Non-Invasive Assessment of Liver Fibrosis

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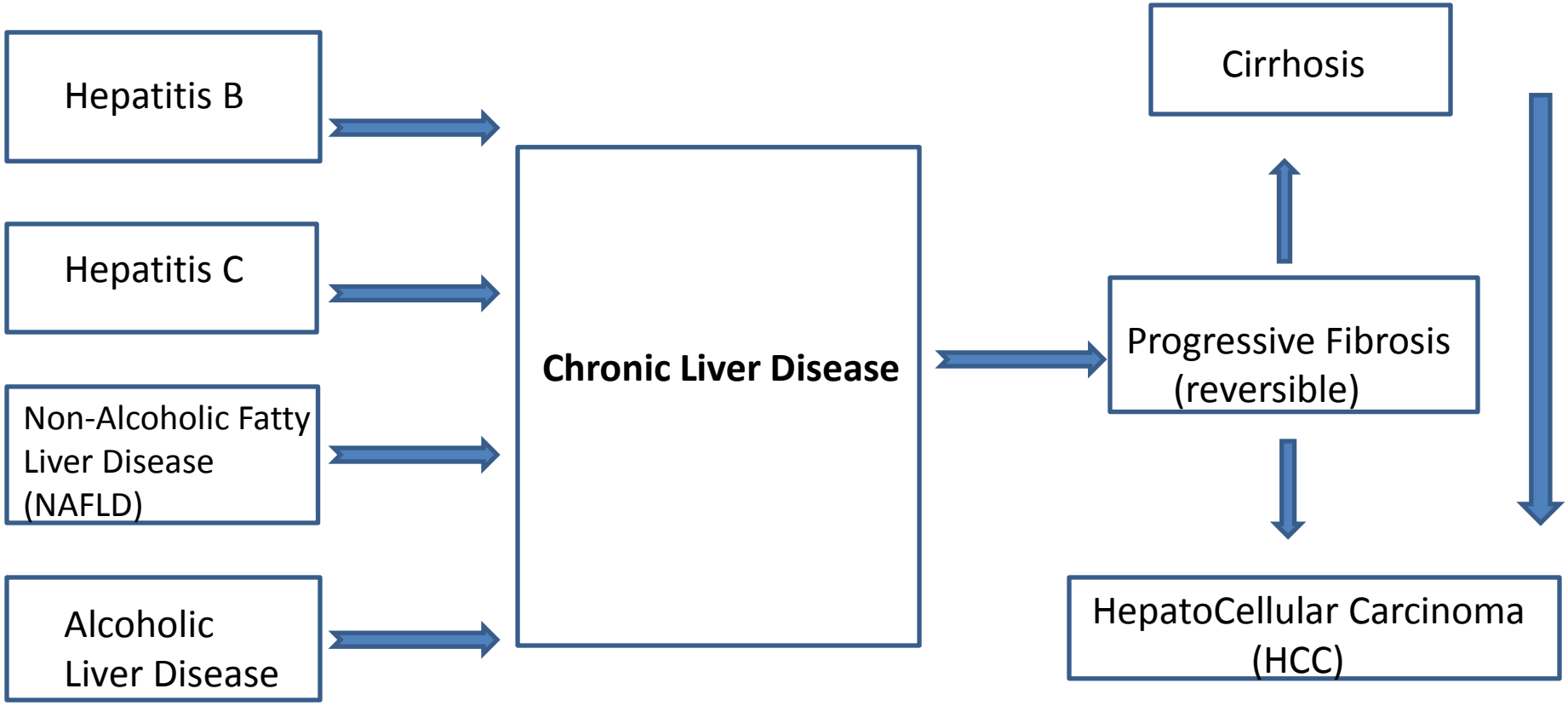
Disclosure

- Patricia Slev has no relevant financial relationships to disclose

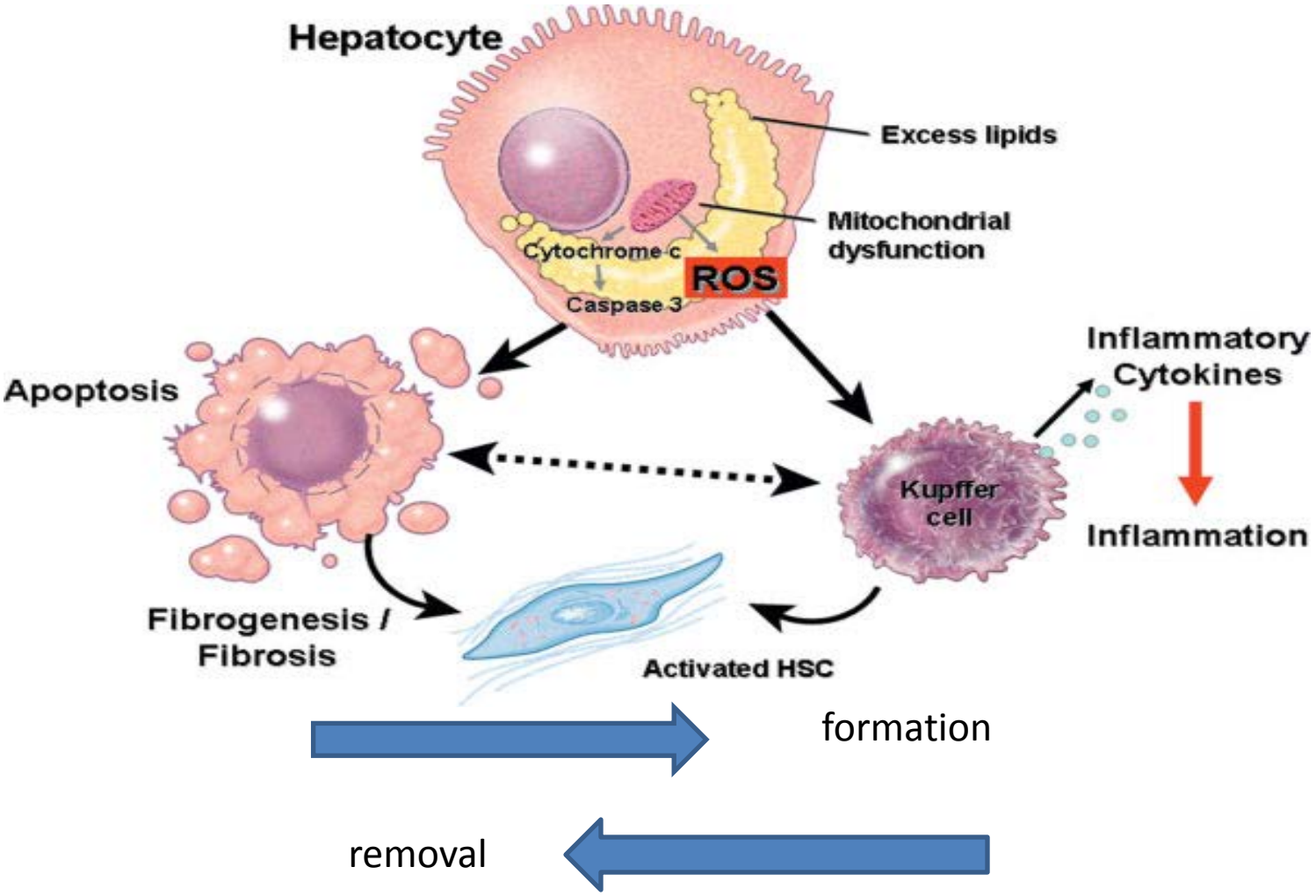
Outline

- Chronic liver disease & pathogenesis of liver fibrosis
- Non-invasive serum markers for assessing liver fibrosis
- Compare and contrast currently available surrogate serum marker assays for different chronic liver disease etiologies
- Combination algorithms of serum biomarkers or serum biomarkers and elastrography for increased accuracy for assessing liver fibrosis

