



Amiens Picardie University Hospital

Les tests non-invasifs dans la Maladie alcoolique du foie: tests sanguins et méthodes physiques



Prof Eric NGUYEN-KHAC

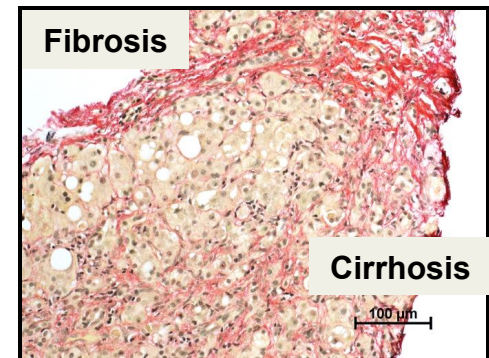
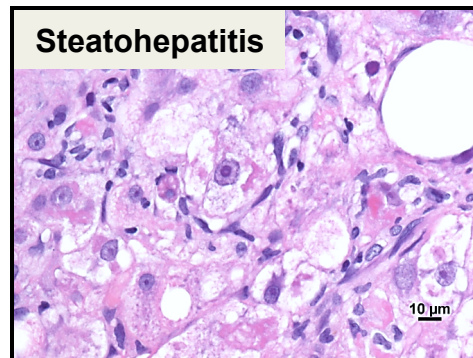
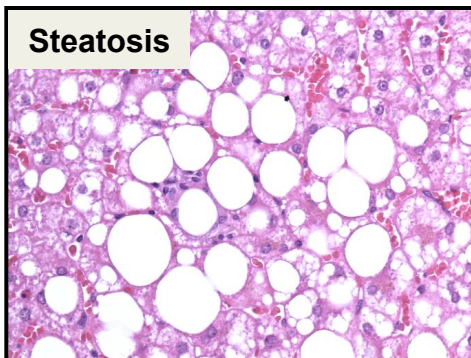
CHU Amiens Picardie

Service d'Hépto-Gastroentérologie

Conflict of interest

No conflict of interest

Steatosis, steatohepatitis, Fibrosis

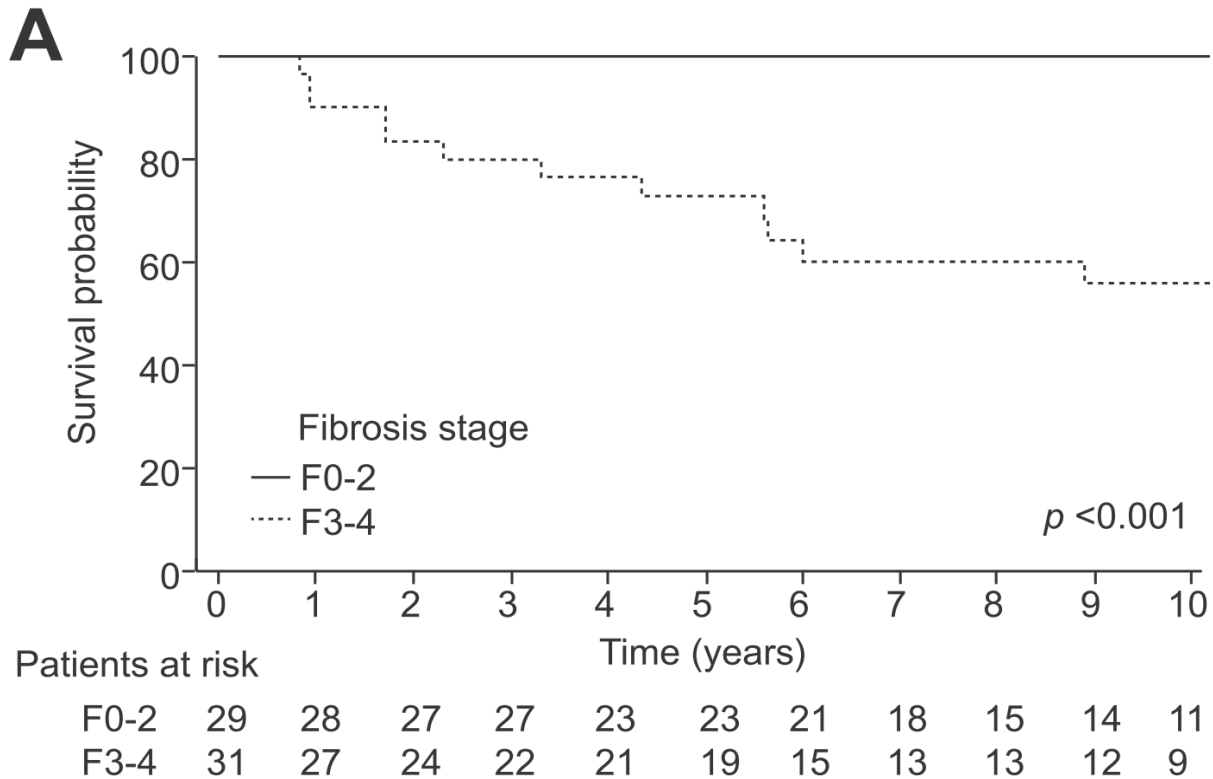


Images provided courtesy of Lackner C.
EASL CPG ALD. J Hepatol 2018;69:154–81

Natural history of ALD

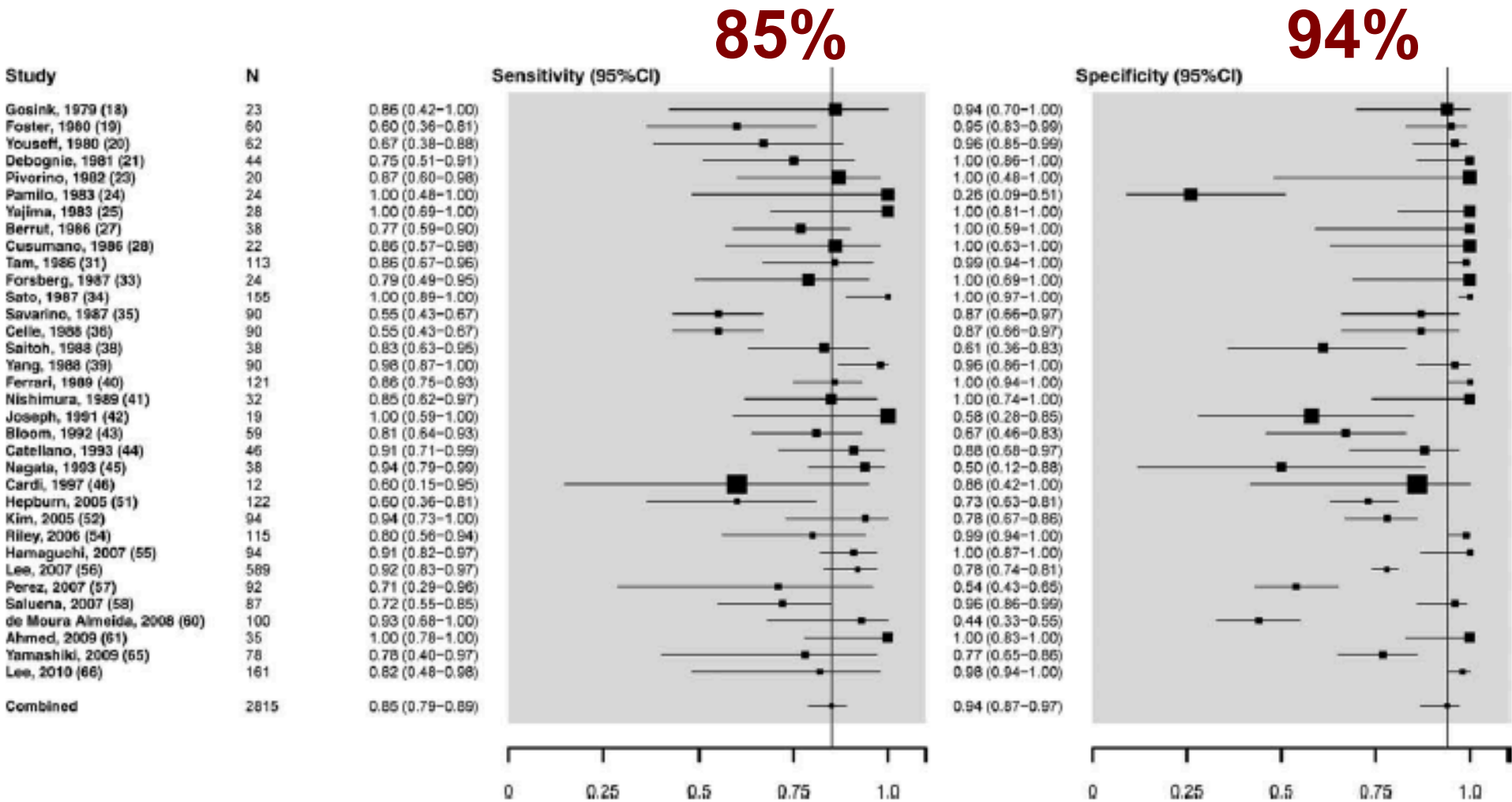
Survival prognosis

only Fibrosis as risk factor in ALD



Steatosis : Ultrasonography

Meta-analysis 49 studies, 4720 patients



Steatosis : Ultrasonography

Meta-analysis 49 studies, 4720 patients

Steatosis > 10%

Concordance (k)

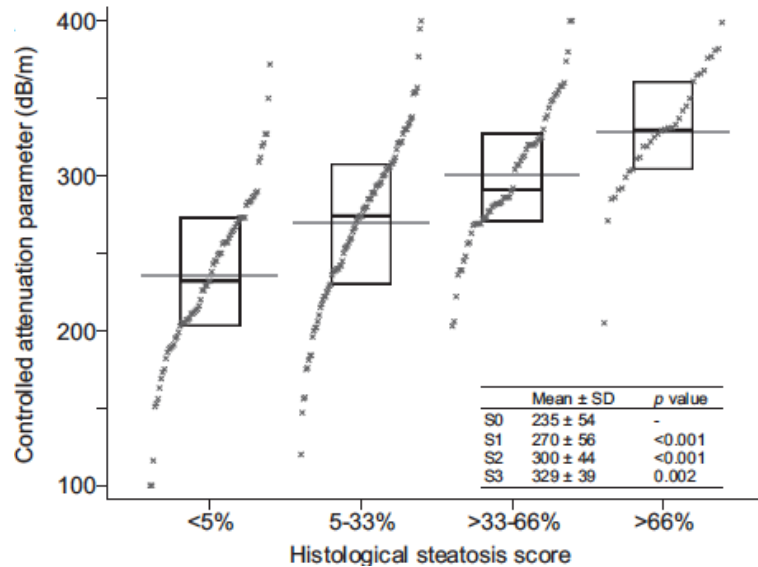
New cut offs	Sensitivity	Specificity
>0-5%	0.65 (0.51, 0.76)	0.81 (0.70, 0.88)
≥10%	0.93 (0.88, 0.97)	0.88 (0.63, 0.97)
≥20-30%	0.91 (0.68, 0.98)	0.99 (0.75, 1.00)
N/R	0.78 (0.71, 0.84)	0.97 (0.92, 0.99)

Intra operator
0.54-0.92

Inter operator
0.44-1.00

Steatosis : Controlled Attenuated Parameter

CAP in 562 ALD patients



AUROC values
non-invasive diagnosis of steatosis in ALD

$S \geq 1$ (5-33% steatosis) : **0.94**
 $S \geq 2$ (33-66% steatosis) : **0.92**
 $S \geq 3$ (>66% steatosis) : **0.84**

Any Steatosis

Rule out

Rule in

$S \geq 1$

Se 90%
NPV 62%

CAP < 220

CAP \geq 290

Sp 88%
PPV 92%

CAP not superior to Ultrasonography (any steatosis $S \geq 1$) ($p=0.051$)

Liver fibrosis : non-invasive tests

Serum Tests



Physical Tests



Liver fibrosis : non-invasive tests

Serum Tests



Physical Tests



Serum tests : *classification*

	Tests	Indirect fibrosis markers Simple biologicals											Direct fibrosis markers						
		Sexe	Age	Transa	Platelet	PT	GGT	Bilirubin	A2M	Apo A1	Haptoglob	Cholesterol	hyaluronate	PIIINP	TIMP				
Simple tests	PGAA					X	X		X	X								1991	
	APRI			X	X													⋮	
	FIB-4		X	X	X														
	hyaluronate												X					⋮	
Composite tests Regression formula	Fibrotest	X	X				X	X	X	X	X								2005
	Fibrometer			X	X	X	X		X				X						⋮
	Hepascore						X	X	X				X						
	Forns		X		X		X						X						
	ELF												X	X	X			2018	

PGAA index : how to assessed it

PGAA	PT	γ -GT	ApoA1	A2M
0	≥ 80	< 36	≥ 200	< 1.25
1	≥ 70 to < 80	≥ 36 to < 89	≥ 175 to < 200	≥ 1.25 to < 1.75
2	≥ 60 to < 70	≥ 89 to < 178	≥ 150 to < 175	≥ 1.75 to < 2.25
3	≥ 50 to < 60	≥ 178 to < 357	≥ 125 to < 150	≥ 2.25 to < 2.75
4	< 50	≥ 357	< 125	≥ 2.75

A2M, α -2-macroglobulin; ApoA1, apolipoprotein A1; γ -GT: γ -glutamyl transpeptidase; PT, prothrombin time.

PGAA : diagnosis performances in ALD

Good AUROCs value
Free access

AUROC	n	F \geq 2	F4
Naveau, Dig Dis Sci 1994	525	0.84 [#]	0.94 [#]
Nguyen Khac, APT 2008	103	0.81	0.83
Naveau, EJGH 2014 [*]	200	0.83	0.80
Voican, Liver Int 2017	193	0.83 (F \geq 3)	0.89

[#]Post hoc

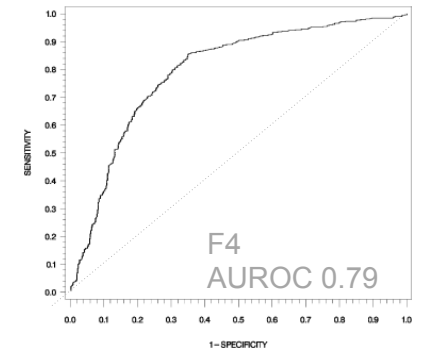
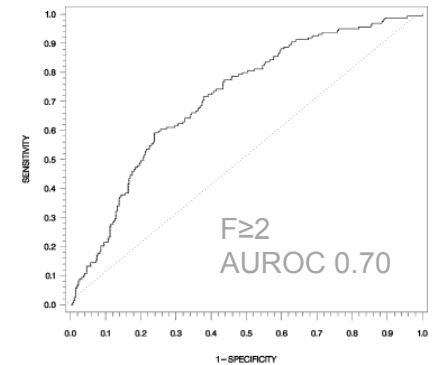
***Cirrhosis F4 : Cut off 10**

Se 80%, Sp 88%, PPV 72%, NPV 92%

APRI : bad diagnosis performances in ALD

Low AUROC values

AUROC	n	F \geq 2	F4
Liber, Am J Gastro 2006	507	0.70 [#]	0.79 [°]
Nguyen Khac, APT 2008	103	0.54	0.56
Naveau, Hepatology 2009	218	0.59	0.67
Fernandez, EJGH 2015	123	0.65 (F \geq 3)	0.75
Voican, Liver Int 2017	193	0.59 (F \geq 3)	0.63
Ducancelle, J Clin Gastro 2017	115	0.55	0.52
Thiele, Gastroenterology 2018	289	0.75	0.85



[#]F \geq 2 : Se 13.2, Spe 77.6, PPV 21.2, NPV 78.9

[°]F4 : Se 16.9, Spe 86.4, PPV 76.7, NPV 63

F1b-4 index : in ALD

Not enough for $F \geq 2$...

