a natural nucleotide and inserts itself into the polymerase acting on the RNA primer strand of HCV thus leading to replication termination. At a fixed oral dose of 90 mg/400 mg taken once daily studies showed varying success based on patient characteristics; i.e. treatment naive or experienced, with or without comorbidities, and with or without cirrhosis [17]. These characteristics also affected duration of treatment with twelve weeks as the least time to achieve viral reduction to within a specified range. In their 2015 study McQuaid et al. found that less than 2% of their 327 participants discontinued treatment due to adverse reactions [20] indicating that HARVONI is
well-tolerated. An overall efficacy of 97% was observed in participants with genotype 1, meaning those who were treatment naïve and diagnosed with HCV but no cirrhosis or comorbid conditions [20]. However, the cost of this treatment is prohibitive ranging from $63,000 to $189,000 depending on the duration of therapy: eight weeks to twenty-four weeks.

The success of DAA therapy raises a question about the continued relevance of improving upon and utilizing noninvasive diagnostic and monitoring methods. While an argument exists against the merit of ongoing disease assessment, initial diagnoses will always be necessary. Improved treatment methods only address existing disease cases, making diagnostic procedures a perpetual need. Fibroscan, Fibrotest, and the other methods discussed can only be obsolete when there are universal vaccines for viral liver diseases, however, this disregards all etiologies which are not viral in nature. Additionally, the cost of direct-acting antiviral therapies will make that treatment modality impossible for some patients, particularly those in underdeveloped nations whose access and resources are already limited. Since the WHO has recognized the public health implications of HCV, most evidence supports a growing global disease burden requiring comprehensive strategies for treatment and containment. Noninvasive diagnostics, transient elastography in particular, remain a viable and necessary option for clinical settings worldwide.
REFERENCES


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• made follow up care plans and coordinated with other facilities
• fulfilled on site and academic department supervision requirements
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- assisted in exercise programs
- kept schedule and appointments
- performed household tasks and errands

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Cardiovascular Health Specialists
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- organized laboratory and prescription records
- performed EKG’s

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- maintained a calm learning environment

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