Chronic Liver Disease

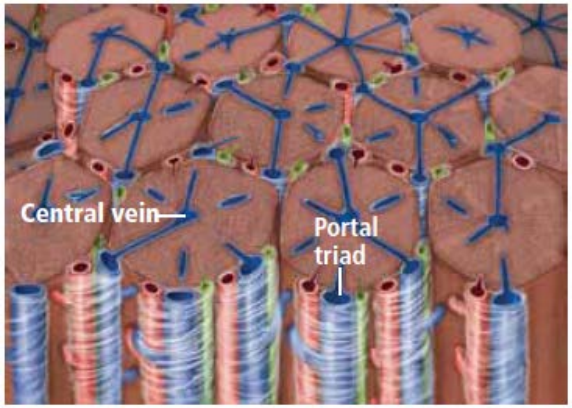


Liver Disease and Pathogenesis

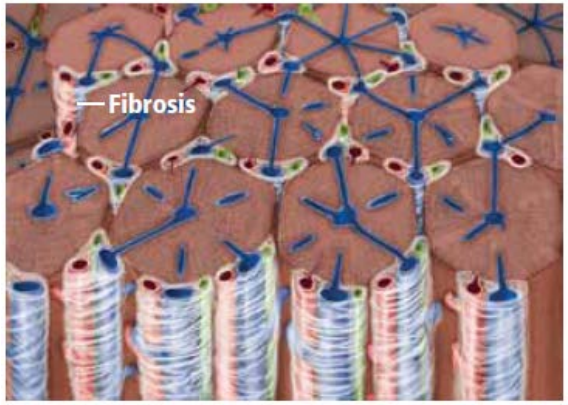


Seminars in Liver Disease/volume 28, Number 4 2008

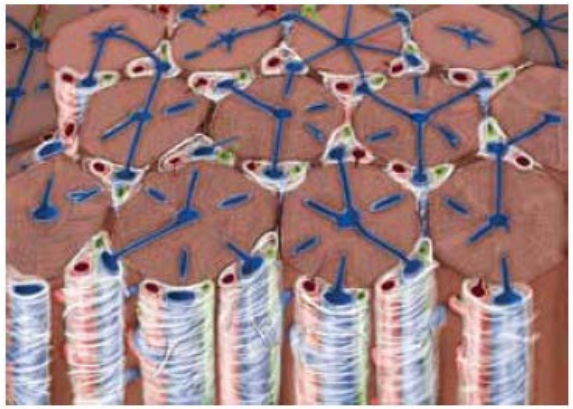
Stages of Fibrosis



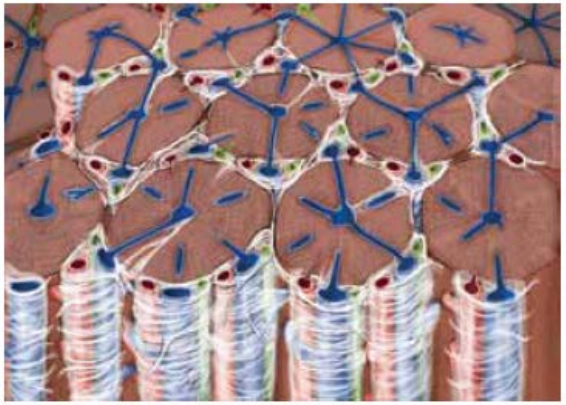
Stage 0 (normal): No fibrosis surrounding portal triads.



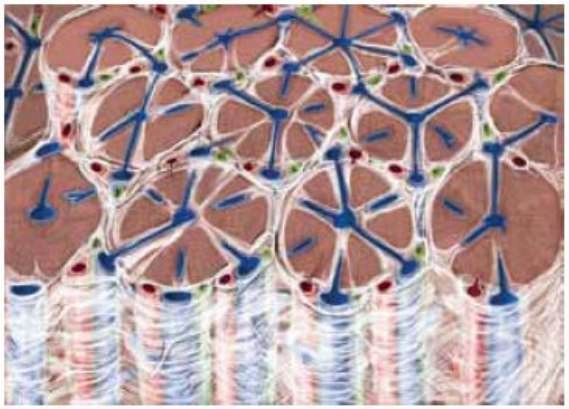
Stage 1 (portal fibrosis): Fibrous connective tissue surrounds portal triads but is limited to those areas.



Stage 2 (periportal fibrosis): Fibers begin to extend into the periportal space but do not connect any portal area to any other.



Stage 3 (septal fibrosis): Fibrous connective tissue now links neighboring portal triads and begins to extend to the central veins and to distort the shape of the lobules.

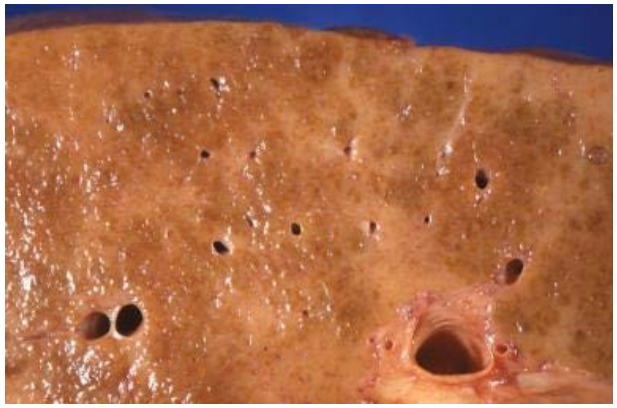


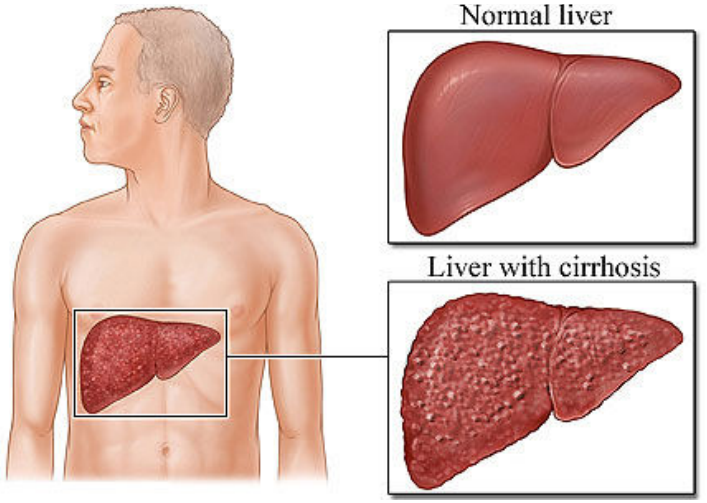
Stage 4 (cirrhosis): Most portal areas connected by fibrous tissue and some portal areas and central veins connected. Hepatocyte clusters surrounded by fibrous tissue producing sclerotic nodules.

Cleve Clin J Med 2010 77(8):519–527

Cirrhosis

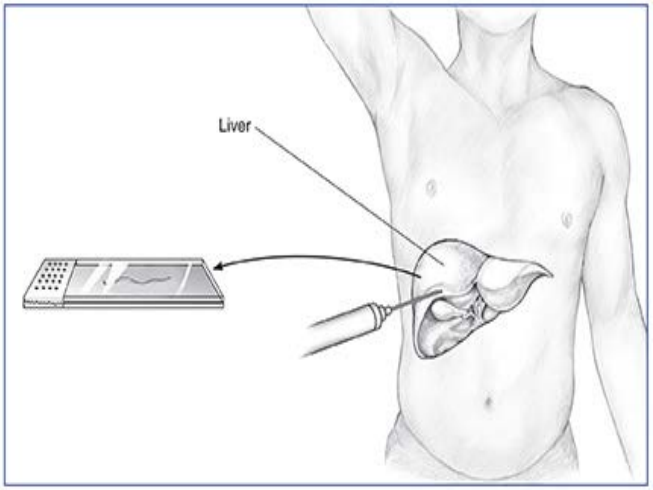
- End stage chronic liver disease
- Irreversible ?
- Portal hypertension, ascites, bleeding disorders and liver failure
- Hepatorenal syndrome





The Reference Standard - Biopsy

- Histological assessment for management of liver disease
  - diagnosis
  - stage
  - prognosis



Role of Liver Biopsy

Etiology	Diagnosis	Staging	Prognosis	Management
Hepatitis B	no	yes ++++	yes +(+)	yes ++
Hepatitis C	no	Yes ++++	Yes +(+)	Yes ++++
NAFLD/NASH	yes +++	yes +++	yes +(+)	yes (+)
Autoimmune Hepatitis /AIH	yes	yes	yes	yes

Scoring Scales
Histological Stage (Fibrosis)

Description	IASL	Metavir (F)	Batts-Ludwig (stage)
No Fibrosis	No Fibrosis	0	0
Portal Fibrosis w/o septa or bridging	Mild Fibrosis	1	1
Portal fibrosis with few septa or bridges	Moderate Fibrosis	2	2
Septal fibrosis with numerous bridges w/o cirrhosis	Severe Fibrosis	3	3
Cirrhosis	Cirrhosis	4	4

Scoring Scales
Histological Grade (Inflammation)

Description	IASL	Metavir	Batts-Ludwig
No inflammation No activity	Minimal chronic hepatitis	A0	0
Mild inflammation Mild activity	Mild chronic hepatitis	A1	1
Moderate inflammation Moderate activity	Moderate chronic hepatitis	A2	2
Severe Inflammation Severe activity	Severe chronic hepatitis	A3	3

Problems with Liver Biopsy

- Invasive
  - Risks include pain, hypotension, bleeding, pneumothorax, infection
  - Contraindicated in certain patient populations

- Sample variation
  - Needle biopsy produces small tissue sample (1/50,000 of liver)
  - Grading/staging accuracy influenced by sample size and location

Differences Between Right and Left Lobes	Number of Patients	% of Total
Identical grade	94	75.8
Different grade (total)	30	24.2
Difference of one grade	28	22.6
Difference of two grades	2	1.6
Grade 1–2 in one lobe *vs* 3–4 in the other	5	4.0

Differences Between Right and Left Lobes	Number of Patients	% of Total
Identical stage	83	66.9
Different stage (total)	41	33.1
Difference of one stage	38	30.6
Difference of two stages	3	2.4
Stage 0–2 in one lobe *vs* 3–4 in the other	12	9.7

Am J Gastroenterol 2002 97(10):2614–2618

- Intraobserver variation
  - Accuracy of biopsy interpretation influenced by pathologist experience

Non-Invasive Tests for Assessment

- Useful in patients who cannot undergo biopsy
- Can limit the number of biopsies performed
- Can be used to serially monitor disease progression
- Imaging
  - Ultrasonography
  - Computed tomography
  - Transient elastography

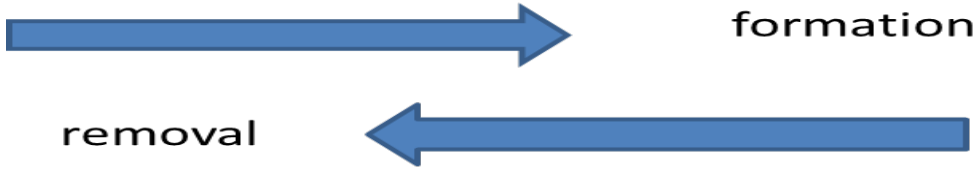
- Non-invasive markers (NIMs)
  - **direct** - fragments of liver matrix components produced by hepatic stellate cells during remodeling
  - **indirect –** markers present in increased concentration due to inflammation or impaired liver function

Biopsy vs. Non-invasive Test Comparison

	Liver biopsy	Non-invasive test
Advantages	Direct; semi-quantitative; evaluation of co-existing pathologies	Measurement of global fibrosis; suitable for serial observations
Limitations	Sampling error; intra-observer variability; possible hospitalization	Indirect
Risks	Pain; bleeding; pneumothorax; hemothorax; infection	None
Cost	Expensive	Varies but usually less than biopsy
Contraindications	Uncooperative patient; severe coagulopathy; extrahepatic biliary obstruction; ascites; morbid obesity	Non-hepatic influences on biomarkers (hemolysis, Gilbert's syndrome; thrombocytopenia, etc.)