AUROC	n	$F \geq 2$	F4
Naveau, Hepatology 2009	218	0.70	0.80
Fernandez, EJGH 2015	123	0.70 ($F \geq 3$)	0.73
Voican, Liver Int 2017	193	0.63 ($F \geq 3$)	0.80
Ducancelle, J Clin Gastroenterol 2017	115	0.72	0.70
Thiele, Gastroenterology 2018	289	0.77	0.89

... good performance for F4

Hyaluronate : in ALD

AUROC	n	F \geq 2	F4
Stickel, EJGH 2003 [#]	95	0.76	0.78
Nguyen Khac, APT 2008	103	0.80	0.83
Naveau, Hepatology 2009	218	0.79	0.93

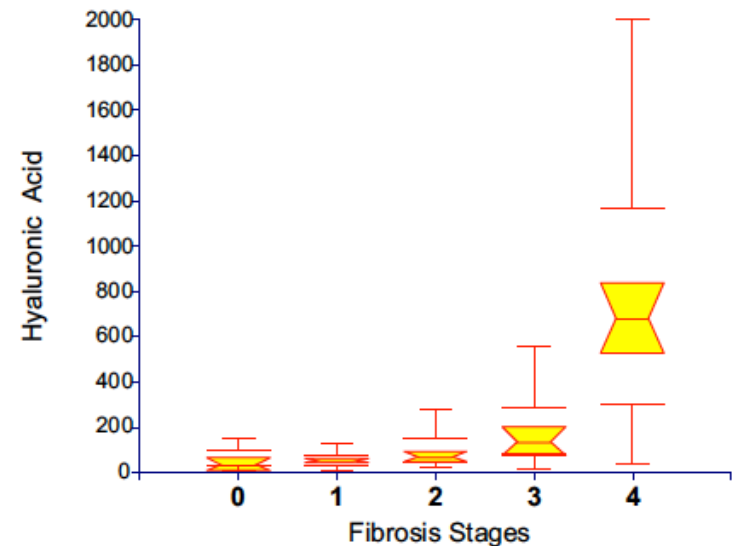
F2 : Cut off 55.5 ng/L

Se 82.8%, Sp 65%, PPV 72%, NPV 92%

Cirrhosis F4 : Cut off 250 ng/L

Se 100%

From Stickel et al. EJGH 2003



From Naveau et al. Hepatology 2009

Fibrotest : the most validated in ALD

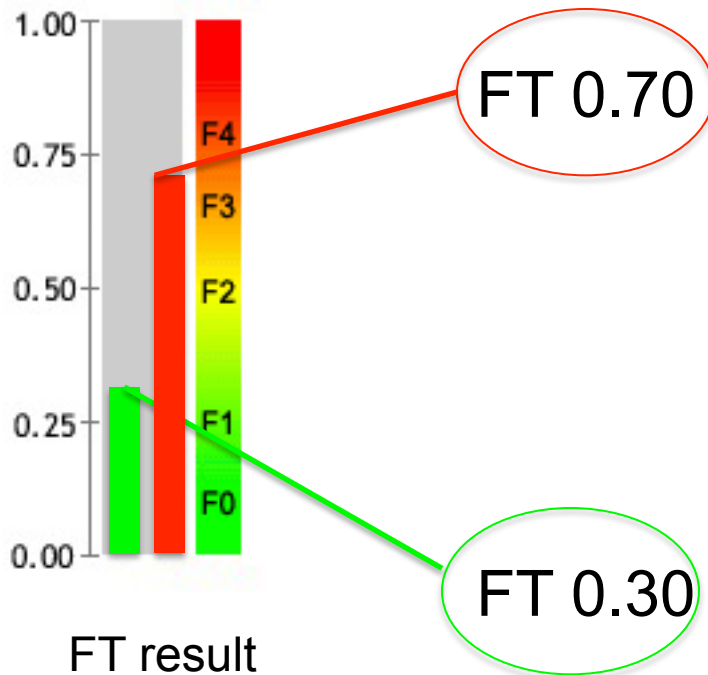
LICENSED

AUROC	n	F \geq 2	F4
Naveau, Clin Gastro Hepatol 2005	221	0.84	0.95
Nguyen Khac, APT 2008	103	0.79	0.84
Naveau, Hepatology 2009	218	0.83	0.94
Naveau, EJGH 2014	200	0.80	0.86
Fernandez, EJGH 2015	123	0.81 (F \geq 3)	0.88
Voican, Liver Int 2017	193	0.85 (F \geq 3)	0.88
Thiele, Gastroenterology 2018	289	0.85	0.88

High diagnosis performances

Use of Fibrotest in ALD

LICENSED



F4 diagnosis by Fibrotest

Sen	Spe	PPV	NPV
0.91	0.87	0.76	0.96

F \geq 2 diagnosis by Fibrotest

Sen	Spe	PPV	NPV
0.84	0.66	0.82	0.70

Fibrometer : in ALD

LICENSED

AUROC	n	F \geq 2	F4	FM	Method
Cales, Hepatology 2005	95	0.88	ND	Fibrometer	Original article
Nguyen Khac, APT 2008	103	0.82	0.85	Fibrometer	Original article
Naveau, Hepatology 2009	218	0.83	0.94	Fibrometer	Original article
Ducancelle, J Clin Gastro 2017	115	0.93	0.92	Fibrometer ^{ALD2G}	¹ Retrospective
Ducancelle, J Clin Gastro 2017	150	0.79	0.82	Fibrometer ^{ALD2G}	² Retrospective
Ducancelle, J Clin Gastro 2017	150	0.73	0.78	Fibrometer ^{V2G}	² Post hoc
Ducancelle, J Clin Gastro 2017	150	0.70	0.73	Fibrometer ^{V3G}	² Post hoc

¹From Cales. Hepatology 2005.

²From Nguyen Khac APT 2008 and Nguyen Khac Lancet Gastro Hepatol 2018

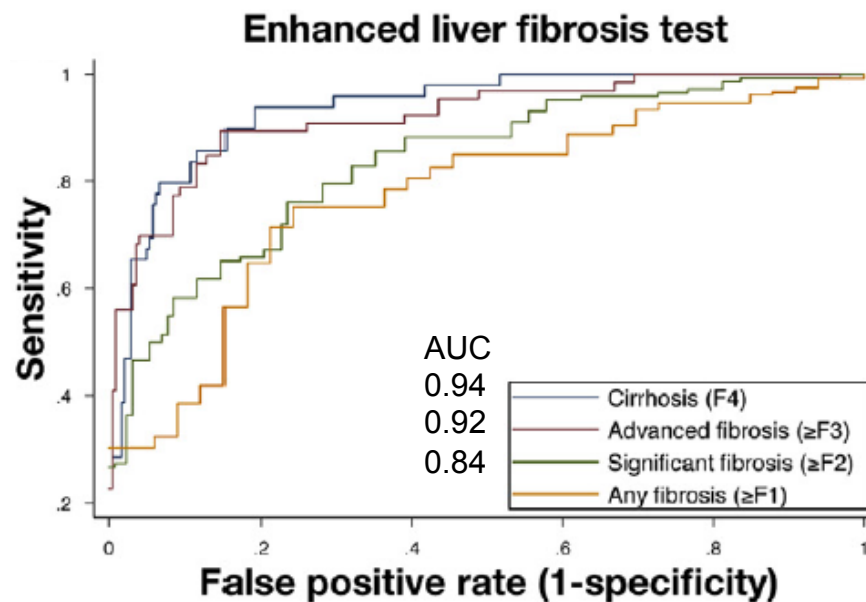
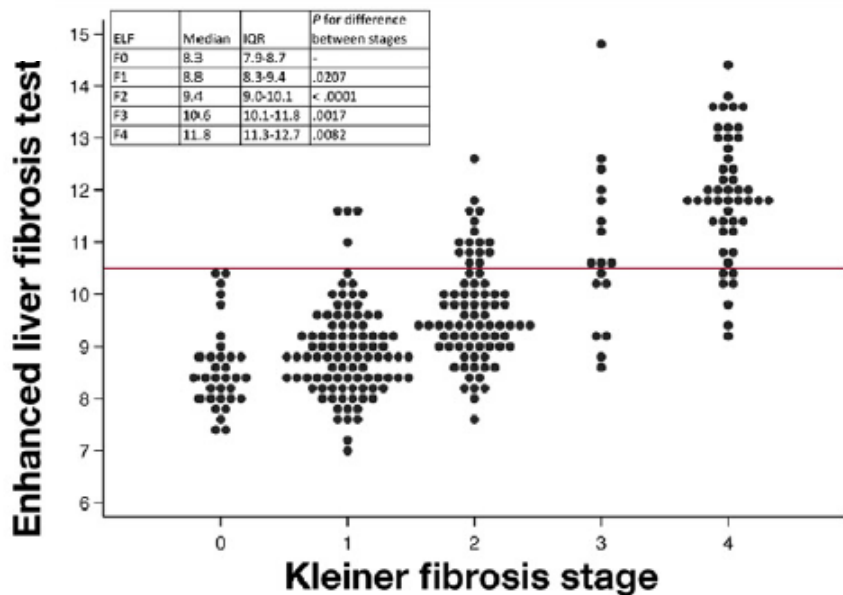
Fibrometer incorporated hyaluronate in 2005

Fibrometer^{ALD2G} (with hyaluronate acid, age added)

Fibrometer^{V3G} (without hyaluronate, with GGT)

ELF : 3 direct markers of fibrosis Hyaluronate, PIINP, TIMP

LICENSED



F_{≥3} : Cut off 10.51 ng/L

Se 79%, Sp 91%, PPV 71%, NPV 94%

N=287 ALD patients

Thiele et al. Gastroenterology 2018

Forns and Hepascore : in ALD

FORNS

AUROC	n	F \geq 2	F4
Naveau, Hepatology 2009	218	0.38	0.38
Voican, Liver Int 2017	193	0.64 (F \geq 3)	0.80
Thiele, Gastroenterology 2018	289	0.80	0.89
Fernandez, EJGH 2015	123	0.64 (F \geq 3)	0.78

Not enough diagnosis performances

HEPAScore

AUROC	n	F \geq 2	F4
Nguyen Khac, APT 2008	103	0.76	0.76
Naveau, Hepatology 2009	218	0.83	0.92
Ducancelle, J Clin Gastroenterol 2017	115	0.92	0.92

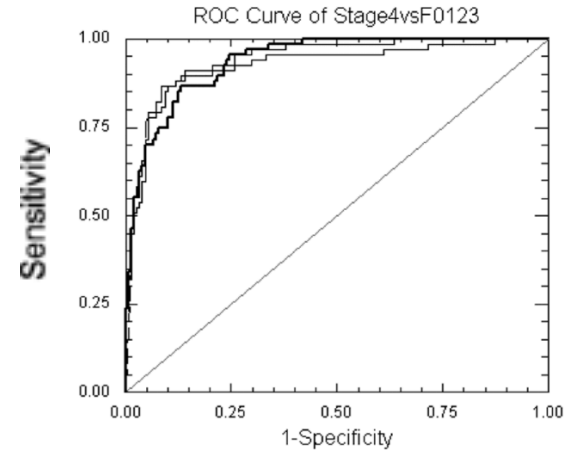
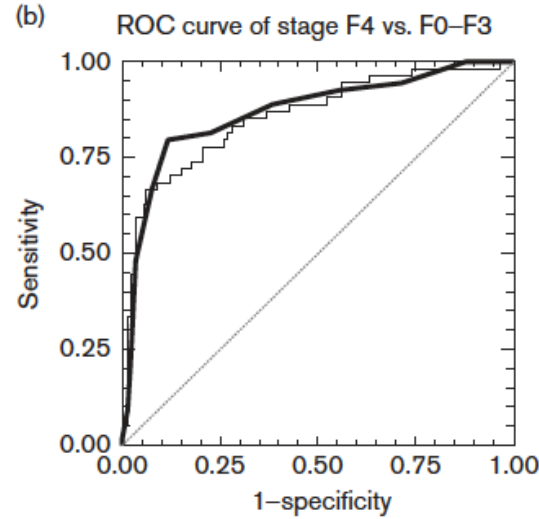
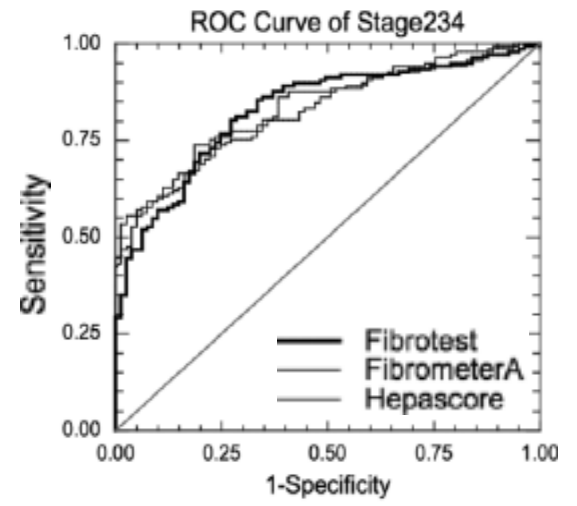
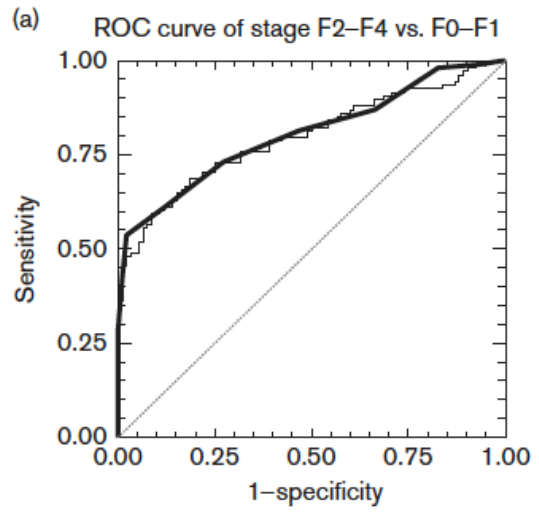
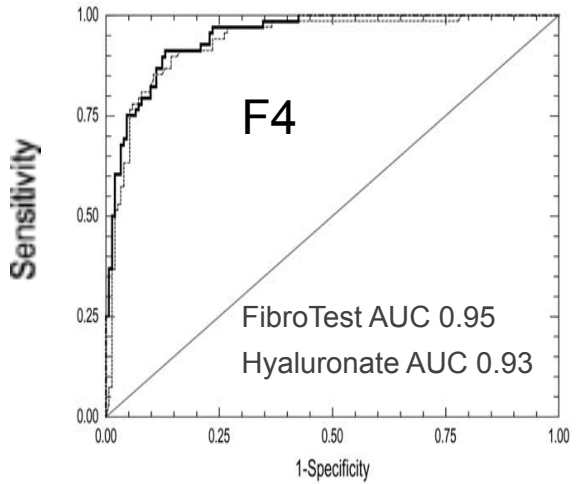
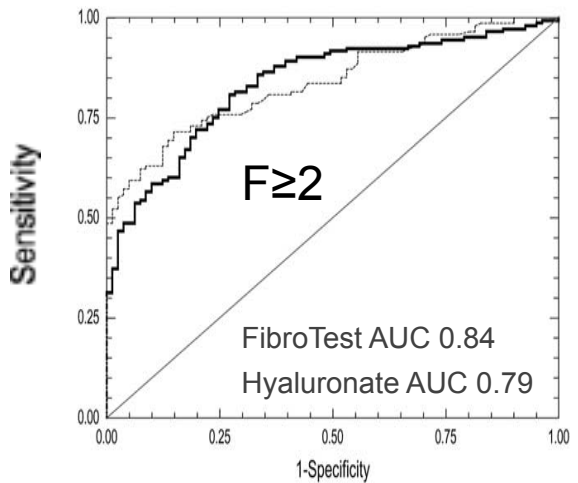
Not enough data

Comparison Head to Head (p=ns)

Fibrotest vs. Hyaluronate

Fibrotest vs. PGAA

Fibrotest vs. FM vs. Hepascore



Naveau et al. CGH 2005

Naveau et al. EJGH 2014

Naveau et al. Hepatology 2009

Combine 2 serum tests ?

Fibrotest plus Fibrometer, not superior to 1 test alone

	FT + FM	FT	FM	p
AUROC F>2	0.85	0.83	0.83	ns
AUROC F4	0.95	0.95	0.95	ns

Scores	Advanced Fibrosis F0F1 versus F2F3F4		Cirrhosis F0F1F2F3 versus F4	
	AUROC	95% CI	AUROC	95% CI
Patented biomarkers (n = 218)				
FibroTest [†]	0.83	0.77-0.88	0.94	0.90-0.96
FibrometerA	0.83	0.77-0.87	0.94	0.90-0.97
Hepascore	0.83	0.77-0.88	0.92	0.87-0.97
Nonpatented scores				
Forns [‡]	0.38	0.30-0.46	0.38	0.27-0.47
APRI	0.59	0.51-0.67	0.67	0.59-0.75
FIB4	0.70	0.62-0.76	0.80	0.72-0.86

Blood tests in the management of ALD: Non-invasive tests to estimate liver fibrosis

- Tests can distinguish mild from severe fibrosis
 - Less well suited to classify intermediate fibrosis stages
- Not helpful in the early diagnosis of ALD

Diagnostic performance of some non-invasive serum fibrosis tests for cirrhosis diagnosis:

Test	Cut-off	F4 prevalence (%)	AUROC (95% CI)	PPV (%)	NPV (%)
Hyaluronic acid	250 µg/L		0.78	35	98
PGAA index*	10	27	0.87 (0.79–0.92)	72	92
FibroTest	≥0.70	31	0.94 (0.90–0.96)	73.4	93.5
	≥0.75	15	0.88 (0.79–0.93)	43.9	92.8
ELF test[†]	≥10.5	23	0.92 (0.89–0.96)	71	94
Fibrometer	≥0.5	31	0.94 (0.90–0.97)	53.7	98.9
FIB-4	<1.45	31	0.80 (0.72–0.86)	NA	NA
	<1.45	15	0.80 (0.71–0.87)	NA	NA

*PGAA index: combines α2alpha-2-macroglobulin, prothrombin time, serum GGT, serum apolipoprotein A1;

[†]ELF combines hyaluronic acid (HA), the N-terminal pro-peptide of collagen type III (PIIINP) and tissue inhibitor of metalloproteinase-1 (TIMP-1). The test is validated for diagnosis of >F3 fibrosis

Liver fibrosis : non-invasive tests

Serum Tests



Physical Tests

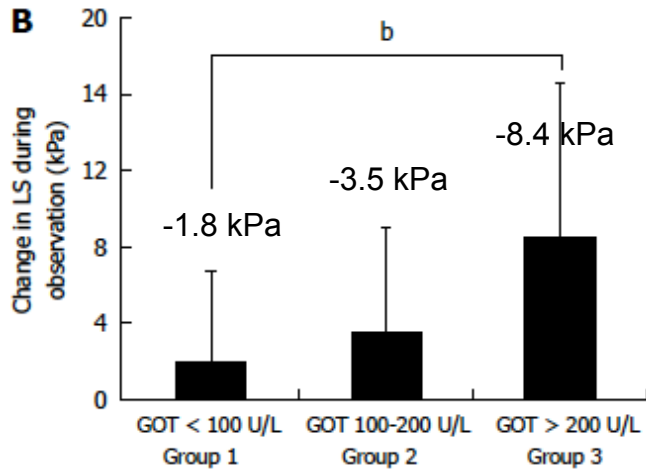
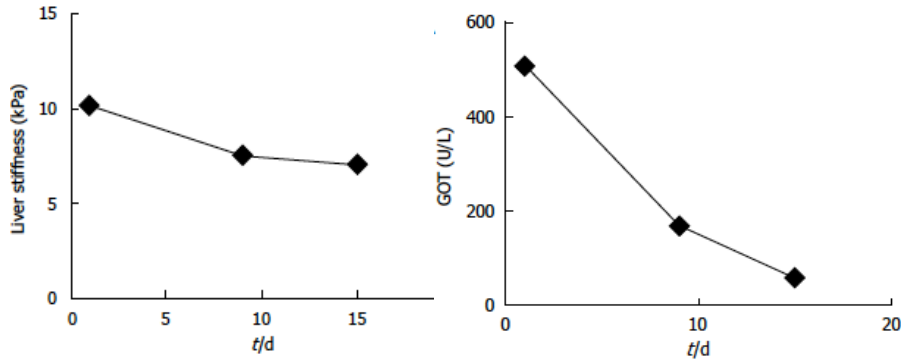


Higher Liver stiffness cut-off for F3 and F4 in Alcoholic Liver Disease

	Etiologies	n	Cut off F \geq 2 (AUROC)	Cut off F \geq 3 (AUROC)	Cut off F=4 (AUROC)
Castera et al	HCV	183	7.1 (0.83)	9.5 (0.90)	12.5 (0.91)
Ziol et al	HCV	327	8.8 (0.79)	9.6 (0.91)	14.6 (0.97)
Marcellin et al	HBV	173	7.2 (0.81)	- (0.93)	11 (0.93)
Corpechot et al	PBC/PSC	95	7.3 (0.92)	9.1 (0.95)	17.3 (0.96)
Yoneda et al	NASH	97	6.65 (0.86)	9.8 (0.90)	17.5 (0.99)
Nguyen-Khac et al	Alcohol	103	7.8 (0.91)	11 (0.89)	19.5 (0.92)
Nahon et al	Alcohol	147	-	11.6 (0.94)	22.6 (0.87)

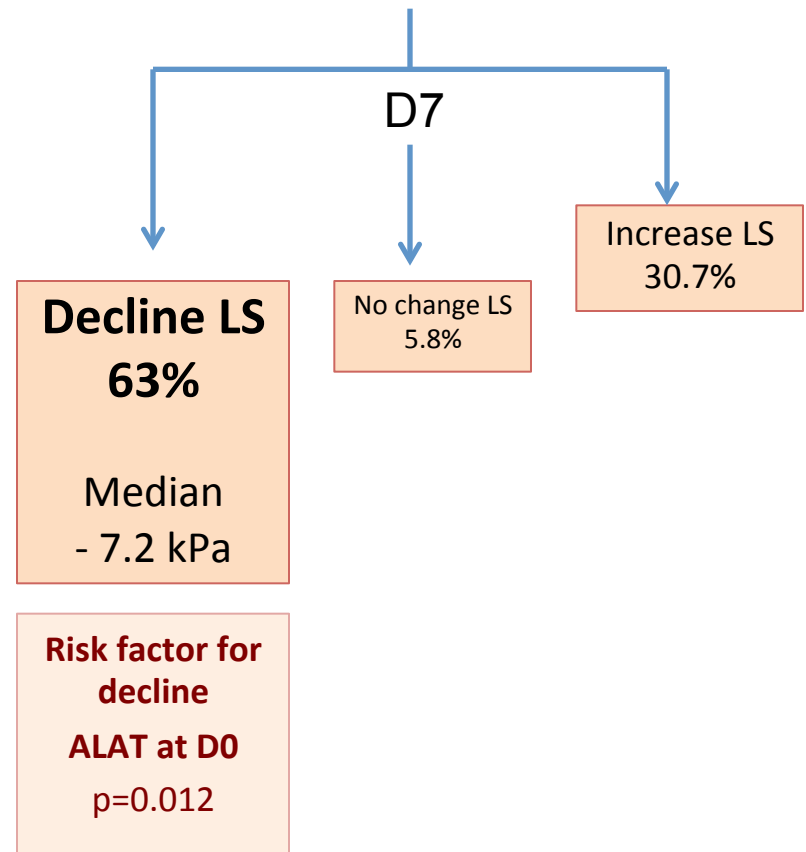
- Cut offs are similars for F \geq 2 et F \geq 3, regardless etiology
- Cut offs higher for F=4, in comparison with viral disease

Decline of liver stiffness after alcohol withdrawal



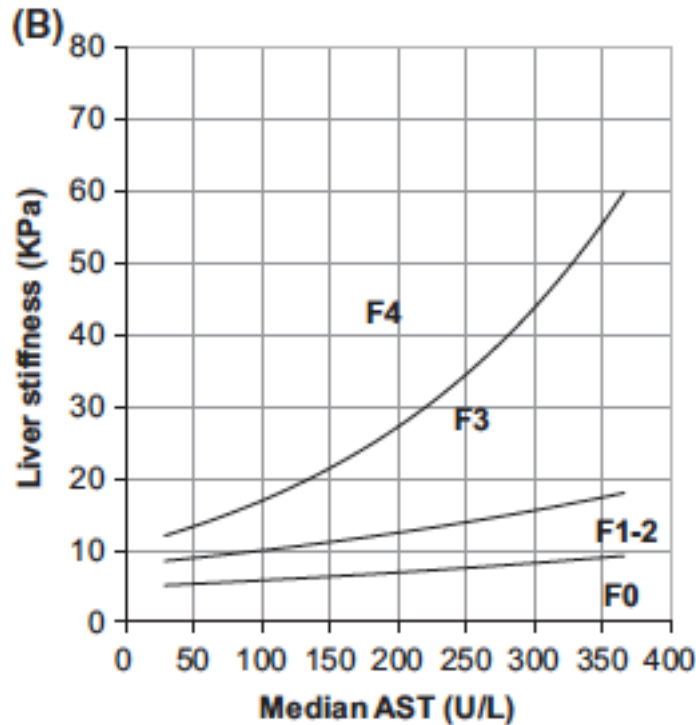
Mueller et al. WJG 2010

137 patients,
Alcohol Withdrawal



Trabut et al. ACER 2012

Elastography in ALD : impact of Transaminase and Bilirubin levels



Misclassified

TE > 22.6 kPa
no cirrhosis

TE < 22.6 kPa
cirrhosis

Patient	Total bilirubin (μmol/L)	HAH (grade)
1	19	2
2	14	2
3	12	1
4	16	2
5	7	1
6	7	1
7	16	2
8	78	3
9	223	1
10	10	2
11	27	1

Patient	Total bilirubin (μmol/L)	HAH (grade)
1	9	0
2	4	0
3	8	0
4	6	0
5	14	0
6	12	0
7	13	1
8	4	0
9	14	1
10	18	1
11	8	0

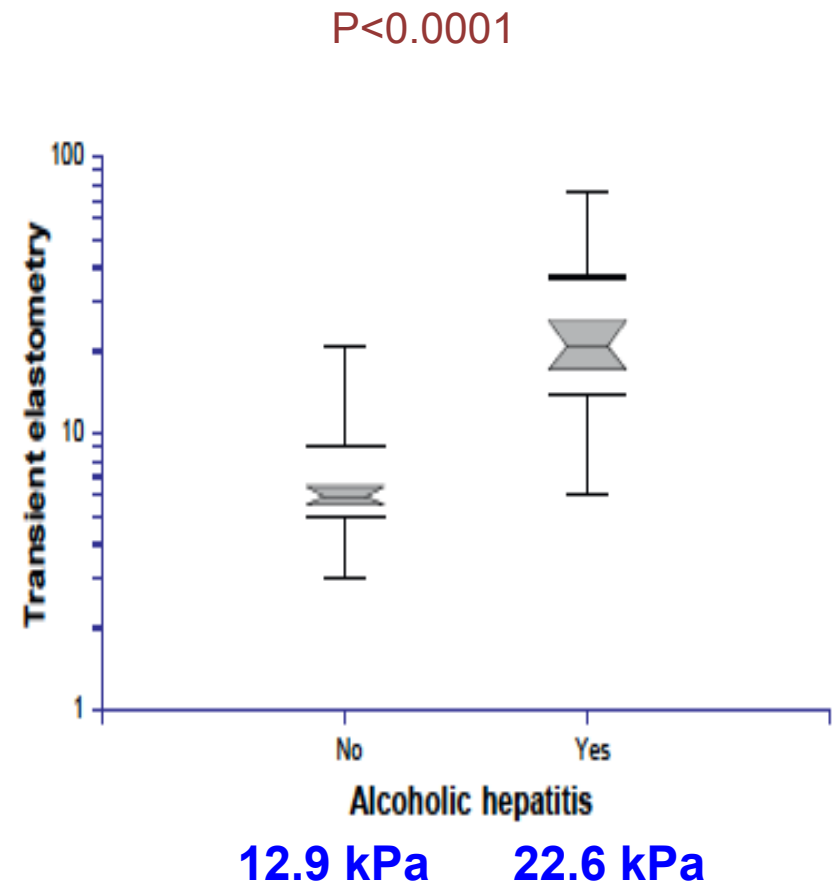
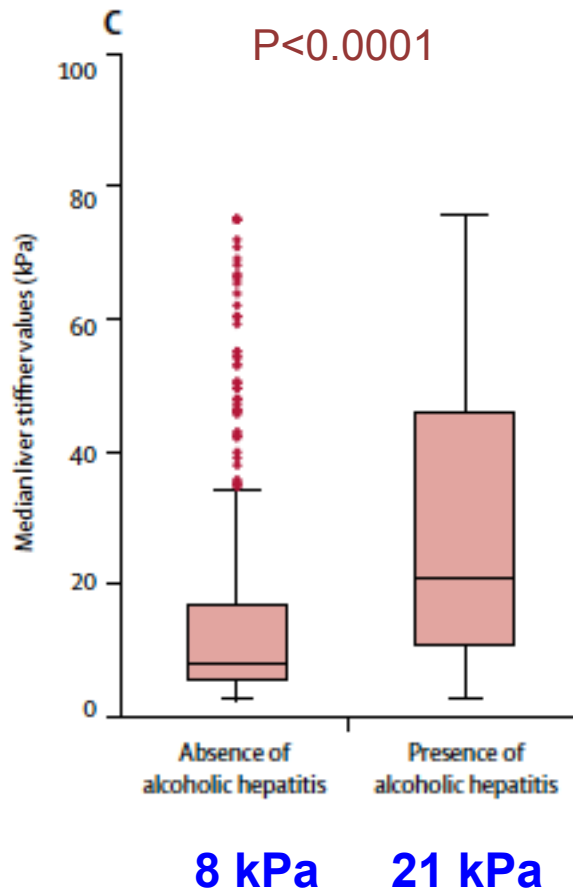
39±64 100%

10±4 20%

→ Role of inflammation on liver stiffness

Histological features of alcoholic hepatitis

Higher Liver stiffness



Meta analysis with individual data

	Total population (n=1026)	Nguyen-Khac et al (n=102) ¹⁵	Nahon et al (n=126) ¹⁶	Fernandez et al (n=112) ¹⁴	Foucher et al (n=53) ¹⁷	Janssens et al (n=48) ¹⁸	Mueller et al (n=73) ¹⁹	Kim et al (n=44) ²⁰	Boursier et al (n=91) ²¹	Voican et al (n=188) ¹⁶	Thiele et al (n=189) ²⁵
Age (years)	54 (11)	59 (10)	54 (9)	55 (10)	57 (11)	55 (9)	54 (10)	49 (8)	56 (10)	55 (11)	49 (10)
Male sex, n (%)	788 (77%)	76 (75%)	99 (79%)	78 (70%)	44 (83%)	32 (67%)	56 (77%)	37 (84%)	78 (86%)	135 (72%)	153 (81%)
Alcohol consumption, g/day	139.1 (84.1)	128.5 (78.6)	146.0 (101.9)	152.3 (60.1)
Body mass index, kg/m ²	26 (5)	28 (6)	26 (5)	26 (5)	26 (5)	26 (6)	..	24 (4)	28 (5)	27 (5)	23 (3)
Prothrombin activity, %	89% (17)	87% (12)	80% (18)	84% (20)	77% (15)	91% (16)	101% (18)	74% (19)	90% (12)	95% (10)	94% (15)
Cholesterolaemia, mmol/L	4.90 (2.00)	5.15 (2.38)	5.03 (1.15)	4.69 (2.16)	5.04 (1.33)	4.50 (2.94)	5.57 (1.55)	3.82 (1.25)
Platelet count, 10 ⁹ per L	213 (101)	251 (126)	192 (98)	221 (110)	221 (110)	197 (95)	195 (87)	172 (130)	195 (75)	246 (89)	211 (89)
AST, IU/L	77 (67)	77 (75)	69 (58)	72 (72)	69 (51)	102 (69)	83 (56)	102 (70)	55 (43)	49 (44)	110 (81)
ALT, IU/L	60 (52)	61 (59)	56 (42)	57 (55)	60 (52)	62 (38)	68 (48)	54 (39)	44 (30)	41 (32)	87 (71)
Bilirubin, µmol/L	16.9 (14.9)	13.6 (10.0)	18.7 (16.4)	17.0 (16.5)	23.9 (19.7)	26.1 (18.9)	18.7 (14.5)	33.0 (22.1)	12.8 (11.4)	12.4 (9.1)	15.0 (12.1)
Alkaline phosphatase, IU/L	111 (68)	103 (58)	116 (59)	119 (80)	122 (49)	105 (42)	nd	104 (41)	94 (47)	106 (60)	124 (95)
Albumin, g/L	39.9 (6.1)	38.5 (7.1)	40.9 (6.3)	41.1 (6.1)	35.1 (5.6)	36.6 (5.6)	42.0 (5.7)	35.7 (6.5)	42.8 (4.5)	40.9 (4.3)	39.3 (6.2)
Liver stiffness, kPa	21.2 (20.8)	20.6 (19.5)	28.0 (22.2)	24.5 (21.7)	37.9 (24.5)	26.4 (18.9)	16.5 (19.8)	30.3 (17.7)	21.6 (19.7)	15.2 (18.6)	14.5 (17.3)
Histological fibrosis assessment system	..	METAVIR	Kleiner-Brunt	METAVIR	METAVIR	METAVIR	Kleiner-Brunt	Batts-Ludwig	METAVIR	Kleiner-Brunt	Kleiner-Brunt

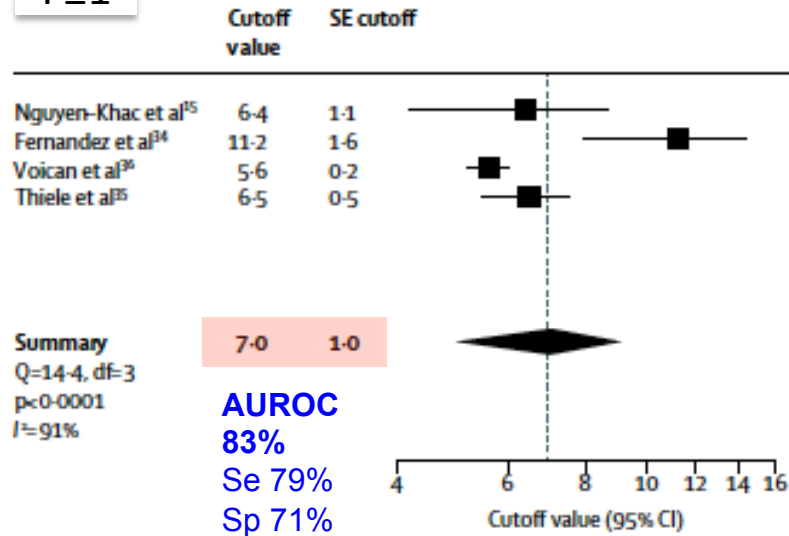
Data are mean (SD) unless stated otherwise. AST=aspartate aminotransferase. ALT=alanine aminotransferase. nd=no data.