Direct Tests

- Tests not routinely performed in clinical lab



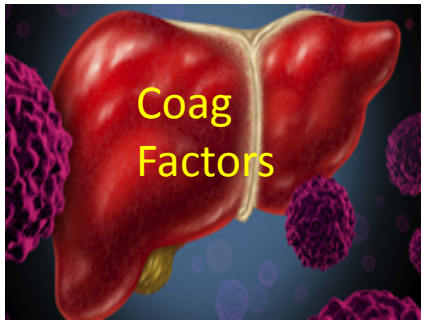
ECM Remodeling

Category	Examples
ECM enzymes	• Prolyl-hydroxylase • Lysyl-oxidase • Collagen peptidase
Fragments of collagen degradation	• Procollagen type I, type III , IV and VI
Glycoproteins & MMPs	• Laminin • MMP-2 • Vitronectin • ICAM • VCAM • TIMP-1 and TIMP-2
Glycosaminoglycans	• Hyaluronic acid
Cytokines	• TGF-β

Indirect Tests

- Markers that reflect the functional alterations of the liver
  - impairment
  - inflamamtion
- Tests commonly performed in clinical lab (some exceptions)

Test name	Constituents
AST/ALT ratio	• AST • ALT
AST/Platelet ratio	• AST • Platelet count
FibroSure (FibroTest)	• GGT • ALT • Bilirubin • Haptoglobin • Apo A1 • α2 macroglobulin
HepaScore	• GGT • Bilirubin • α2 macroglobulin • Hyaluronic acid
FibroMeter (viral/ALD/NAFLD)	• Platelet count • PT index • ALT • AST • GGT • α2 macroglobulin • Hyaluronic acid • Ferritin • Glucose • Urea



Combined Biomarkers & Algorithms

- APRI
- Fibrotest/Fibrosure
- Fibrospect II
- Fibrometer
- Others – HepaScore, Fib-4, Forns and European Liver Fibrosis (ELF)

AST/Platelet Ratio Index (APRI)

- Derived and validated from chronic HCV

$$APRI = \frac{(AST/ULN)}{PLT x 10^9/L} x100$$

	Significant Fibrosis (47% prevalence)	Cirrhosis (15% prevalence)
Rule in	>1.5 (PPV 88%)	>2.0 (PPV 57%)
Rule out	<0.5 (NPV 86%)	<1.0 (NPV 98%)

Hepatology 2003 38(2):518–526

Test Threshold and Outcome	Number of Studies (Patients)	Summary Sensitivity (95% CI)	Summary Specificity (95% CI)
Significant Fibrosis			
~ 0.4 (0.38-0.42)	4 (717)	86% (54-97%)	54% (49-59%)
0.5	16 (3,277)	81% (76-86%)	50% (47-52%)
0.7	3 (438)	84% (78-88%)	70% (63-76%)
1.0	2 (473)	59% (48-70%)	86% (81-89%)
1.5	15 (3,146)	35% (30-41%)	91% (89-92%)
Cirrhosis			
1.0	9 (2,057)	76% (68-82%)	71% (69-73%)
2.0	8 (1,946)	49% (43-55%)	91% (90-93%)

Hepatology 2007 46(3):912-921

- Best at excluding significant fibrosis and cirrhosis

@50% prevalence NPV is 75%
@30% prevalence NPV is 86%

@15% prevalence NPV is 91%

Avoids ~30% of biopsies

Fibrosure Test Family

FibroSure Test	Age	Gender	Height	Weight	α2-macroglobulin	Haptoglobin	Apo A1	Bilirubin	GGT	ALT	AST	Cholesterol	Triglyceride	Glucose
HCV	✔	✔			✔	✔	✔	✔	✔	✔				
ASH & NASH	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

- Each test type utilizes proprietary algorithms that evaluates surrogate biomarker concentration and provide a score indicative of fibrosis stage and grade

Fibrosure Scale

FibroTest	METAVIR Fibrosis stage estimate
0.75-1.00	**F4**
0.73-0.74	**F3-F4**
0.59-0.72	**F3**
0.49-0.58	**F2**
0.32-0.48	**F1-F2**
0.28-0.31	**F1**
0.22-0.27	**F0-F1**
0.00-0.21	**F0**

Comp Hepatol 2004 3(1):8

Fibrosure Results

	Fibrosis Stage	Fibrosis Grade	Steatosis Grade	Alcoholic Steatohepa-itis Grade	NASH Assessment
HCV	0.0-1.0 (Metavir F0-F4)	0.0-1.0 (Metavir A0-A3)			
ASH	0.0-1.0 (Metavir F0-F4)		0.0-1.0 (S0-S3)	0.0-1.0 (ASH 0-ASH 3)	
NASH	0.0-1.0 (Metavir F0-F4)		0.0-1.0 (S0-S3)		No, Borderline, Yes (N0-N2)

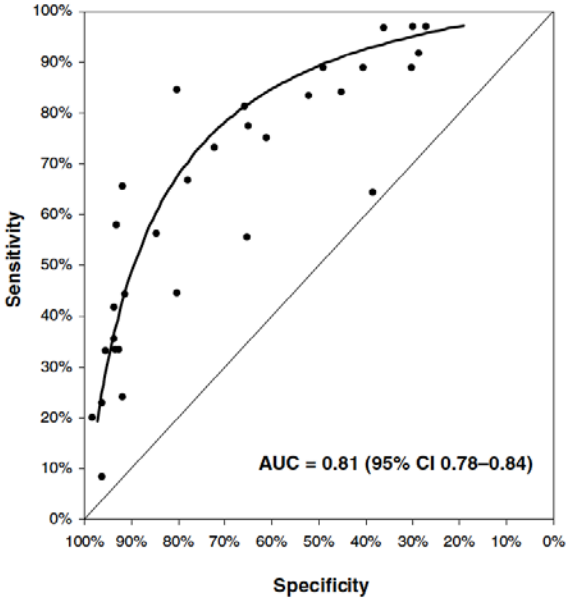
Fibrosure Performance by Panel

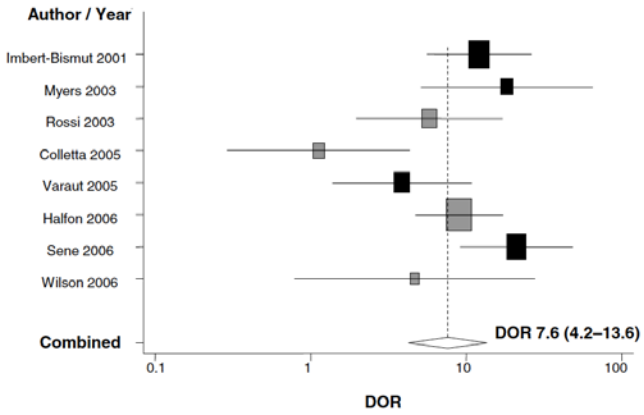
Fibrosure Panel	AUROC		Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	≥F2	F4	≥F2	F4	≥F2	F4	≥F2	F4	≥F2	F4
HCV	0.74-0.87	0.71-0.87	65-77	50-87	72-91	70-93	76-80	58-93	67-87	44-91
HBV	0.78-0.85	0.76	54-81	56	80-90	96	53-96	90	64-81	87
ASH	0.79-0.89	0.94-0.95	55-84	91-100	66-93	50-87	82-93	47-76	70-53	96-99
NASH	0.75-0.86	NA	71-83	NA	74-78	NA	53-56	NA	84-94	NA
HIV/HCV HIV/HBV	0.77-0.85	0.87	66-97	75-100	65-92	65-85	80-86	30-50	61-93	94-99

Clin Chem Lab Med 2011 49(1):13-32

Fibrosure: Review

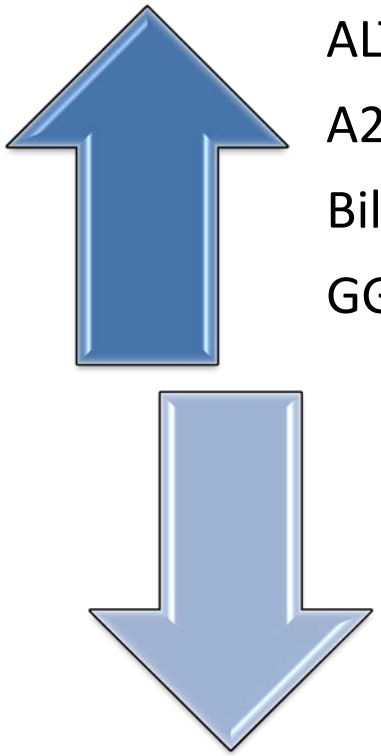
- 71 studies of Fibrosure identified (62 excluded)
  - 9 studies included (4 by developers of Fibrosure)
- Population included 1,679 patients with HCV
  - 45% significant fibrosis (F2-F3)
  - 9% cirrhosis (F4)
- Reasonably accurate for detecting significant fibrosis
  - Low result excludes significant fibrosis
- Better at non-invasive diagnosis of cirrhosis
  - AUROC = 0.90
  - DOR = 16.3
- Intermediate Fibrosure results are common and poorly differentiate fibrosis stage