Table 1: Baseline characteristics of the study population

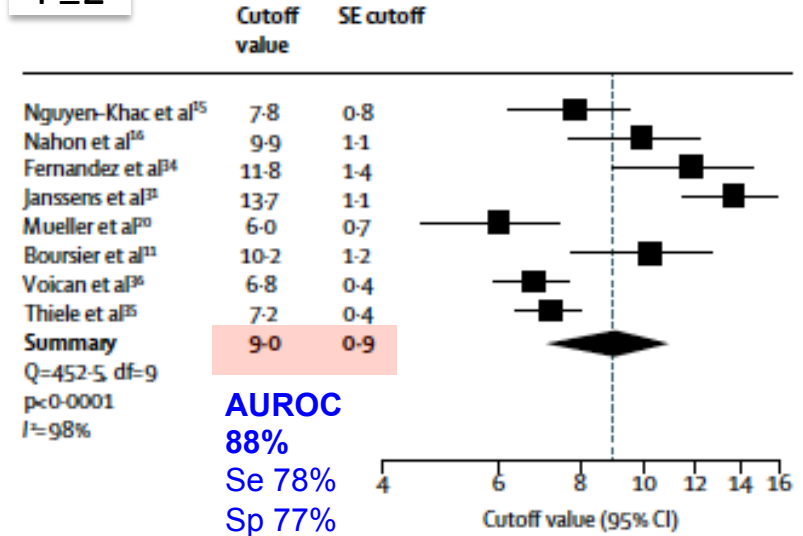
10 prospective studies, 1036 patients
 France (n=5), Belgium (n=2), Germany (n=1),
 Denmark (n=1), Korea south (n=1)

Confirmation of Higher Liver stiffness cut-offs in ALD

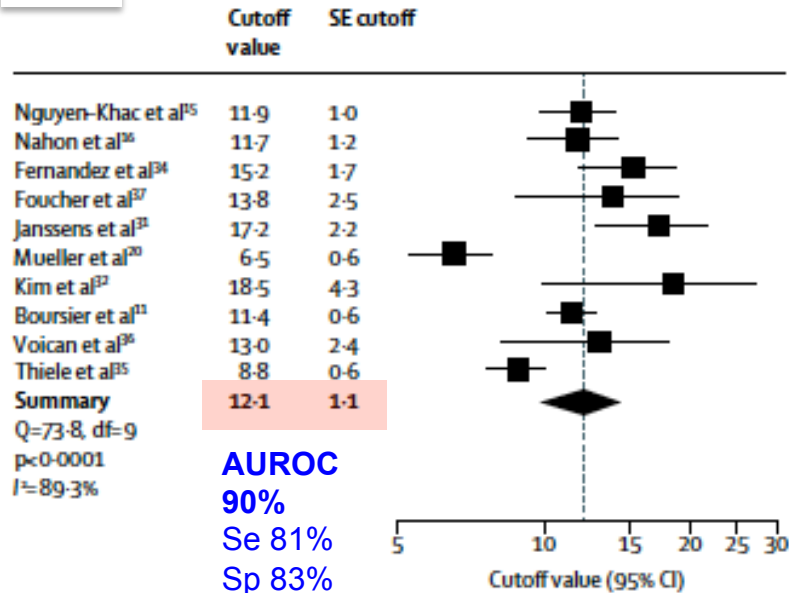
F \geq 1



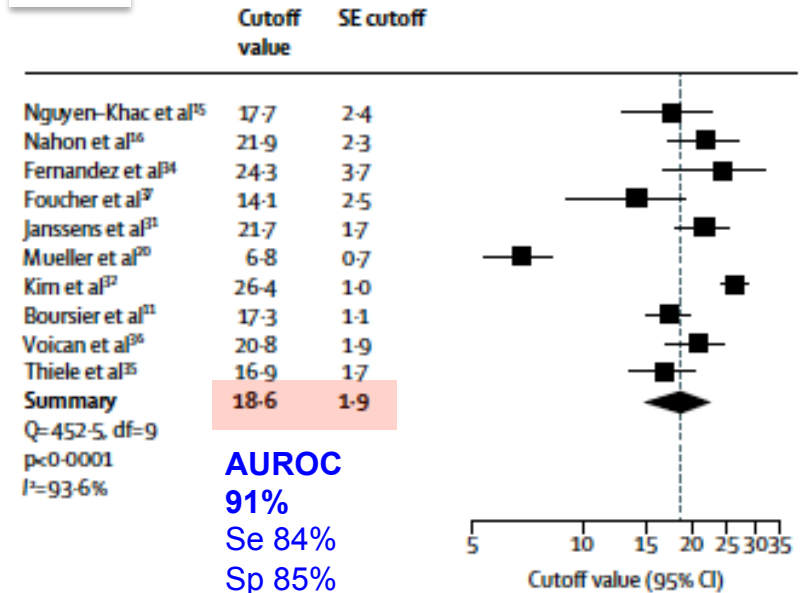
F \geq 2



F \geq 3

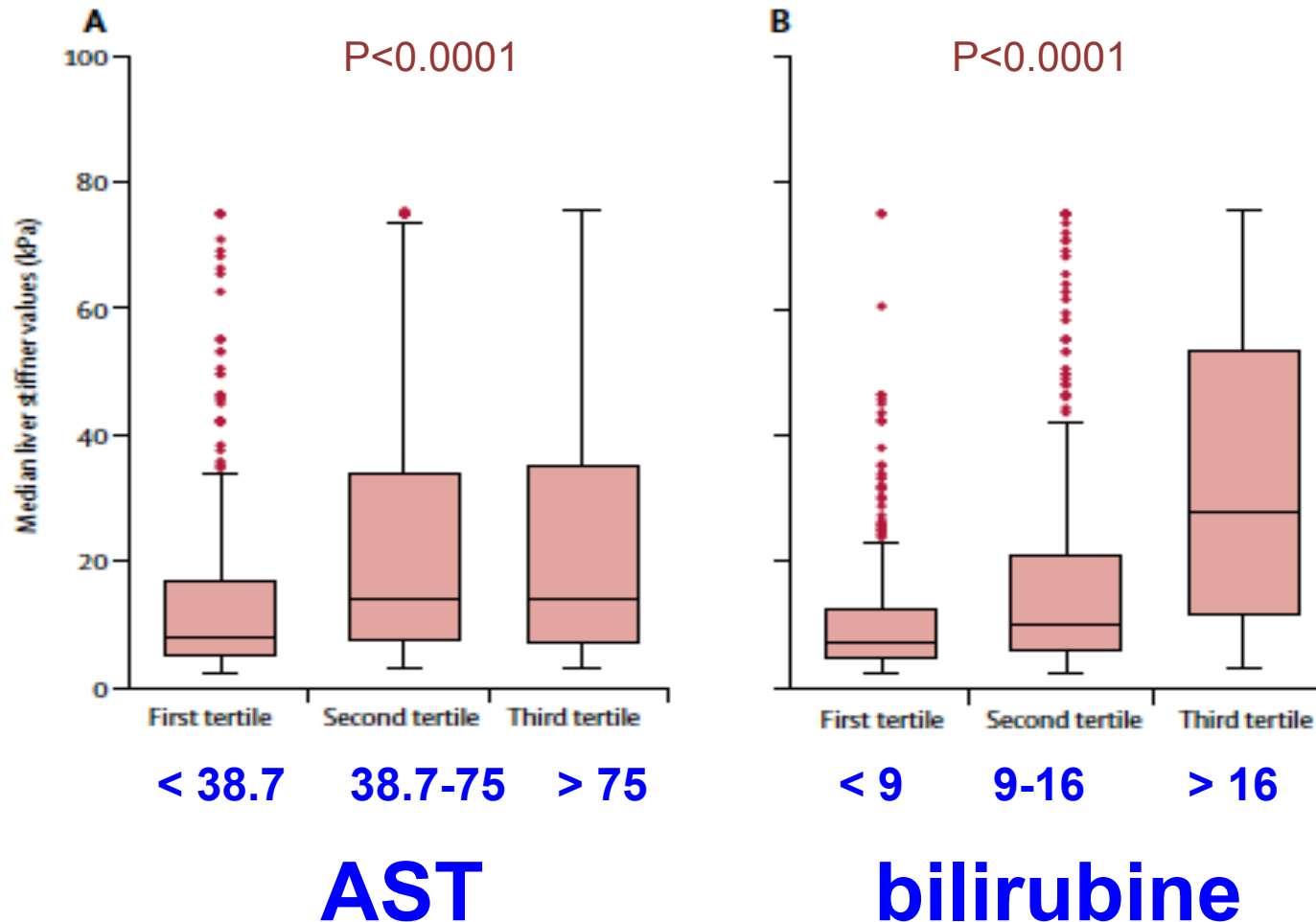


F=4



Higher AST and Bilirubin levels

Higher Liver stiffness



Specific liver Stiffness cut offs

according to AST or bilirubine levels

Fibrosis stage	First tertile bilirubin <9.0 (μmol/L)	Second tertile bilirubin: 9.0 – 16.0 (μmol/L)	Third tertile bilirubin >16.0 (μmol/L)	p
F _{≥1}	5.8 [5.4 – 6.3]	7.2 [6.1 – 8.3]	12.7 [9.8 – 15.6]	<0.0001
	5.8 [5.4 – 6.3]	8.8 [7.4 – 10.2]		<0.0001
F _{≥2}	6.7 [6.00 – 7.5] I ² =27%	8.0 [7.3 – 8.7] I ² =25%	12.3 [10.5 – 14.0] I ² =51%	<0.0001
	6.7 [6.00 – 7.5]	9.4 [8.5 – 10.3]		<0.0001
F _{≥3}	9.0 [7.9 – 10.1] I ² =18%	10.8 [9.7 – 11.9] I ² =93%	16.2 [13.4 – 19.0] I ² =84%	<0.0001
	9.0 [7.9 – 10.1]	12.4 [11.3 – 13.6]		<0.0001
F=4	10.9 [9.1 – 12.6] I ² =57%	14.8 [12.1 – 17.5] I ² =87%	25.1 [21.2 – 29.0] I ² =93%	<0.0001
	10.9 [9.1 – 12.6]	19.2 [17.2 – 21.2]		<0.0001

ALD : specific LS cut-offs according to AST and bilirubin levels

	AST <38.7 IU/L and bilirubin <9 µmol/L (n=160)	AST 38.7-75 IU/L and bilirubin <9 µmol/L or AST <38.7 IU/L and bilirubin 9-16 µmol/L (n=518)	AST 38.7-75 IU/L and bilirubin 9-16 µmol/L (n=135)	AST >75 IU/L and bilirubin >16 µmol/L (n=184)
F≥1				
Cutoffs, kPa*	5.6 (4.9-6.3)	6.9 (5.6-8.2)	8.4 (6.9-9.9)	9.6 (4.9-14.3)
AUROC	0.82 (0.74-0.89)	0.86 (0.81-0.91)	0.90 (0.82-0.98)	0.98 (0.88-1.00)
F≥2				
Cutoffs, kPa†	6.9 (5.8-8.0)	8.1 (7.3-8.9)	8.8 (7.6-10.1)	11.6 (8.5-14.7)
AUROC	0.87 (0.82-0.93)	0.88 (0.85-0.91)	0.90 (0.84-0.96)	0.89 (0.82-0.95)
F≥3				
Cutoffs, kPa†	8.8 (7.1-10.0)	11.2 (10.3-12.1)	12.3 (10.0-14.5)	16.1 (11.7-20.5)
AUROC	0.92 (0.87-0.96)	0.91 (0.89-0.94)	0.90 (0.84-0.95)	0.92 (0.88-0.96)
F=4				
Cutoffs, kPa†	12.1 (8.1-16.1)	15.4 (13.0-17.8)	19.9 (16.2-23.7)	25.9 (20.2-31.7)
AUROC	0.92 (0.87-0.97)	0.93 (0.91-0.95)	0.92 (0.87-0.97)	0.90 (0.86-0.95)
Obuchowski index	0.93 (0.91-0.95)	0.95 (0.94-0.96)	0.95 (0.93-0.97)	0.96 (0.95-0.97)

Data are mean (95% CI) unless stated otherwise. Data missing from 29 patients across both variables. AST=aspartate aminotransferase. AUROC=area under the receiver operating characteristic curve. PPV=positive predictive value. NPV=negative predictive value. LR=likelihood ratio. *p=0.0417. †p<0.0001.

Table 3: Diagnosis performances by combined AST and bilirubin concentrations, in a one-stage meta-analysis

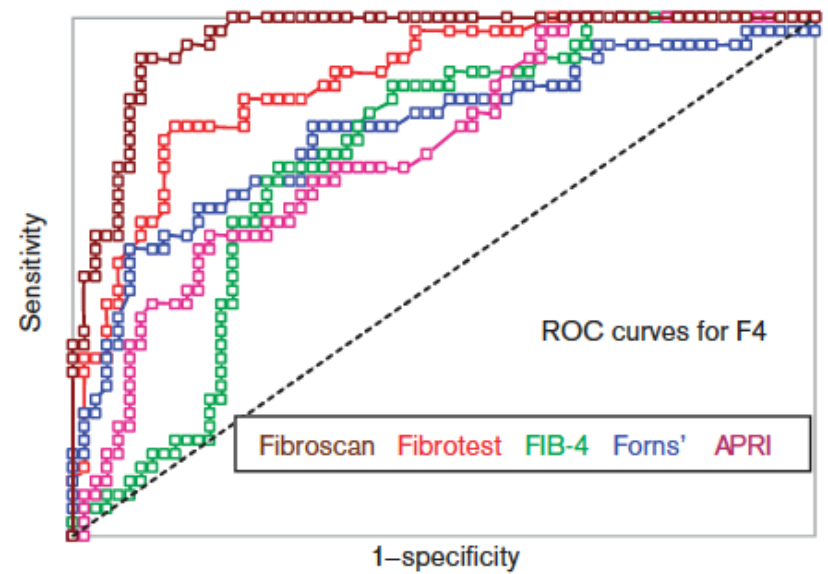
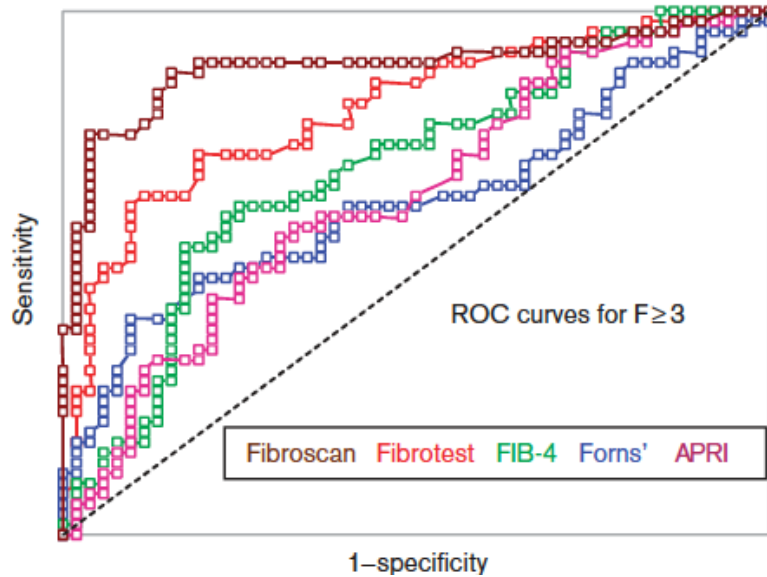
Liver fibrosis : non-invasive tests

Serum Tests \geq or \leq **Physical Tests**
?



Comparison Head to Head

Fibroscan superior vs. FT, APRI, FIB-4, Forns



Test	F \geq 3: F012 VS F34 AUROC (95% CI) N=123	F4: F0123 VS F4 AUROC (95% CI) N=123
Fibroscan®	0.89 (0.83-0.95)	0.93(0.90-0.97)
FT	0.81 (0.73-0.89)	0.88(0.81-0.94)
APRI	0.65(0.54-0.75)	0.75(0.65-0.84)
Forns' index	0.64 (0.53-0.74)	0.78(0.68-0.88)
FIB-4	0.70 (0.60-0.80)	0.73(0.63-0.82)

Comparison Head to Head : Fibroscan vs. 7 Serum tests in ALD

Fibroscan is better for $F \geq 2$

Table 6. Areas under ROC curves and 95% CIs for Fibroscan and comparison with seven non-invasive laboratory tests

	Liver fibrosis (METAVIR)			
	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F = 4$
Fibroscan	0.84 (0.73–0.95)	0.91 (0.85–0.98)	0.90 (0.82–0.97)	0.94 (0.87–0.98)
Fibrometer	0.72 (0.57–0.87)	0.82 (0.72–0.93)	0.88 (0.80–0.95)	0.85 (0.74–0.96)
Fibrotest	0.77 (0.63–0.90)	0.79 (0.69–0.90)*	0.80 (0.70–0.91)	0.84 (0.72–0.97)
Hepascore	0.70 (0.51–0.89)	0.76 (0.64–0.88)†	0.83 (0.74–0.93)	0.76 (0.63–0.90)†
Hyaluronic acid	0.76 (0.58–0.94)	0.80 (0.70–0.92)	0.83 (0.74–0.92)	0.80 (0.68–0.92)
PGA	0.66 (0.50–0.82)*	0.78 (0.68–0.89)‡	0.84 (0.74–0.94)	0.89 (0.82–0.97)
PGAA	0.74 (0.60–0.88)	0.81 (0.71–0.91)	0.86 (0.76–0.96)	0.83 (0.73–0.93)
APRI	0.76 (0.58–0.95)	0.54 (0.4–0.68)§	0.43 (0.30–0.56)§	0.56 (0.38–0.73)§

Comparison vs. Fibroscan: * $P = 0.04$; † $P = 0.02$; ‡ $P = 0.03$; § $P < 0.001$.

Liver fibrosis : Non-invasive tests

Serum Tests



+

Physical Tests

?



Combination :

Fibroscan *plus* Serum tests in ALD ?

Fibroscan alone *vs.* Combinations
same performances

Table 7. Areas under ROC curves and 95% CIs for Fibroscan alone and its combinations with non-invasive laboratory tests

	Liver fibrosis (METAVIR)			
	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F = 4$
Fibroscan (FS)	0.84 (0.73–0.95)	0.91 (0.85–0.98)	0.90 (0.82–0.97)	0.94 (0.87–0.98)
FS and Fibrometer	0.82 (0.72–0.93)	0.91 (0.85–0.98)	0.92 (0.86–0.98)	0.92 (0.86–0.98)
FS and Fibrotest	0.86 (0.77–0.95)	0.91 (0.84–0.97)	0.91 (0.85–0.97)	0.94 (0.87–0.98)
FS and Hepascore	0.84 (0.73–0.95)	0.91 (0.84–0.98)	0.92 (0.85–0.98)	0.92 (0.85–0.98)
FS and hyaluronic acid	0.85 (0.75–0.95)	0.92 (0.86–0.98)	0.91 (0.85–0.97)	0.90 (0.83–0.97)
FS and PGA	0.84 (0.74–0.94)	0.91 (0.84–0.97)	0.90 (0.84–0.97)	0.93 (0.88–0.98)
FS and PGAA	0.83 (0.74–0.93)	0.90 (0.84–0.97)	0.91 (0.85–0.98)	0.93 (0.87–0.98)

Combination :

FibroMeter^{VC1E2G/3G} in ALD ?

Table S8. Accuracy of non-invasive tests in the ALD population #6 (150 patients).

Target	Obuchowski AUROC			
	All F	Significant fibrosis	Severe fibrosis	Cirrhosis
APRI	0.616	0.587	0.549	0.643
Fib-4	0.634	0.627	0.602	0.732
Fibrotest	0.654	0.663	0.706	0.757
Hepascore	0.704	0.770	0.805	0.772
FibroMeter ^{V2G}	0.705	0.731	0.715	0.780
CirrhoMeter ^{V2G}	0.704	0.760	0.755	0.858
FibroMeter ^{V3G}	0.680	0.701	0.666	0.735
CirrhoMeter ^{V3G}	0.680	0.723	0.689	0.818
FibroMeter ^{ALD2G}	0.720	0.795	0.802	0.820
Zeng score	0.750	0.786	0.771	0.770
LSM	0.833	0.922	0.861	0.909
FibroMeter ^{VC1E2G}	0.820	0.901	0.868	0.919
FibroMeter ^{VC1E3G}	0.808	0.890	0.855	0.910
p	-	0.988	0.056	0.015

Shear Wave Elastography

	Fibrosis (Ishak ≥ 3)		Cirrhosis (Ishak ≥ 5)	
	TE	2D-SWE	TE	2D-SWE
Per-protocol analyses				
Overall				
Prevalence, n (%)	84/199 (42)		36/199 (18)	
AUC (95% CI)	0.95 (0.91–0.98)	0.94 (0.91–0.97)	0.96 (0.93–0.98)	0.95 (0.92–0.98)
Optimal cutoff, kPa	9.6	10.2	19.7	16.4
Correctly classifies	87 (82–92)	88 (83–93)	93 (89–97)	93 (88–97)
Sensitivity	83 (73–91)	82 (71–91)	97 (83–100)	94 (79–99)
Specificity	91 (84–96)	93 (87–97)	90 (84–94)	91 (86–96)
PPV	87 (78–92)	90 (81–95)	68 (57–78)	71 (58–81)
NPV	88 (82–93)	88 (81–92)	99 (95–100)	99 (95–100)
Pretest odds	0.73		0.22	
Post-test odds (+)	6.57 (3.61–11.97)	8.94 (4.33–18.43)	2.13 (1.31–3.47)	2.42 (1.40–4.18)
Post-test odds (-)	0.13 (0.23–0.08)	0.14 (0.32–0.08)	0.01 (0.06–0.00)	0.02 (0.06–0.00)

199 patients ALD, **same performances**
cut offs different (TE and SWE) for F4

ARFI (Acoustic Radiation Force Impulse)

Table 3. Comparative diagnosis values of LS measurements by means of ARFI elastography and APRI for each fibrosis stage in patients with ALD

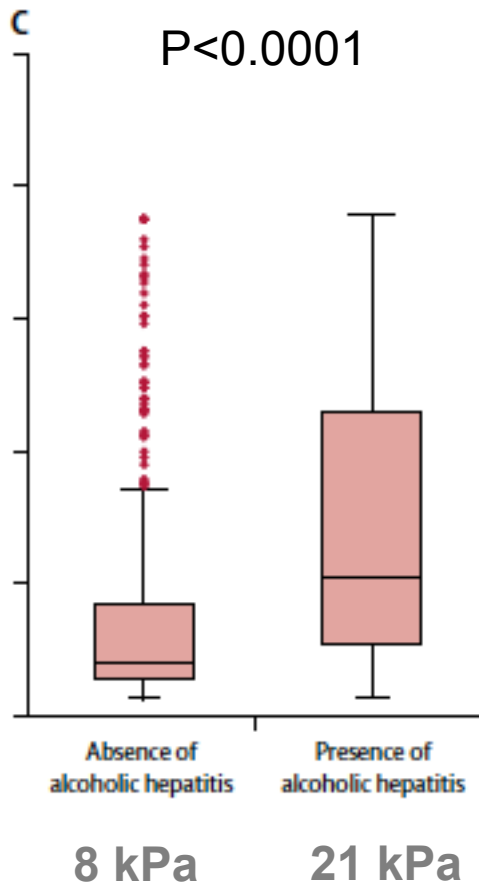
All patients (<i>n</i> = 99)	<i>S</i> ≥ 2		<i>S</i> ≥ 3		<i>S</i> = 4	
	ARFI (m/s)	APRI	ARFI (m/s)	APRI	ARFI (m/s)	APRI
Cutoff (m/s)	1.27	0.59	1.40	0.95	1.65	1.10
Sensitivity	0.77	0.47	0.84	0.40	0.89	0.44
Specificity	0.85	0.95	0.82	0.97	0.84	0.94
PPV	0.89	0.93	0.62	0.83	0.36	0.44
NPV	0.70	0.54	0.93	0.83	0.99	0.94
AUROC	0.846	0.763	0.875	0.688	0.893	0.648
SE	0.038	0.047	0.043	0.067	0.035	0.114
95% CI	0.772–0.921	0.672–0.855	0.791–0.959	0.557–0.818	0.824–0.961	0.425–0.871
<i>P</i> ^a	0.171		0.019		0.041	

99 patients ALD
Good Diagnosis performances

Conclusions

- Liver fibrosis due to alcohol-related disease can be assessed by non-invasive tests.
 - **Serum tests**
 - PGAA, Fibrotest, FibrometerALD1G, hyaluronate (for F4).
 - **Transient Elastography**
 - according to inflammation (AST and Bilirubin levels)
- There is no added value to associate tests
- Other tests are promising : ELF, SWE, ARFI

Influence sur l'élasticité hépatique de l'hépatite alcoolique histologique



Variable	Univariate analysis		Multivariate analysis	
	Odds ratio [95%CI]	p	Odds ratio [95%CI]	p
Age (n=789)	1.01 [1.00 - 1.03]	0.0841		
Gender (F vs. M) (n=790)	1.33 [0.94 - 1.89]	0.1025		
BMI (n=693)	1.04 [1.01 - 1.08]	0.0152		
Bilirubin (n=783)	1.05 [1.03 - 1.06]	<0.0001	1.03 [1.01 - 1.05]	0.0002
Cholesterolaemia (n=361)	1.10 [0.98 - 1.23]	0.0893		
Albumin (n=714)	0.94 [0.91 - 0.97]	<0.0001		
Alkaline phosphatases (n=747)	1.005 [1.003 - 1.008]	<0.0001		
AST (n=768)	1.008 [1.005 - 1.011]	<0.0001	1.006 [1.004 - 1.009]	<0.0001
ALT (n=772)	1.004 [1.002 - 1.007]	0.0027		
Prothrombin Time (n=774)	0.975 [0.965 - 0.985]	<0.0001	0.984 [0.972 - 0.996]	0.0109
Platelet count (n=784)	0.998 [0.996 - 0.999]	0.0030		

BMI, Alkaline phosphatases and Cholesterolaemia were excluded from the multivariate analysis due to a high proportion of missing data

Comparison

Elastometry vs. Serum tests in ALD

AUROC: Fibroscan vs. 7 serum tests

	F \geq 1	F \geq 2	F \geq 3	F=4
Fibroscan	0.84 [0.73-0.95]	0.91 [0.85-0.98]	0.90 [0.82-0.97]	0.94 [0.87-0.98]
Fibrometre				
Fibrotest		0.79 [0.69-0.90] ^a		
Hepascore		0.76 [0.64-0.88] ^b		0.76 [0.63-0.90] ^b
Hyaluronate				
PGA	0.66 [0.50-0.82] ^a	0.78 [0.68-0.89] ^c		
PGAA				
APRI		0.54 [0.40-0.68] ^d	0.43 [0.30-0.56] ^d	0.56 [0.38-0.73] ^d

Méthode de Hanley et McNeil :^ap=0,04; ^bp=0,02; ^cp=0,03; ^dp<0,001

Combine Elastometry plus Serum tests in ALD ?

AUROC Fibroscan vs. Combinations (FS + 1 serum test, regression formula)

	F \geq 1	F \geq 2	F \geq 3	F=4
Fibroscan (FS)	0.84 [0.73-0.95]	0.91 [0.85-0.98]	0.90 [0.82-0.97]	0.94 [0.87-0.98]
FS + Fibrometer	0.82 [0.72-0.93]	0.91 [0.85-0.98]	0.92 [0.86-0.98]	0.92 [0.86-0.98]
FS + Fibrotest	0.86 [0.77-0.95]	0.91 [0.84-0.97]	0.91 [0.85-0.97]	0.94 [0.87-0.98]
FS + Hepascore	0.84 [0.73-0.95]	0.91 [0.84-0.98]	0.92 [0.82-0.98]	0.92 [0.85-0.98]
FS + hyaluronate	0.85 [0.75-0.95]	0.92 [0.86-0.98]	0.91 [0.85-0.97]	0.90 [0.83-0.97]
FS + PGA	0.84 [0.74-0.94]	0.91 [0.84-0.97]	0.90 [0.84-0.97]	0.93 [0.88-0.98]
FS + PGAA	0.83 [0.74-0.93]	0.90 [0.84-0.97]	0.91 [0.85-0.98]	0.93 [0.87-0.98]

Comparison Elastometry vs. Serum tests in ALD

AUROC: Fibroscan vs. 7 serum tests

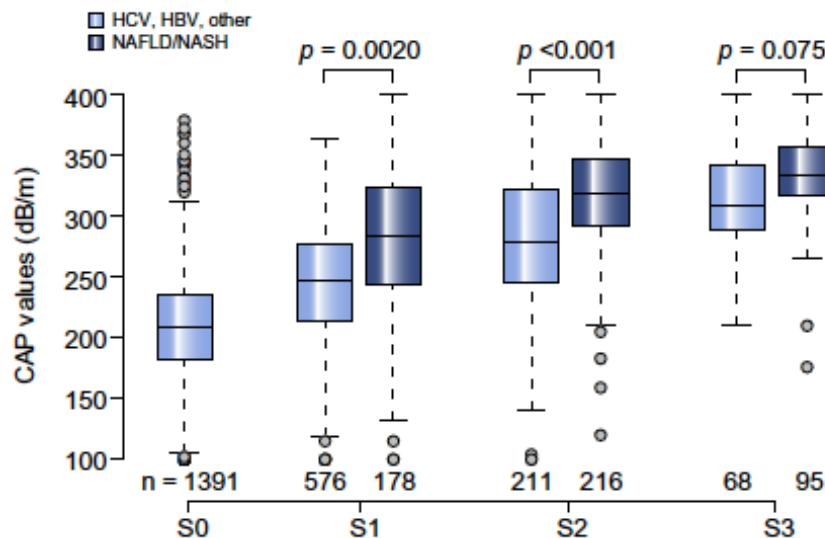
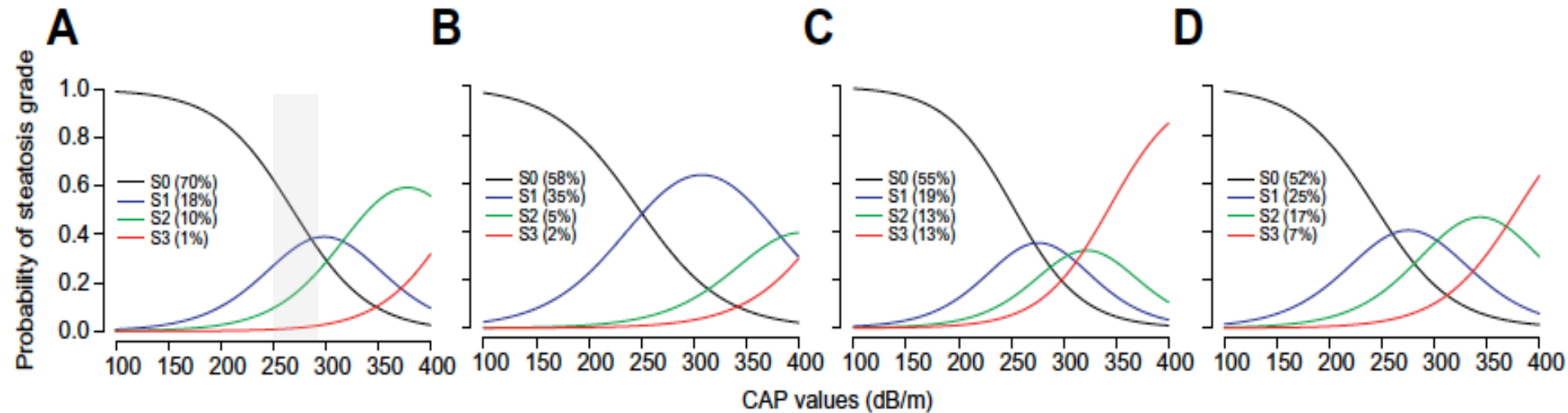
	F \geq 1	F \geq 2	F \geq 3	F=4
Fibroscan	0.84 [0.73-0.95]	0.91 [0.85-0.98]	0.90 [0.82-0.97]	0.94 [0.87-0.98]
Fibrometer	0.72 [0.57-0.87]	0.82 [0.72-0.93]	0.88 [0.80-0.95]	0.85 [0.74-0.96]
Fibrotest	0.77 [0.63-0.90]	0.79 [0.69-0.90]	0.80 [0.70-0.91]	0.84 [0.72-0.97]
Hepascore	0.70 [0.51-0.89]	0.76 [0.64-0.88]	0.83 [0.74-0.93]	0.76 [0.63-0.90]
Hyaluronate	0.76 [0.58-0.94]	0.80 [0.70-0.92]	0.83 [0.74-0.92]	0.80 [0.68-0.92]
PGA	0.66 [0.50-0.82]	0.78 [0.68-0.89]	0.84 [0.74-0.94]	0.89 [0.82-0.97]
PGAA	0.74 [0.60-0.88]	0.81 [0.71-0.91]	0.86 [0.76-0.96]	0.83 [0.73-0.93]
APRI	0.76 [0.58-0.95]	0.54 [0.40-0.68]	0.43 [0.30-0.56]	0.56 [0.38-0.73]

Agenda

- **Non-invasive Diagnosis of Liver Fibrosis, in ALD**
 - Liver Stiffness (Fibroscan)
 - Serum markers
 - Liver Stiffness vs. Serum Tests
- **Combinations**
 - Association of two serum markers ?
 - Liver stiffness (Fibroscan) plus serum markers ?