Am J Gastroenterol 2007 102(11):2589-2600

Fibrosure Limitations



ALT

A2-macroglobulin

Bilirubin

GGT

Apo A1

Haptoglobin

- False positive results
  - Hemolysis
    - Decreased haptoglobin
    - Ribavirin therapy for HCV
  - Extrahepatic cholestasis; Gilbert's syndrome
    - Increased bilirubin
  - Inflammation
    - Increased α2-macroglobulin
  - Acute hepatitis
- False negative results
  - Inflammation
    - Increased haptoglobin

Fibrometer

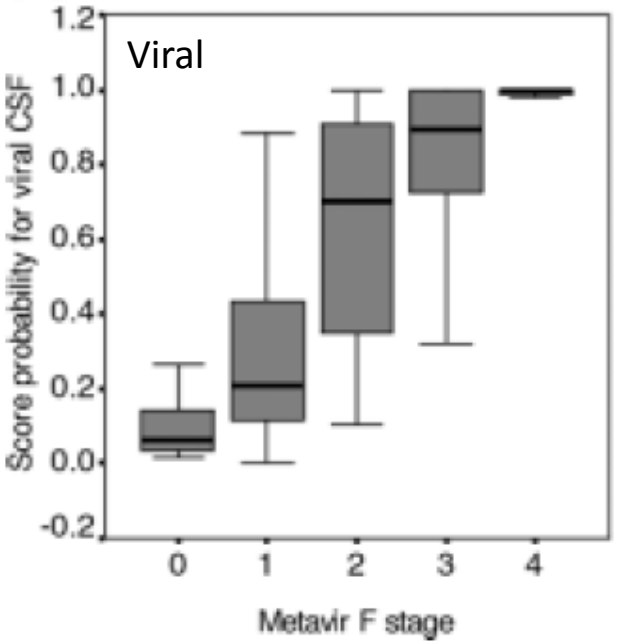
- Developed at the University of Angers (France) and first described in 1997
  - 2nd generation test in 2005
  - 3rd generation test in 2010
- Available only in Europe and now in the US
  - Lab performs the tests and send results to Echosens for score calculation
- 3 FibroMeter Assays
  - Chronic viral hepatitis (HBC, HCV, HIV-coinfection)
  - Alcoholic liver disease
  - Non-alcoholic fatty liver disease
- Provides scores for
  - Fibrosis stage (Metavir)
  - Inflammation
  - Area of fibrosis (percent)
- Results evaluated by an “expert system” to detect discordant results of component tests
  - Eliminates analyte from algorithm to correct possible false-positive/negative results

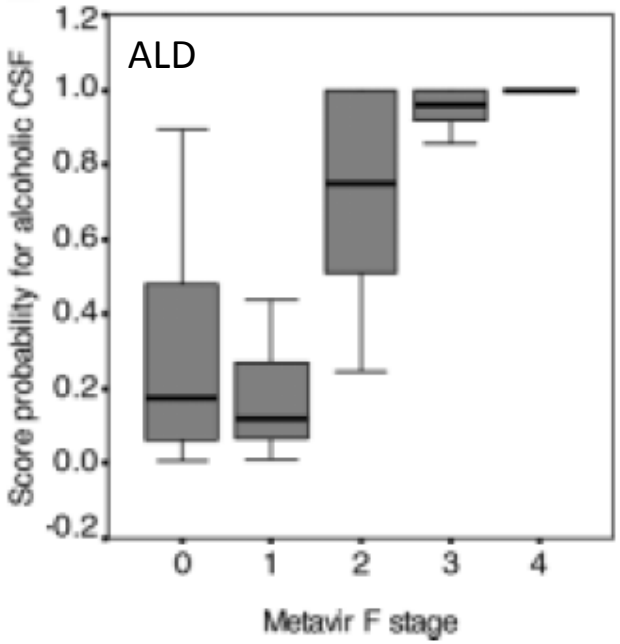
Fibrometer Test Family

FibroMeter	Parameter	Age	Gender	Weight	α2 macro	Hyaluronic acid	PT Index	Platelets	AST	Urea	GGT	ALT	Ferritin	Glucose
Viral	Fibrosis score	✔	✔		✔		✔	✔	✔	✔	✔	✔		
	Cirrhosis score	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔		
	Activity score				✔		✔	✔				✔		
ALD	Fibrosis score	✔	✔		✔	✔	✔							
	Area of fibrosis				✔	✔	✔	✔						
NAFLD	Fibrosis score	✔	✔	✔				✔	✔			✔	✔	✔
	Area of fibrosis					✔	✔	✔	✔			✔		✔

- Hyaluronic acid is used for NAFLD for estimating liver fibrosis area

Fibrometer Performance





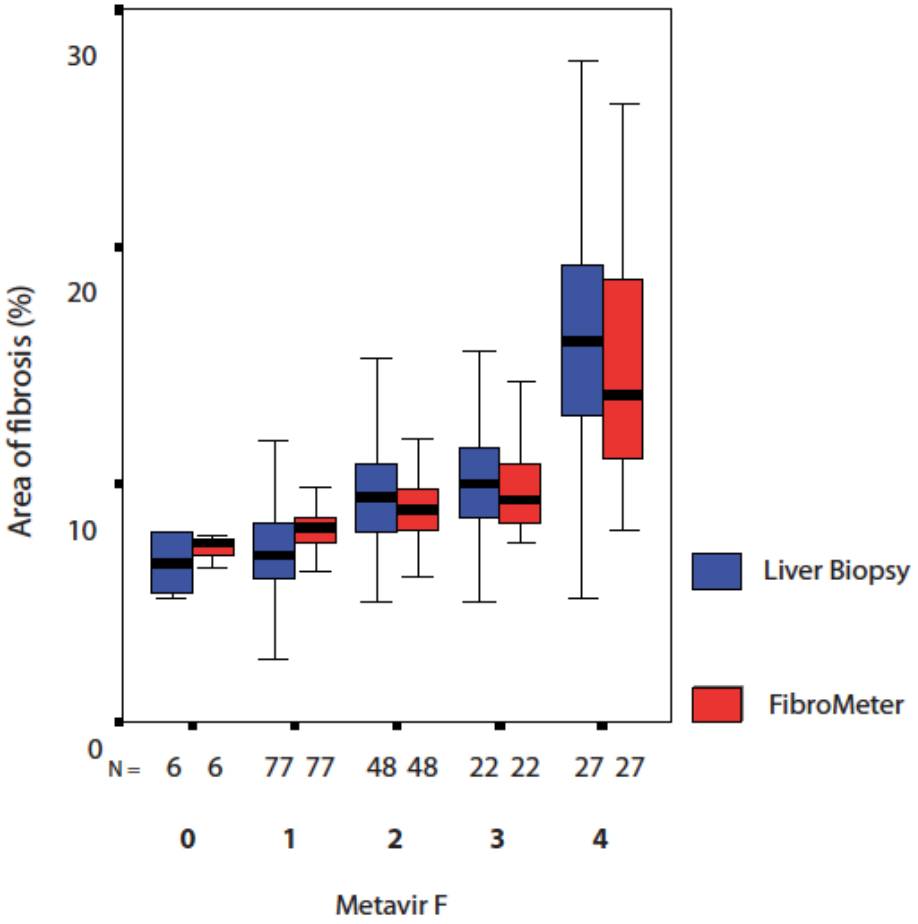
0.5 cutoff	Viral	ALD
Sens (%)	81	92
Spec (%)	84	93
PPV (%)	86	97
NPV (%)	78	83
For stage ≥F2		

AUROC

Test	Exploratory Population			Validation Population		
	Personal	Native	r_{ic}	Personal	Native	r_{ic}
Fibrometer	0.883 ± 0.019	–	–	0.907 ± 0.027	0.892 ± 0.029*,†	0.88, $P < 10^{-4}$
Fibrotest[8]	0.820 ± 0.026‡	0.808 ± 0.027§	0.95, $P < 10^{-4}$	0.857 ± 0.036‖	0.871 ± 0.034¶,#	0.89, $P < 10^{-4}$
APRI[10]	–	0.794 ± 0.028**	–	–	0.822 ± 0.037††	–
Fibrospect[12]	–	–	–	0.869 ± 0.034‡‡	–	–
ELFG[11]	–	–	–	0.834 ± 0.037§§	–	–
Forns[9]	–	0.820 ± 0.030‖‖	–	–	0.864 ± 0.059¶¶	–

Hepatology 2005 42(6):1373-1381

Fibrometer Performance



- Area of fibrosis estimated by FM showed less variability than when done by biopsy