CAP meta analyse à données individuelles

21 études, 2735 patients



Serum tests for steatosis

$$\text{FLI} : \frac{[e^{0.953 * \log_e(\text{triglycerides}) + 0.139 * \text{BMI} + 0.718 * \log_e(\text{GGT}) + 0.053 * \text{WC} - 15.745}]}{[1 + e^{0.953 * \log_e(\text{triglycerides}) + 0.139 * \text{BMI} + 0.718 * \log_e(\text{GGT}) + 0.053 * \text{WC} - 15.745}]} * 100.^9$$

$$\text{NAFLD-LFS} : -2.89 + 1.18 * \text{metabolic syndrome}(\text{yes} = 1/\text{no} = 0) + 0.45 * \text{type 2 diabetes}(\text{yes} = 2/\text{no} = 0) + 0.15 * \text{insulin}(\text{mU/L}) + 0.04 * \text{AST}(\text{U/L}) - 0.94 * \text{AST/ALT}.^{10}$$

$$\text{HSI} : 8 * \text{ALT/AST} + \text{BMI}(+2, \text{if type 2 diabetes}; +2, \text{if female}).^{11}$$

$$\text{VAI} : [\text{WC}/39.68 + (1.88 * \text{BMI})] * (\text{triglycerides}/1.03) * (1.31/\text{HDL}), \text{for males}; [\text{WC}/36.58 + (1.89 * \text{BMI})] * (\text{triglycerides}/0.81) * (1.52/\text{HDL}), \text{for females}.^{18}$$

$$\text{TyG index} : \log[(\text{triglycerides})(\text{mg/dL}) \times \text{glucose}(\text{mg/dL})/2].^{17}$$

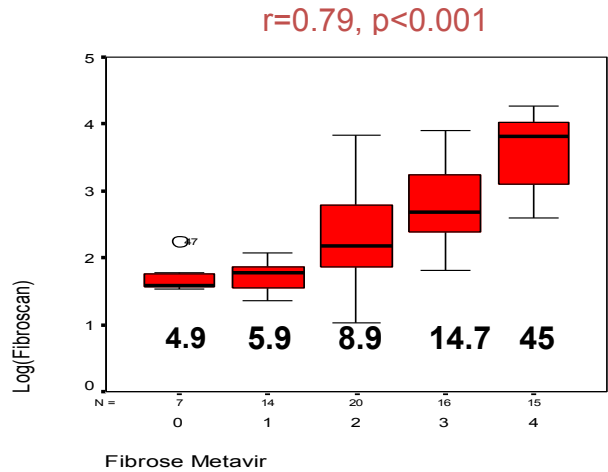
Serum tests for steatosis

Table 2 | Diagnostic values of steatosis biomarkers for predicting steatosis in comparison to liver biopsy

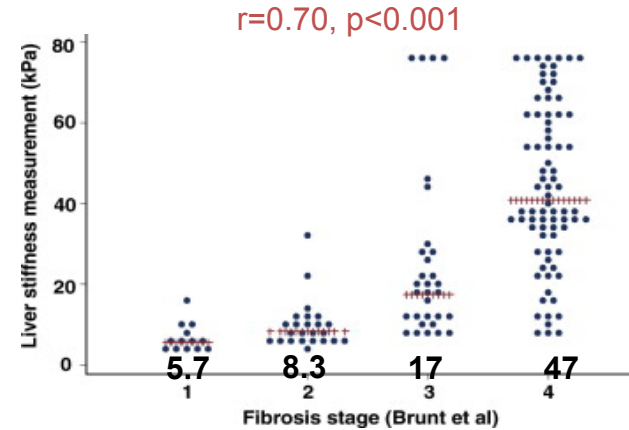
	AUROC (95% CI)	Sensitivity,%	Specificity,%	PPV,%	NPV,%	LR
Steatosis >5%						
FLI (>60)	0.83 (0.72–0.91)	76	87	99	15	5.7
VAI (>1.25)	0.92 (0.85–0.95)	79	92	99	16	9.4
NAFLD liver fat score (>0.16)	0.80 (0.69–0.88)	65	87	99	11	4.9
TyG (>8.38)	0.90 (0.84–0.94)	80	92	99	16	10.4
HSI (>41.6)	0.81 (0.71–0.88)	61	93	99	10	9.2

Significant Correlation between Liver stiffness & Fibrosis stages

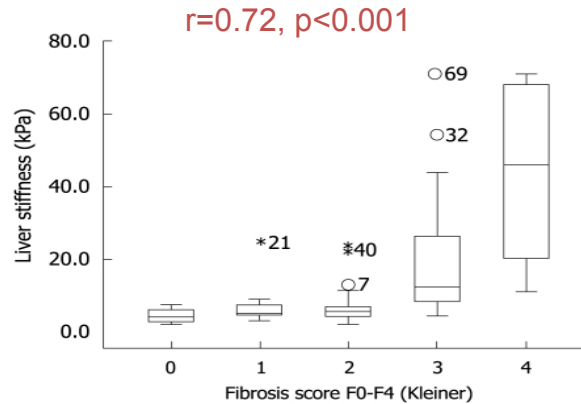
1



2



3



- 1 Nguyen-Khac et al. Aliment Pharmacol Ther 2008
- 2 Nahon et al. J Hepatol 2008
- 3 Mueller et al. World J Gastroenterol 2010

Assessment of specific liver stiffness cut offs for non-invasive diagnosis of Alcoholic Liver Fibrosis

Characteristics	Results (mean ± s.d.)		P
	Included patients (n = 103)	Non-included patients (n = 57)	
Males n (%)	76 (74%)	43 (75.4)	0.85
Age (years)	52.6 ± 9.6	50.5 ± 9.9	0.196
BMI	27.7 ± 5.9	31	0.58
Alcohol intake (g/day)	128.4 ± 78.3	137.5 ± 79.1	0.47
Duration of alcohol abuse (years)	18.7 ± 8.8	14.9 ± 6.8	0.07
White blood cells (4–10.10 ³ /mm ³)	7092 ± 2482	6952.8 ± 2394	0.74
Mean corpuscular volume (μ ³)	97.9 ± 7.7	95.4 ± 13.6	0.15
Platelets (150–400.10 ³ /mm ³)	250.5 ± 126	230 ± 123	0.35
PT (70–100%)	87 ± 11.8	87.8 ± 10.2	0.65
ASAT (<35 IU/L)	79.8 ± 78.7	72.6 ± 67.6	0.57
ALAT (<50 IU/L)	61.7 ± 59.3	55.6 ± 48.8	0.52
Gamma-GT (<51 IU/L)	484.9 ± 811.9	374.3 ± 545	0.38
Alkaline phosphatase (<117 IU/L)	114 ± 122.6	104.5 ± 104.5	0.64
Total bilirubin (<21 μmol/L)	17.6 ± 42	13.3 ± 7.6	0.65
Gamma globulins (10–20 g/L)	11.3 ± 3.5	9.9 ± 3.7	0.052
Serum albumin (37–53 g/L)	38.5 ± 7.2	40.4 ± 7.3	0.18
Total cholesterol (<5.2 mmol/L)	5.1 ± 2.4	5.15 ± 1.8	0.99
Triglycerides (<1.8 mmol/L)	1.7 ± 1.3	1.4 ± 0.9	0.23
Hyaluronic acid (<75 μg/L)	80.4 ± 106	66.4 ± 111	0.66
Apolipoprotein A1 (1.1–2.0 g/L)	1.56 ± 0.45	1.6 ± 0.48	0.82
Haptoglobin (0.34–2.0 g/L)	1.53 ± 0.81	2.18 ± 1.21	0.051
α2 Macroglobulin (1.1–2.5 g/L)	2 ± 0.86	1.8 ± 0.66	0.32
Serum iron (9–30.4 μmol/L)	19.7 ± 11.1	19.5 ± 9.8	0.91
Transferrin saturation (20–40%)	37.8 ± 21.75	40.8 ± 18	0.46
Serum ferritin (20–300 μg/L)	641.9 ± 580.5	523.4 ± 338.1	0.22

PT, prothrombin time; ASAT, aspartate aminotransferase.

Characteristics of included patients

Characteristics	Included (n = 147)
Age (years)	54.4 ± 8.9
Gender (male)	112 (76.1)
Diabetes mellitus	4 (2.7)
Body mass index (kg/m ²)	25.6 ± 4.4
AST (IU/L)	75.7 ± 61.4
ALT (IU/L)	56.9 ± 40.8
γ-Glutamyl transferase (IU/L)	415.7 ± 452.0
Serum albumin (g/L)	39.5 ± 7.0
γ-Globulins (g/L)	14.7 ± 7.5
Prothrombin time (%)	75.0 ± 21.6
Platelet count (×1000/mm ³)	188.0 ± 97.8
Total bilirubin (μmol/L)	47.1 ± 81.9

Note: Results are given as means ± standard deviation or n (%).

Assessment of specific liver stiffness cut offs for non-invasive diagnosis of Alcoholic Liver Fibrosis

Characteristics	Results (mean ± s.d.)		P
	Included patients (n = 103)	Non-included patients (n = 57)	
Males n (%)	76 (74%)	43 (75.4)	0.85
Age (years)	52.6 ± 9.6	50.5 ± 9.9	0.196
BMI	27.7 ± 5.9	31	0.58
Alcohol intake (g/day)	128.4 ± 78.3	137.5 ± 79.1	0.47
Duration of alcohol abuse (years)	18.7 ± 8.8	14.9 ± 6.8	0.07
White blood cells (4–10.10 ³ /mm ³)	7092 ± 2482	6952.8 ± 2394	0.74
Mean corpuscular volume (μ ³)	97.9 ± 7.7	95.4 ± 13.6	0.15
Platelets (150–400.10 ³ /mm ³)	250.5 ± 126	230 ± 123	0.35
PT (70–100%)	87 ± 11.8	87.8 ± 10.2	0.65
ASAT (<35 IU/L)	73.8 ± 78.7	72.8 ± 87.8	0.57
ALAT (<50 IU/L)	61.7 ± 59.3	55.6 ± 48.8	0.52
Gamma-GT (<51 IU/L)	484.9 ± 811.9	374.3 ± 545	0.38
Alkaline phosphatase (<117 IU/L)	114 ± 122.6	104.5 ± 104.5	0.64
Total bilirubin (<21 μmol/L)	17.6 ± 42	13.3 ± 7.6	0.65
Gamma globulins (10–20 g/L)	11.3 ± 3.5	9.9 ± 3.7	0.052
Serum albumin (37–53 g/L)	38.5 ± 7.2	40.4 ± 7.3	0.18
Total cholesterol (<5.2 mmol/L)	5.1 ± 2.4	5.15 ± 1.8	0.99
Triglycerides (<1.8 mmol/L)	1.7 ± 1.3	1.4 ± 0.9	0.23
Hyaluronic acid (<75 μg/L)	80.4 ± 106	66.4 ± 111	0.66
Apolipoprotein A1 (1.1–2.0 g/L)	1.56 ± 0.45	1.6 ± 0.48	0.82
Haptoglobin (0.34–2.0 g/L)	1.53 ± 0.81	2.18 ± 1.21	0.051
α2 Macroglobulin (1.1–2.5 g/L)	2 ± 0.86	1.8 ± 0.66	0.32
Serum iron (9–30.4 μmol/L)	19.7 ± 11.1	19.5 ± 9.8	0.91
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Serum ferritin (20–300 μg/L)	641.9 ± 580.5	523.4 ± 338.1	0.22

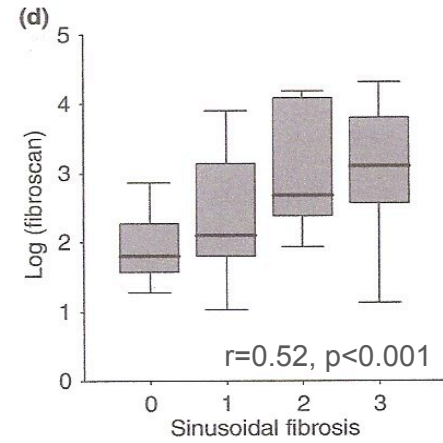
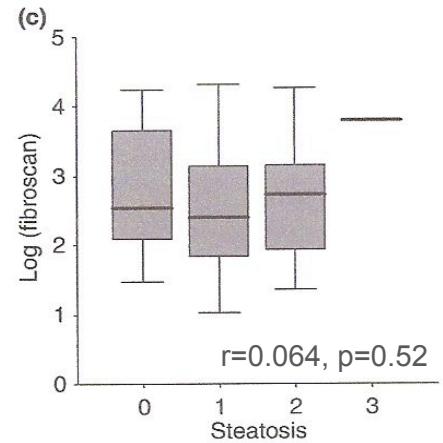
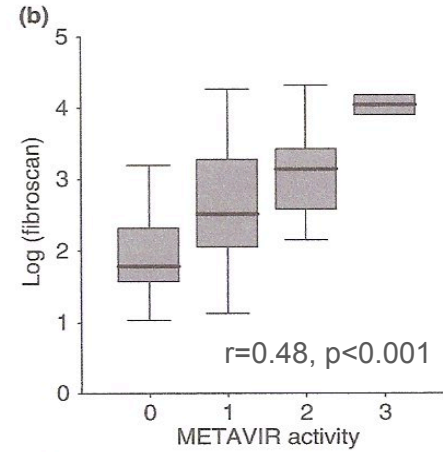
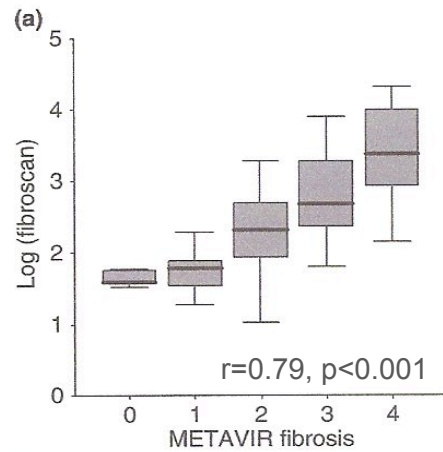
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Assessment of specific liver stiffness cut offs for non-invasive diagnosis of Alcoholic Liver Fibrosis



Liver Stiffness

Specific cut offs for diagnosis of alcoholic liver fibrosis

	Cut Offs (kPa)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	AUROC (CI 95%)	
$F \geq 1$	5.9	83	86	97.6	35.3	0.84	0.73-0.95
$F \geq 2$	7.8	80	90.5	93	70	0.91	0.85-0.98
$F \geq 3$	11	86.7	80.5	81.8	84.3	0.89	0.82-0.97
$F = 4$	19.5	85.7	84.2	68.6	87.9	0.92	0.87-0.98

Liver Stiffness

Specific cut offs for diagnosis of alcoholic liver fibrosis

	Cut Offs (kPa)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	AUROC (CI 95%)	
F ≥ 1	5.9	83	86	97.6	35.3	0.84	0.73-0.95
F ≥ 2	7.8	80	90.5	93	70	0.91	0.85-0.98
F ≥ 3	11	86.7	80.5	81.8	84.3	0.89	0.82-0.97
F ≥ 3	12.9	81	89	95	62	0.94	0.90-0.97
F = 4	19.5	85.7	84.2	68.6	87.9	0.92	0.87-0.98
F = 4	22.6	84	80	83	82	0.87	0.81-0.93