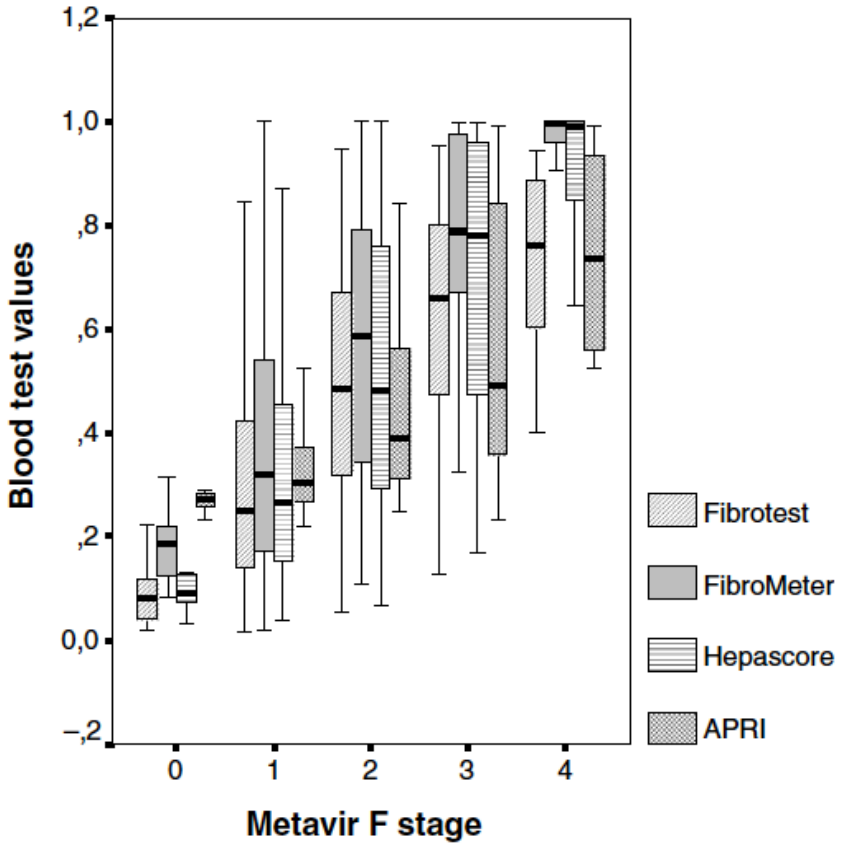
Hepatology 2005 42(6):1373-1381

Fibrometer Performance by Panel

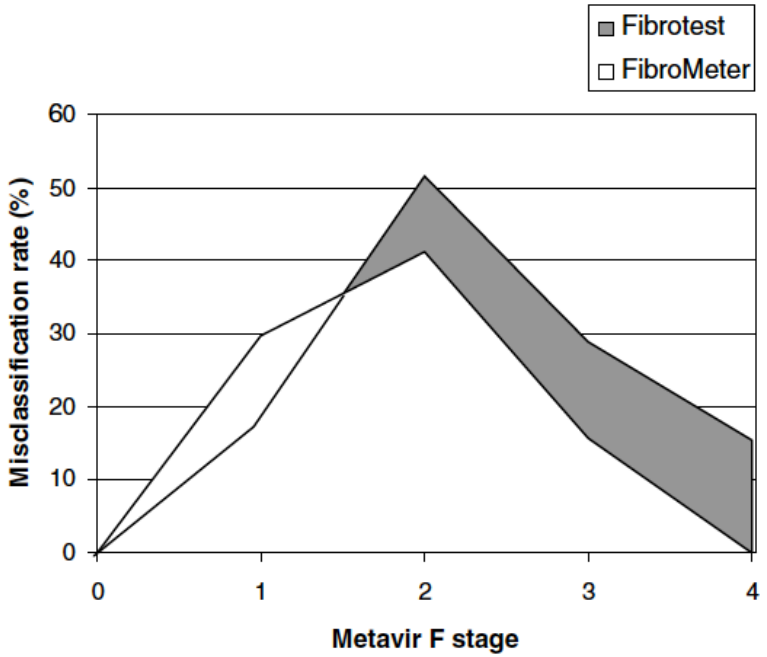
FibroMeters Panel	AUROC		Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	≥F2	F4	≥F2	F4	≥F2	F4	≥F2	F4	≥F2	F4
HCV/HBV	0.85-0.95	0.91	81-89	94	84-90	88	82-86	68	78-83	95
ALD	0.82-0.88	0.85-0.94	92	NA	93	NA	97	NA	83	NA
NAFLD	0.94	0.90	79	NA	96	NA	88	NA	92	NA
HIV/HCV HIV/HBV	0.74-0.89	0.89	73	81	68	85	78	52	62	96

Clin Chem Lab Med 2011 49(1):13-32

Fibrometer vs Fibrotest(sure)



- Tests that include HA (FM and HS) had highest likelihood ratios and narrower score ranges for stages F3 and F4



- FT better than FM at stage F1 (19 vs. 30% misclassification rate)
- FM better than FT at all other stages, particularly F4

J Hepatol 2007 46(3):395-402

AUROC for Liver Fibrosis Biomarkers

Marker	Type of Chronic Liver Disease (CLD)			AUROC Advanced Fibrosis	AUROC cirrhosis	Number of Studies
	CHC	CHB	NAFLD			
Fibrometer	0.892		0.943	0.88-0.96	0.94	4
Fibrospect II	0.77			0.77-0.83		3
ELF	0.773		0.873	0.77-0.98		2

Chronic Hepatitis C - CHC
Chronic Hepatitis B – CHB
Non-Alcoholic Fatty Liver Disease (NAFLD)
Alcoholic Liver Disease - ALD

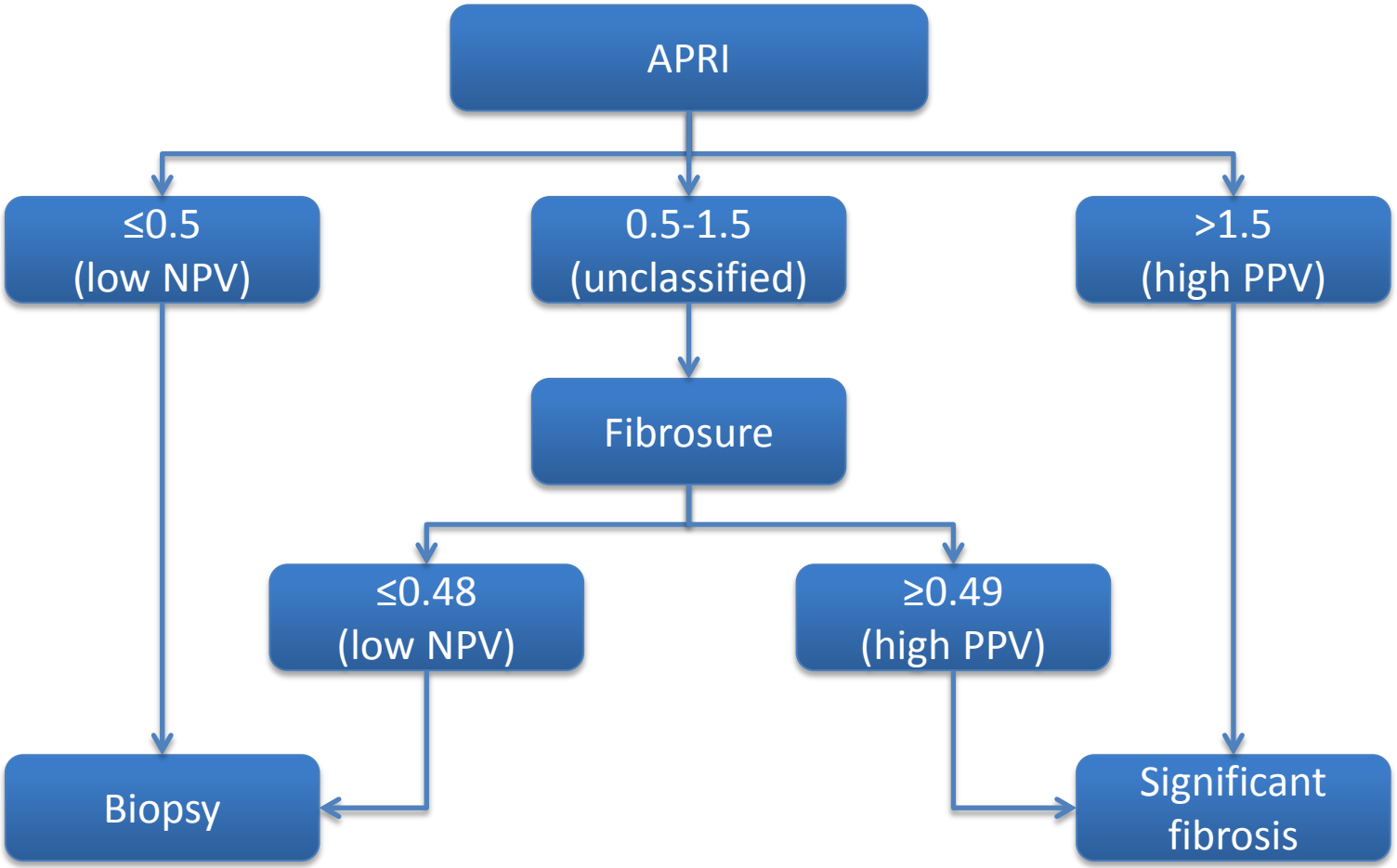
Non-invasive test algorithms

- Non-invasive markers of do not surpass 75–80% diagnostic accuracy which limits their implementation in clinical practice

- Accuracy may be improved by combining non-invasive tests into diagnostic algorithms
  - Limit biopsy to those patients in which noninvasive markers have reduced accuracy

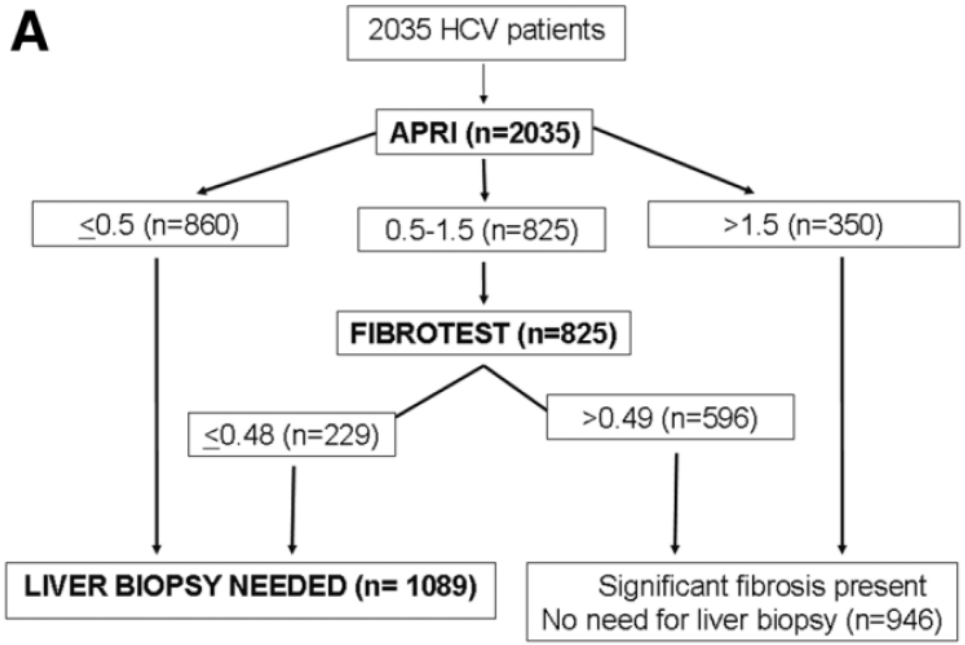
- Sequential Algorithm for Fibrosis Evaluation (SAFE)
  - 2,035 HCV patients undergoing biopsy
    - 46% with significant fibrosis
    - 9% with cirrhosis
  - APRI + Fibrosure performed on blood collected at biopsy

“Safe” biopsy for significant fibrosis



Adapted from *J Hepatol* 2006 44(4):686-693

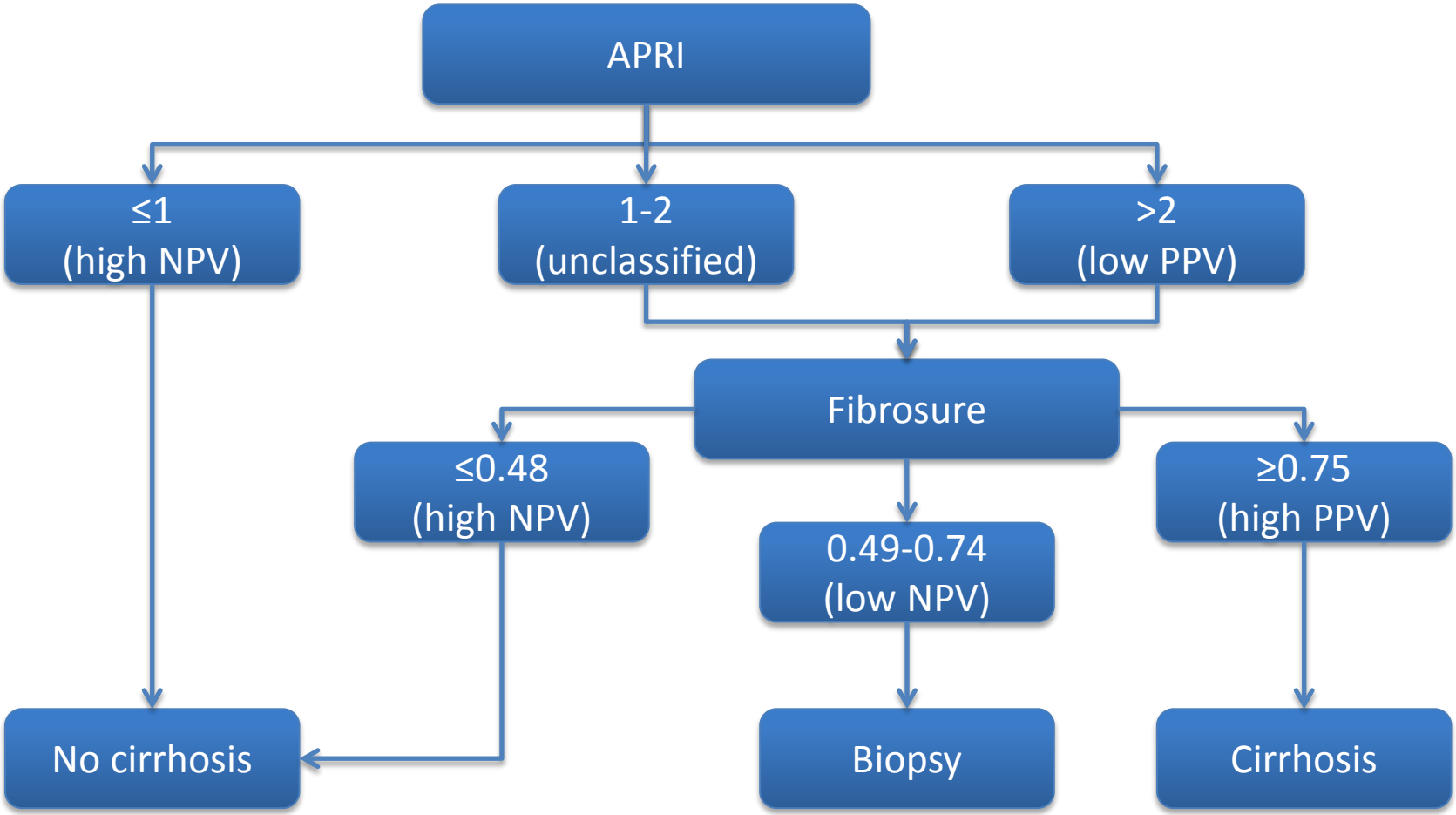
“Safe” biopsy for significant fibrosis



- AUROC of 0.89 (95% CI 0.87-0.90)
- 1,089 (54%) would require biopsy
- 202 (9.9%) had discordant results compared to biopsy

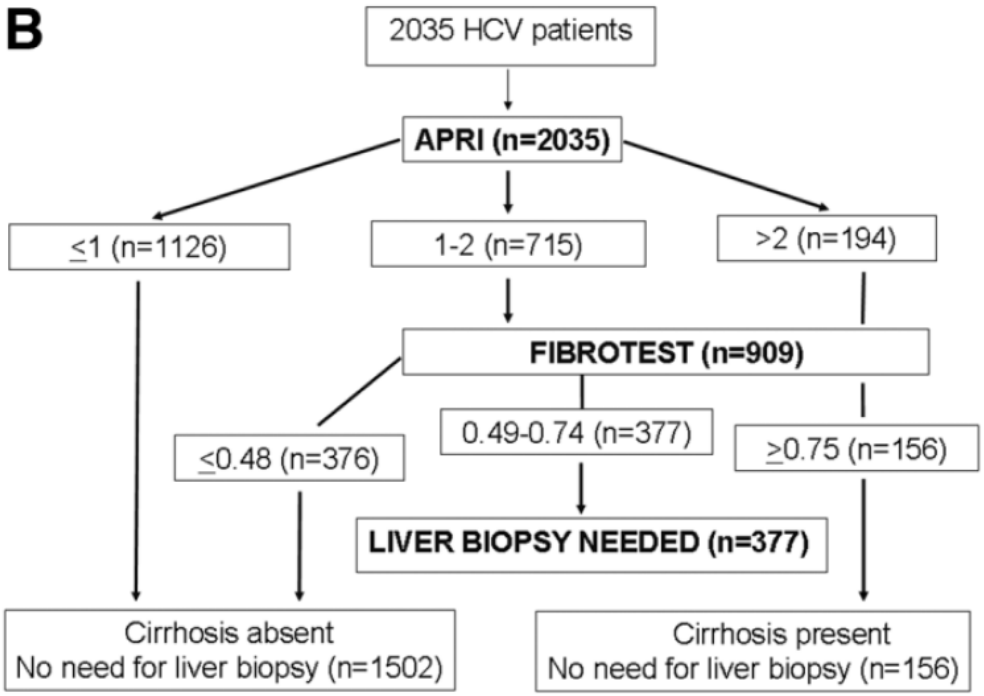
Hepatology 2009 49(6):1821-1827

“Safe” biopsy for cirrhosis



Adapted from *J Hepatol* 2006 44(4):686-693

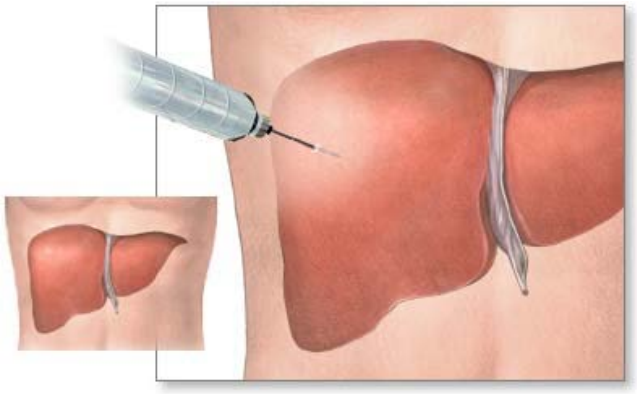
“Safe” biopsy for cirrhosis



- AUROC of 0.92 (95% CI 0.89-0.94)
- 377 (18%) would require biopsy
- 153 (7.5%) had discordant results compared to biopsy