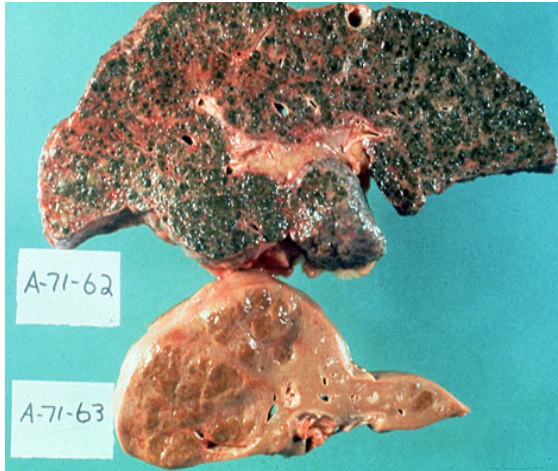
Nahon et al. J Hepatol 2008

Nguyen-Khac et al. Aliment Pharmacol Ther 2008

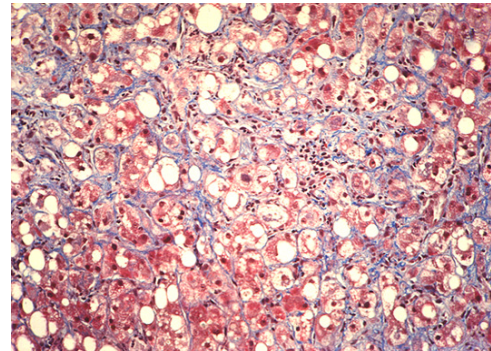
Comment 1 : higher cut off for F4 in Alcoholic Liver Disease

A different distribution of Fibrosis in ALD

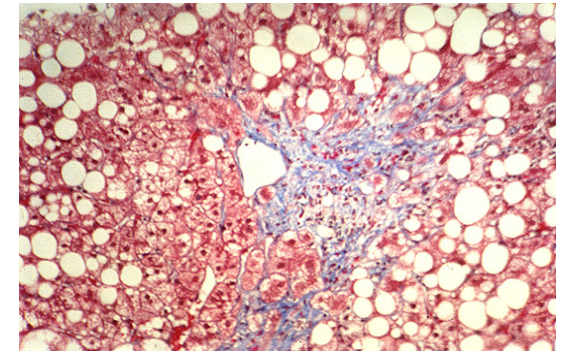
Alcoholic Cirrhosis



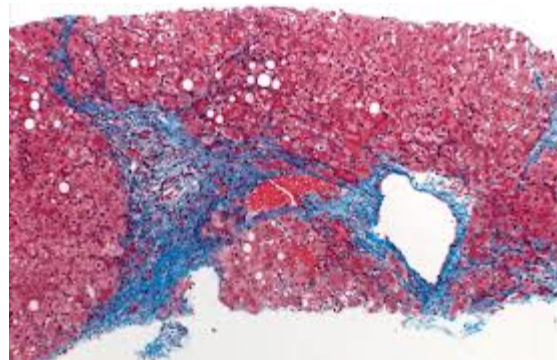
Viral Cirrhosis



Perisinusoidal fibrosis



Centro lobular fibrosis



Portal fibrosis

Comment 1 : higher cut off for F4 in Alcoholic Liver Disease

	Exploratory Population				Validating Population
	Whole	Alcohol	Virus	P Value*	
No. of patients	478	95	383	-	120
Age (yrs)	44.8 ± 12.5	49.8 ± 11.2	43.5 ± 12.5	<10 ⁻⁴	44.1 ± 12.2
Sex (% male)	64.9	71.6	63.2	.13	62.5
Metavir F stage (%):					
F0	5.9	12.6	4.2		16.7
F1	35.6	17.9	40.1		35.8
F2	25.2	16.8	27.3	<10 ⁻⁴	23.3
F3	11.9	11.6	11.9		11.7
F4	21.4	41.1	16.4		12.5
CSF (%)	58.5	69.5	55.6	.01	47.5
Metavir F score (mean)	2.1 ± 1.3	2.5 ± 1.5	2.0 ± 1.2	<10 ⁻⁴	1.7 ± 1.2†
AOF (%)	13.2 ± 10.0	20.1 ± 13.5	10.3 ± 6.2	<10 ⁻⁴	-
Liver specimen length (mm)	18.2 ± 6.4	18.4 ± 6.0	18.1 ± 6.5	.68	20.7 ± 4.9†
ALT and AST < ULN (%)	22.6	30.5	20.7	.04	18.3

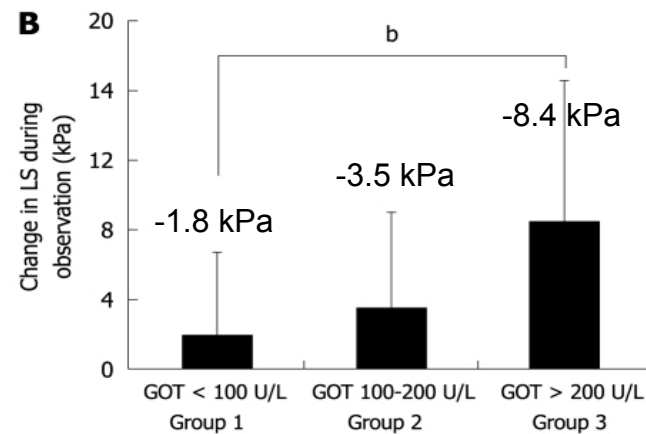
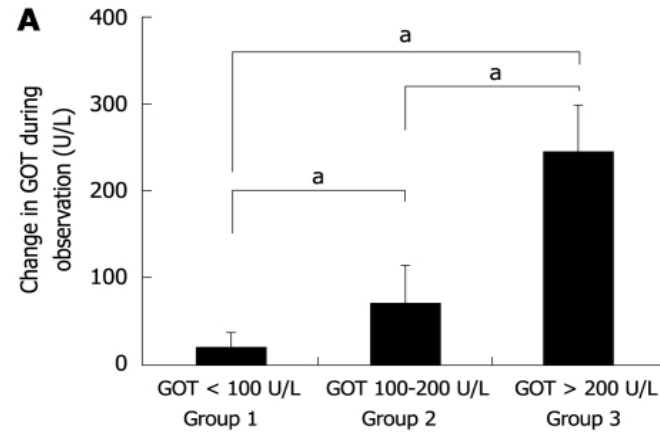
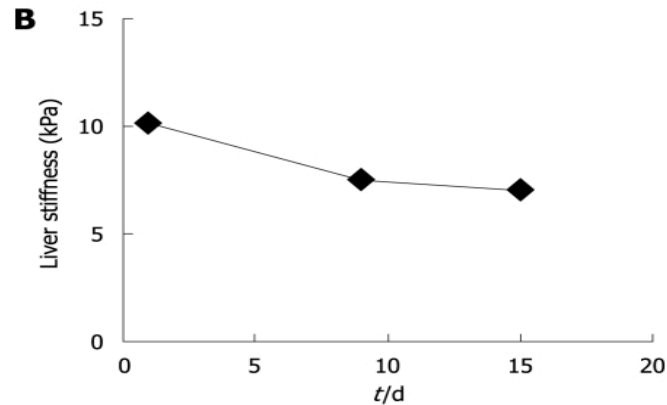
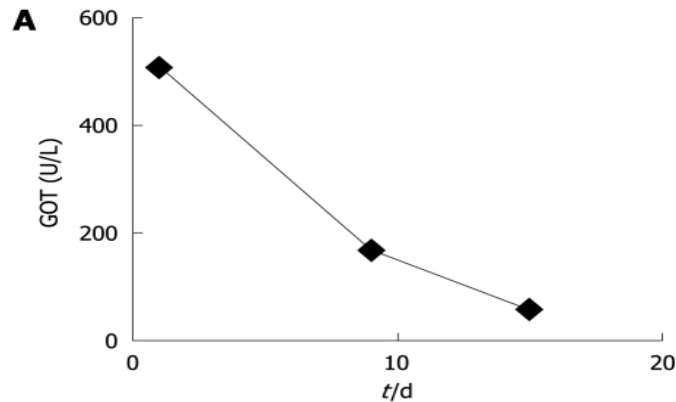
Higher Area of Fibrosis in ALD (F4)

Comment 1 : higher cut off for F4 in Alcoholic Liver Disease

Liver Disease	Area of Fibrosis (%)		p
	F0-F1	F3-F4	
Viral Liver Disease (n=383)	7.3 ± 2.1	12.8 ± 7.2	<0.0001
Alcoholic Liver Disease (n=99)	7.8 ± 3.9	26.5 ± 12.2	<0.0001

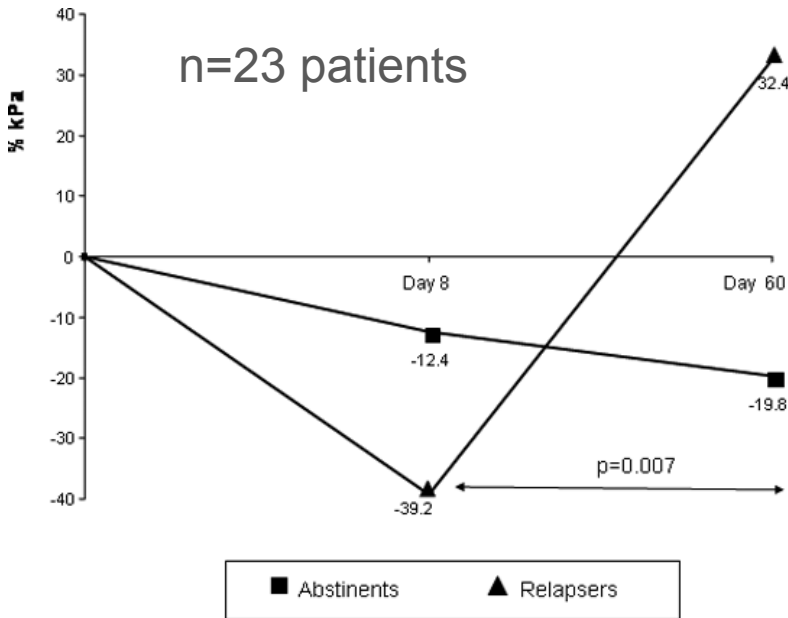
Higher Area of Fibrosis in ALD (F4)

Decline of liver stiffness after alcohol withdrawal



Comment 2 : Decline of liver stiffness after alcohol withdrawal

Decrease stiffness



D8 from alcohol withdrawal

56.5% of patients has decrease stiffness

Mean Stiffness decrease
- 21.67% ± -27.6 % kPa

Comment 2 : Decline of liver stiffness After alcohol withdrawal

137 patients, Alcohol Withdrawal

- D7 : decline of Liver Stiffness
– 7.2 kPa (mean)
- 63% (87) : decrease LS
- 5.8% (8) : no change
- 30.7% (42) : increase LS

Risk factor for decrease

ALAT

p=0.012

OR 1.01 (CI 95%: 1.002-1.018)

Comments 2 : Decline of liver stiffness After alcohol withdrawal

Impact on Fibrosis stage Diagnosis

Whole population

no fibrosis Change	66.4% (91)
- 1 fibrosis grade	15.3% (21)
- 2 fibrosis grades	8% (11)
+ 1 fibrosis grade	8% (11)
+ 2 fibrosis grades	2.2% (3)

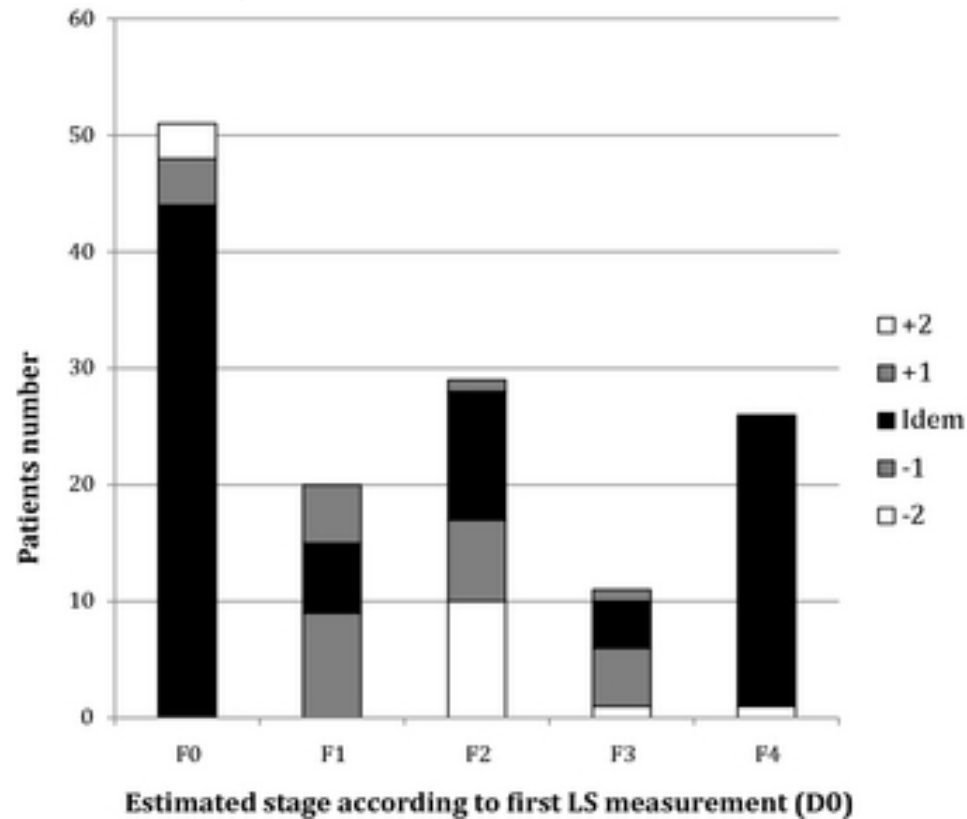
Sub group patients

n = 29, F2 patients
→ 17 (58%) decrease at F1
or F0

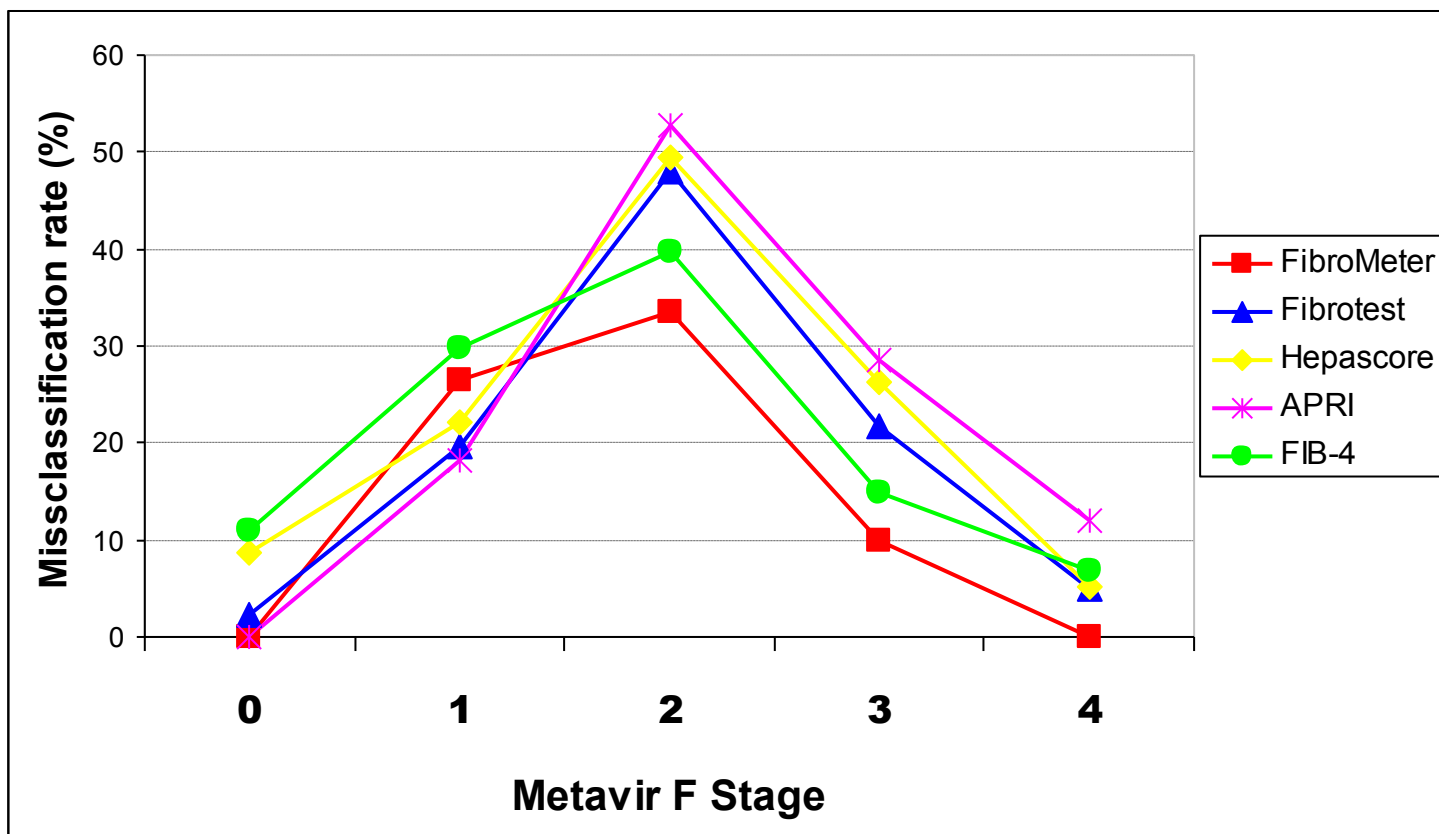
n = 28, F4 patients
→ 23 (82%), F4
→ 5 (17.9%), F3
→ 1 (3.5%), F2