Hepatology 2009 49(6):1821-1827

Liver Fibrosis Assessment



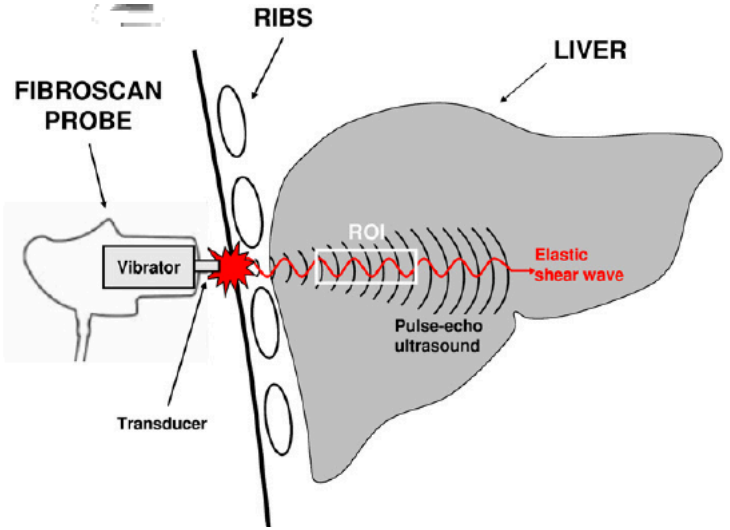
biopsy

serum biomarkers

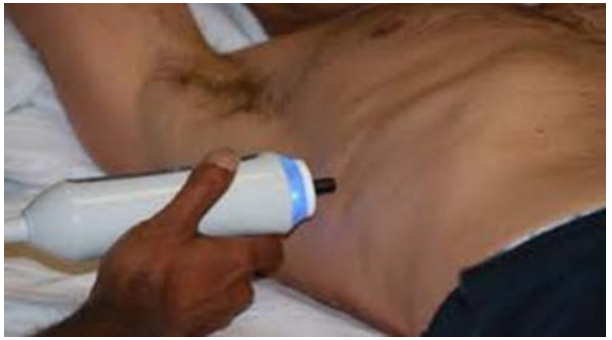
transient elastography

Transient Elastography

- Ultrasound-based measurement of liver stiffness
- Transducer probe mounted on axis of a vibrator
- Vibrator induces an elastic shear wave that propagates through underlying tissue



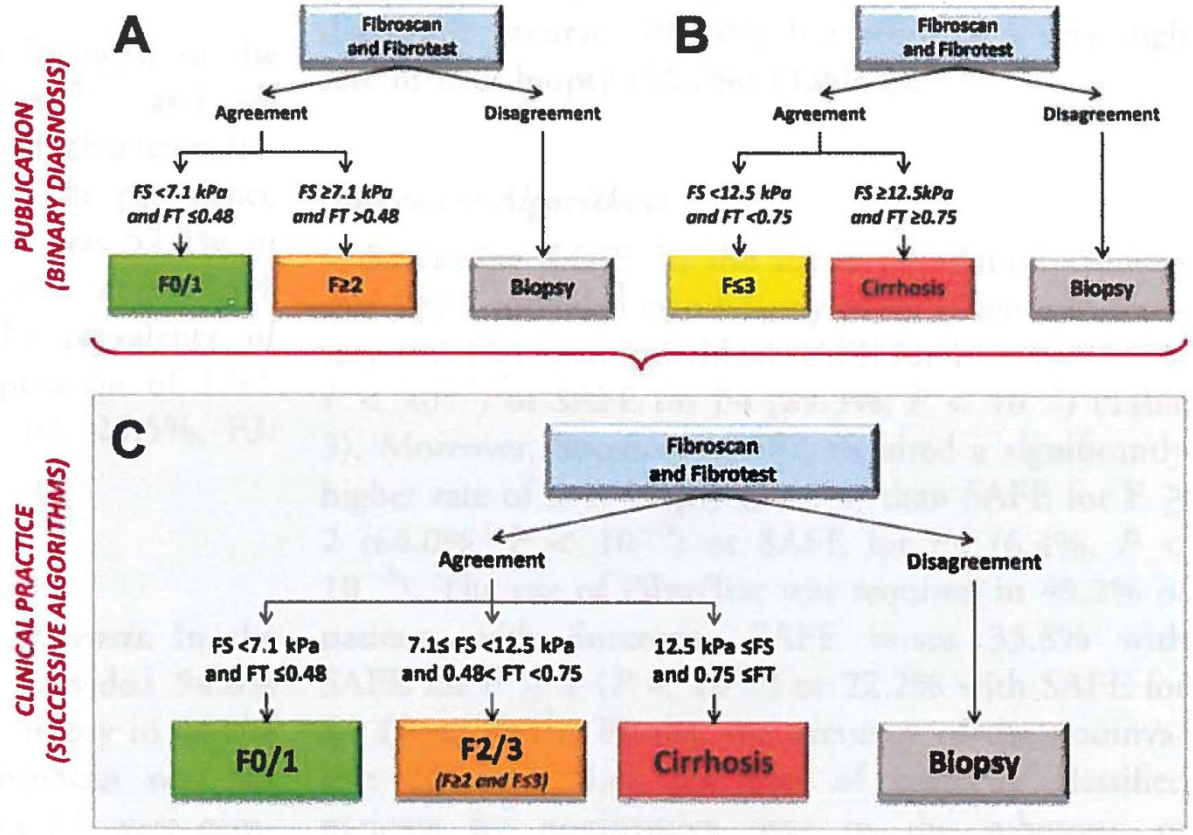
- Pulse-echo ultrasound measures velocity of shear wave which is directly related to tissue stiffness
- The stiffer the tissue, the faster the shear wave propagates
- Patented device marketed as FibroScan (Echosens, Paris, France)
- FDA-cleared



Transient Elastography

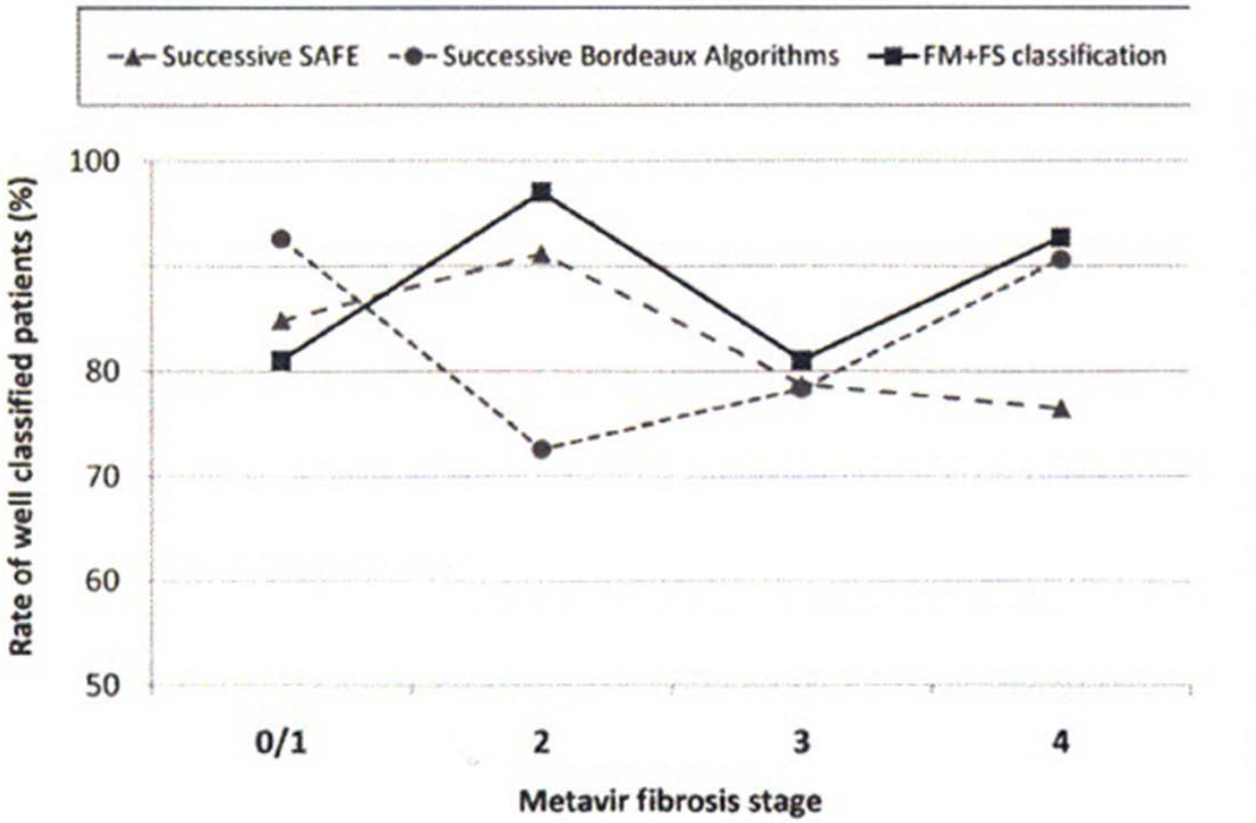
- Liver stiffness values range from 2.5 to 75 kPa
- Result interpreted against cut-offs (vary by study)
  - No fibrosis <5 kPa
  - Significant fibrosis 7.1–8.7 kPa
  - Cirrhosis 12.5–14.5 kPa
- FibroScan accuracy similar to blood-based tests and is best for the diagnosis of cirrhosis
  - Meta-analysis concluded that TE is not sufficiently sensitive for the diagnosis of significant fibrosis (*J Heptaol* 2011 45:650-659)
- Measurement limitations
  - Difficult in obese patients or in those with narrow intercostal space
  - Impossible in patients with ascites

Fibroscan with Fibrotest (Bordeaux Algorithm)



Hepatology. 2012;55:58-67.

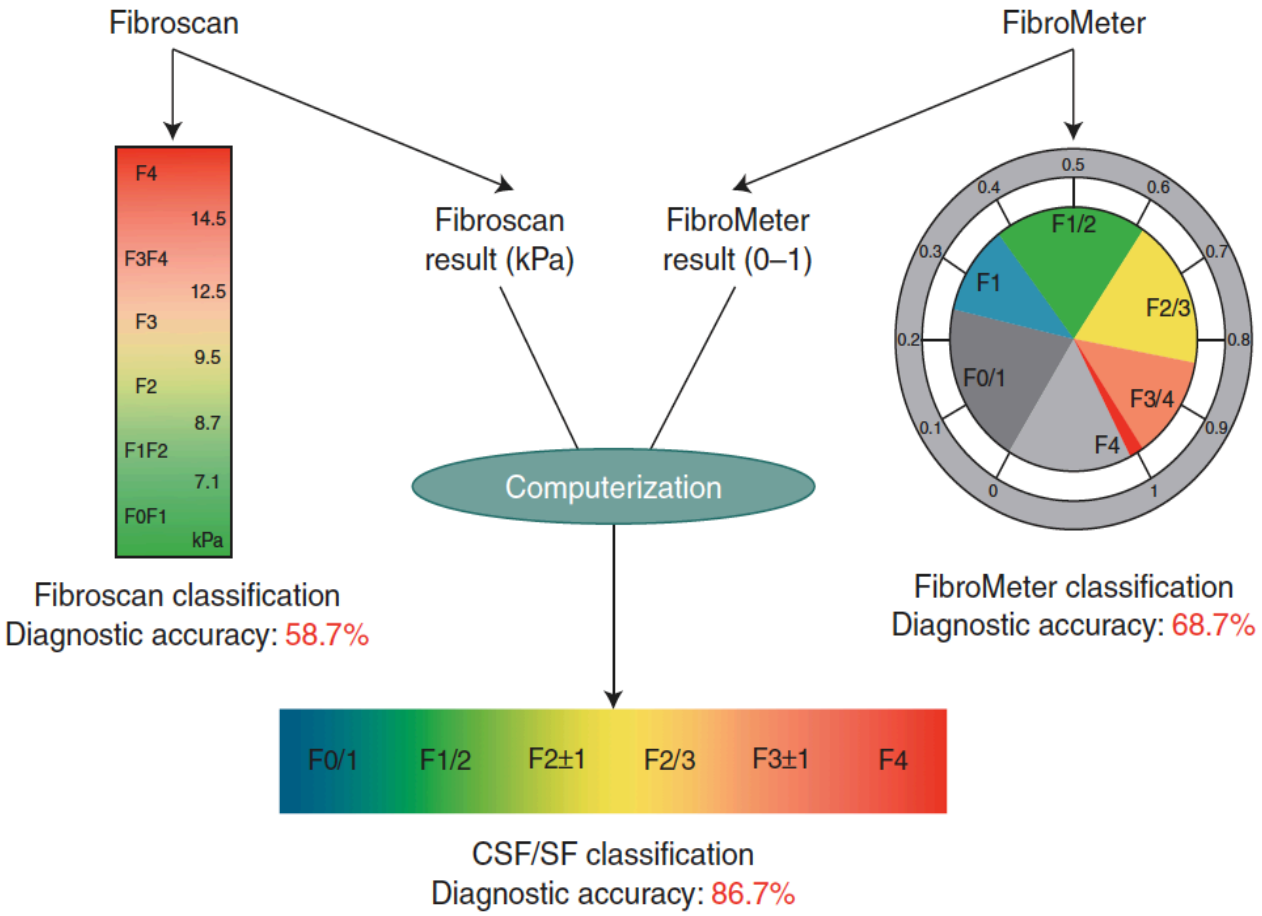
Fibrometer and Fibroscan



Fibrometer + Fibroscan

Hepatology. 2012;55:58-67.

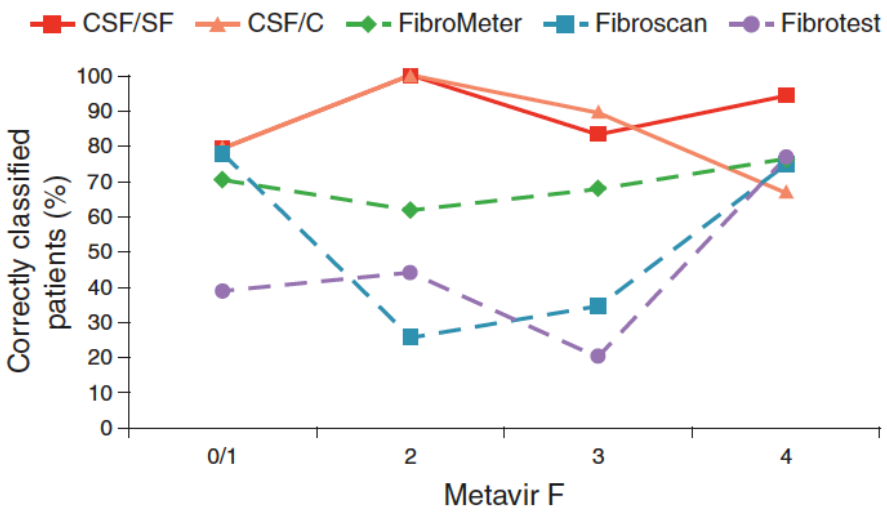
Combining non-invasive tests for improved accuracy



Am J Gastroenterol 2011 106(7):1255-1263

Combining Non-invasive Tests for Improved Accuracy

Diagnosis	Test	AUROC
Significant fibrosis (≥F2)	FibroScan	0.791
	FibroMeter	0.813
	CSF-index	0.846
Severe fibrosis (≥F3)	FibroScan	0.847
	FibroMeter	0.829
	SF-index	0.875
Cirrhosis (F4)	FibroScan	0.905
	FibroMeter	0.861
	C-index	0.921



- Combined tests (indexes) performed better than individual components

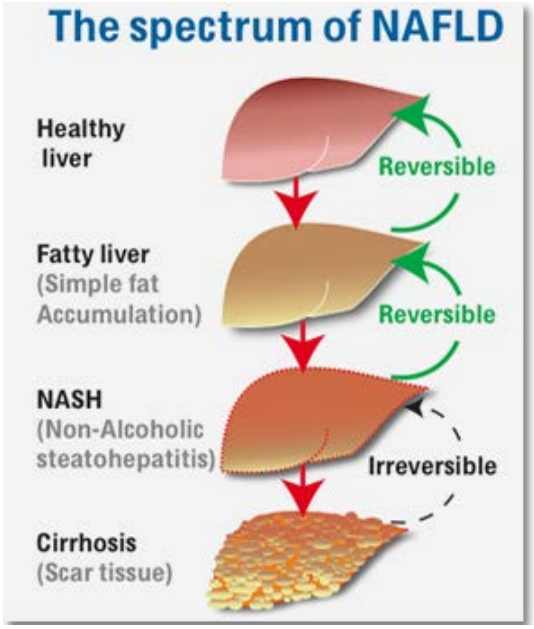
Am J Gastroenterol 2011 106(7):1255-1263

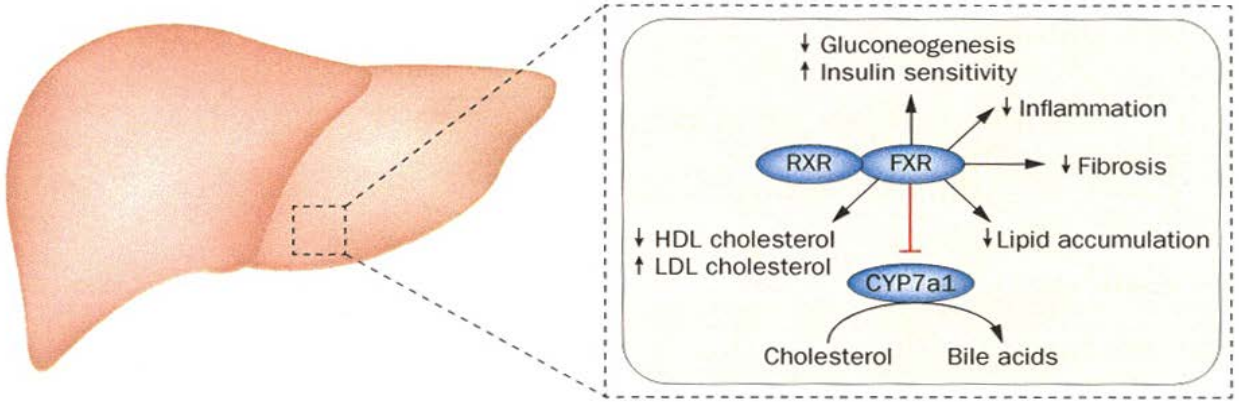
HCV Management Guidelines

- AASLD/IDSA guidance[1]
  - Most efficient strategy combines serum biomarkers and transient liver elastography[2]
  - Consider biopsy for any patient with discordant results between 2 testing methods if the information will affect clinical decisions

1. AASLD/IDSA HCV Management Guidance. October 2014.
2. Boursier J, et al. Hepatology. 2012;55:58-67.

Non Alcoholic Fatty Liver Disease (NAFLD)





Nature Reviews.Gastroenterology&Hepatology. 2014

Accuracy of Diagnostic Panels for Advanced Fibrosis in NAFLD

Author	Test	N	AUROC	Se	SPE
Rosenberg	ELF	61	0.87	89	96
Ratziu	Fibrotest	267	0.81	77	77
Cales	Fibrometer	235	0.943	78.5	95.9

Biomarker Research, 2013.1:7

Summary

- Liver biopsy is the cornerstone of managing patients with chronic liver disease and remains the reference method for assessing liver fibrosis
- Non-invasive biomarker panels do not have sufficient accuracy to replace biopsy
- Non-invasive biomarker assays combined with transient elastography provides increased accuracy
- Algorithms that combine two or more serum biomarker assays or biomarker assay and transient elastography can be used to provide enough accuracy for staging liver fibrosis and reduce the number of biopsies needed

Acknowledgements

- Dr. David Grenache