Comments 2 : Decline of liver stiffness After alcohol withdrawal

No change for F0 and F4 Fibrosis stage



Misclassified fibrosis stage in 1056 chronic hepatitis C



Comment 3 : Impact of Alcoholic Hepatitis on Liver Stiffness

	F4 at 19.5 kPa		F4 at 22.7 kPa	
Amiens	Asymptomatic ALD Obvious cirrhose excluded		Symptomatic ALD ± Cirrhose	
	Cirrhose	32%	50.4%	Cirrhose
	F3+F4	51%	75%	F3+F4
	Bilirubin	17.6 ± 42	47.1 ± 81.9	Bilirubin
	PT	87 ± 11.8	75 ± 21.6	TP
	Impact AH	∅	↑ Stiffness	Impact AH

Bili: r=0,36, p=0,001

TP: r=-0,58, p<0,0001

Comment 3 : Impact of Alcoholic Hepatitis on Liver Stiffness

Symptomatic AH impact Liver Stiffness

Amiens		Bondy	
21 patients with histological Features of AH		88 patients with histological Features of AH	
	LS (kPa)	AH	LS (kPa)
AH	12.5 ± 19.2	Absent	11.3 (7.7-35.3)
no AH	11.35 ± 18.7	Mild	23.9 (11.5-47.2)
	p=0.52	Moderate	38.1 (28.5-52.6)
		Marked	48.3 (21.8-73.5)
			p<0.0001

Agenda

- **Non-invasive Diagnosis of Liver Fibrosis, in ALD**
 - Liver Stiffness (Fibroscan)
 - Serum markers
 - Liver Stiffness vs. Serum tests
- **Combinations**
 - Association of two serum markers ?
 - Liver stiffness (Fibroscan) plus serum markers ?

Serum tests in Alcoholic Liver Disease

Tests	Biological Index			Regression formula		
	PGA	PGAA	APRI	Hepascore	Fibrotest	Fibrometer
PT	x	x			x	x
Bilirubin				x	x	
Apolipo A1	x	x			x	
Haptoglobin					x	
A2 Macroglobulin		x		x	x	x
Hyaluronate Acid				x		x
ALAT			x			
Platelets			x			x
GGT	x	x		x	x	
Age				x	x	x
Sexe				x	x	

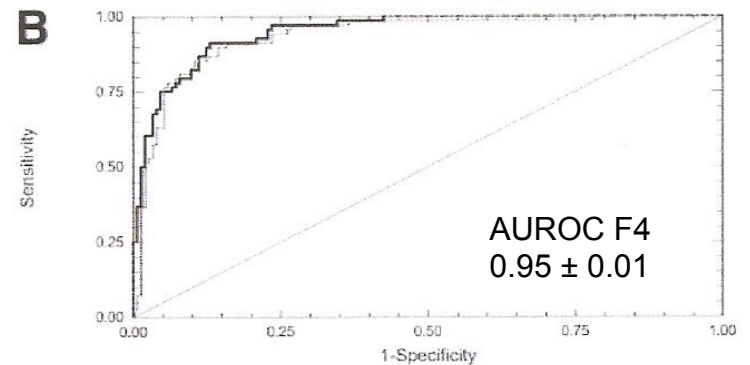
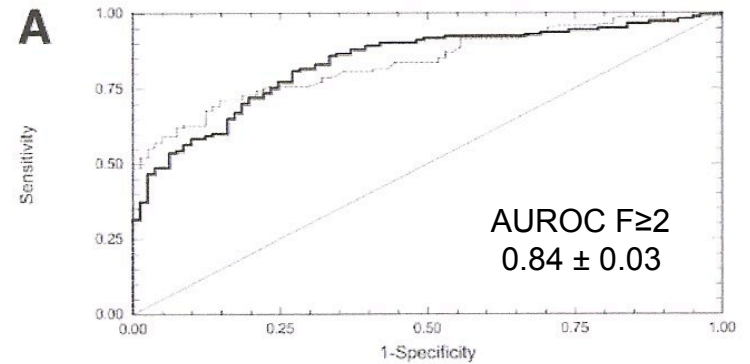
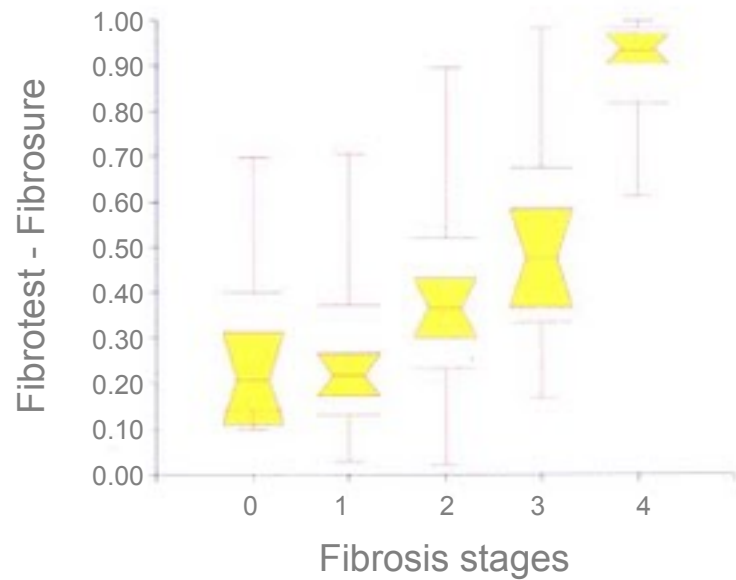
1991 ←

→2005

Licensed tests

Comparison Head to Head

Fibrotest vs. Hyaluronate

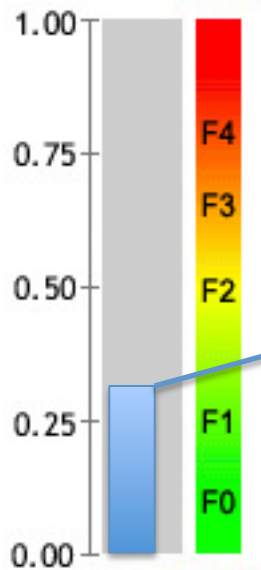


Fibrotest in ALD (n=221 patients)



Predictive

F \geq 2 diagnosis by Fibrotest



FT 0.30

Sen	Spe	PPV	NPV
0.84	0.66	0.82	0.70

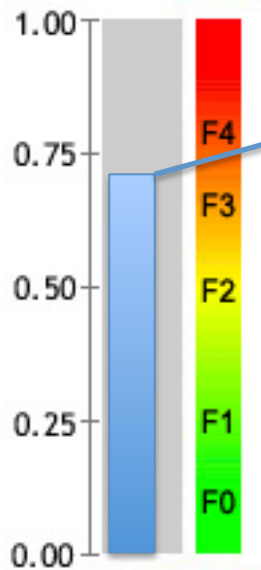
FT result

Fibrotest in ALD (n=221 patients)



Predictive

F \geq 2 diagnosis by Fibrotest



FT 0.70

Sen	Spe	PPV	NPV
0.55	0.93	0.93	0.53

FT 0.30

Sen	Spe	PPV	NPV
0.84	0.66	0.82	0.70

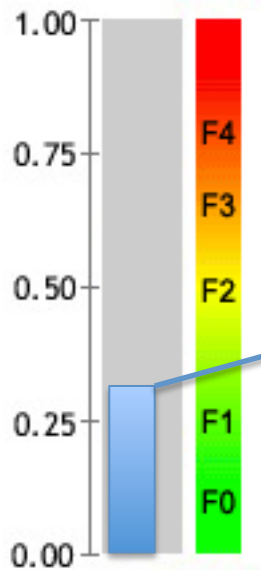
FT result

Fibrotest in ALD (n=221 patients)



Predictive

F4 diagnosis by Fibrotest



FT 0.30

Sen	Spe	PPV	NPV
1.00	0.50	0.47	1.00

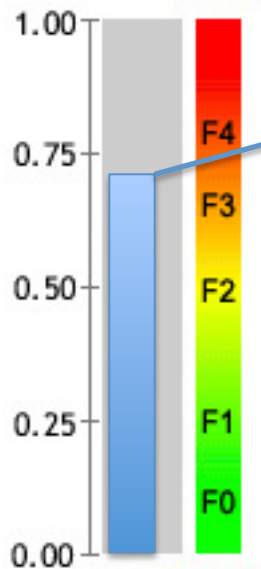
FT result

Fibrotest in ALD (n=221 patients)



Predictive

F4 diagnosis by Fibrotest



FT 0.70

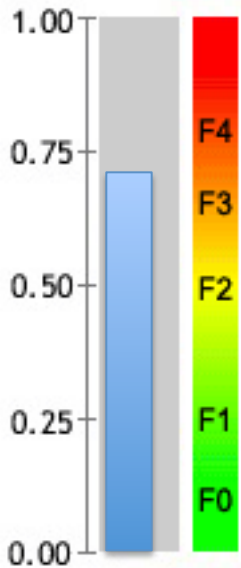
Sen	Spe	PPV	NPV
0.91	0.87	0.76	0.96

FT 0.30

Sen	Spe	PPV	NPV
1.00	0.50	0.47	1.00

FT result

Fibrotest in ALD (n=221 patients)



FT result

Algorithm FT in ALD

FT > 0.70

high risk of cirrhosis

FT 0.30-0.70

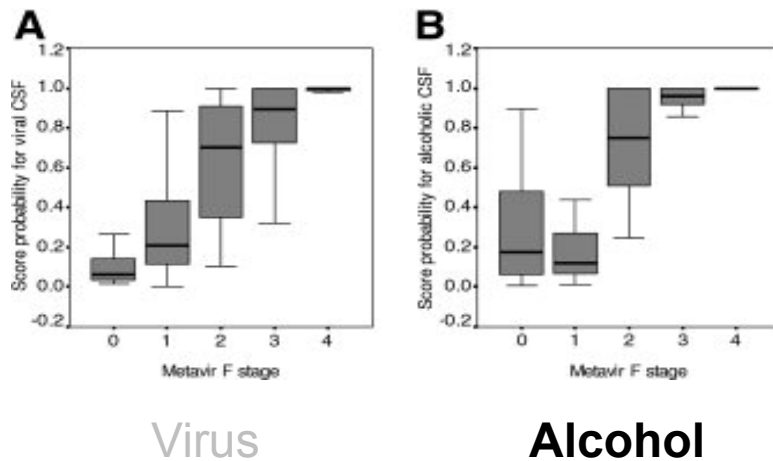
no conclusions

alcohol withdrawal recommended

FT < 0.30

cirrhosis can be excluded

Fibrometer : a specific test for alcoholic liver fibrosis



Specific regression formula

$$0.169 \text{ PI} + 0.015 \text{ A2M (g/L)} + 0.032 \text{ HA (ug/L)} - 0.140 \text{ age (years)} + 16.541$$

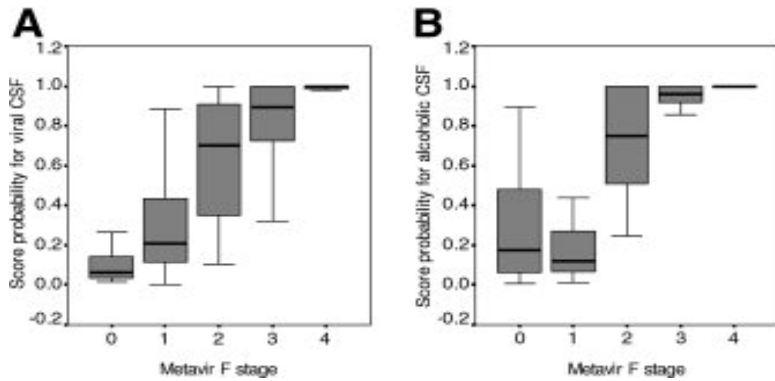
Diagnosis performances

Sensitivity	91.8 %	(CI 95% : 84.9-98.7)
Specificity	92.6 %	(82.7-100)
PPV	96.6 %	(91.9-100)
NPV	83.3 %	(70-96.7)
DA	92 %	(86.4-97.7)

	Training	Validation
AUROC _{alc}	0.883 ± 0.019	0.907 ± 0.027

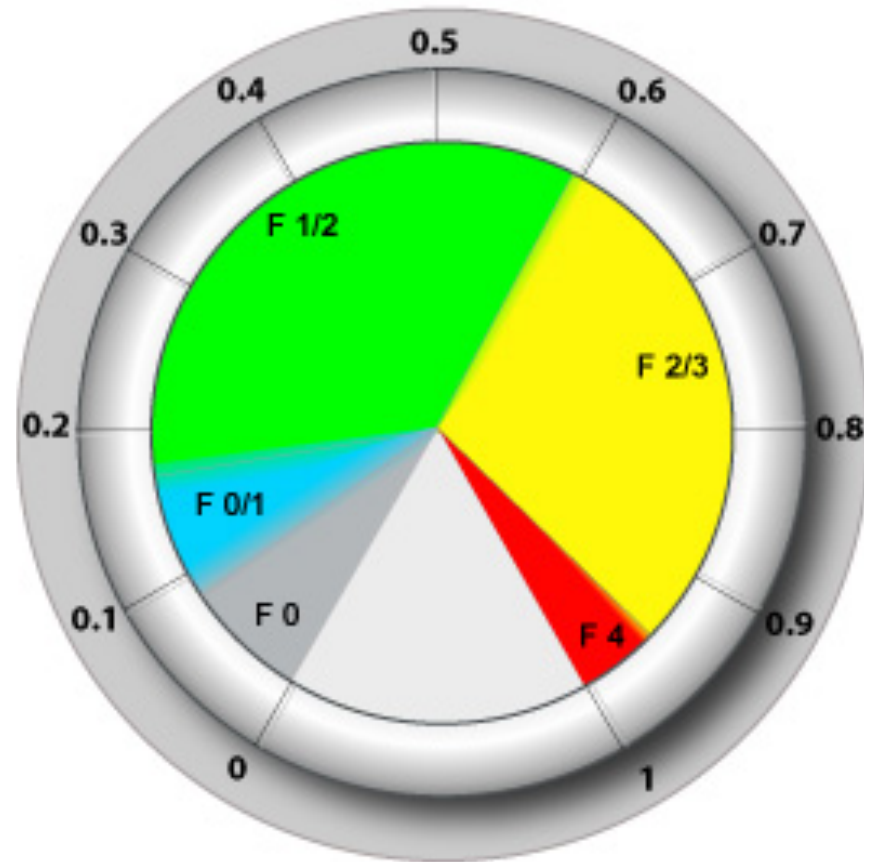
Clinical significant Fibrosis (F>2)

Fibrometer : a specific test for alcoholic liver fibrosis



Virus

Alcohol

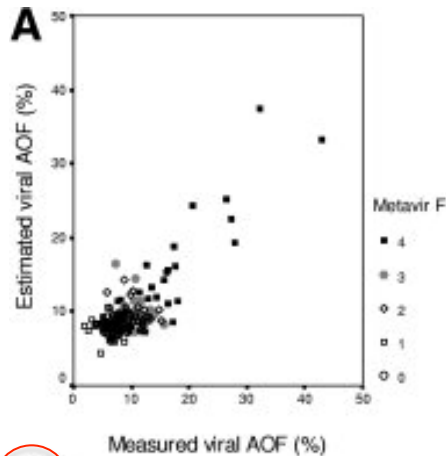


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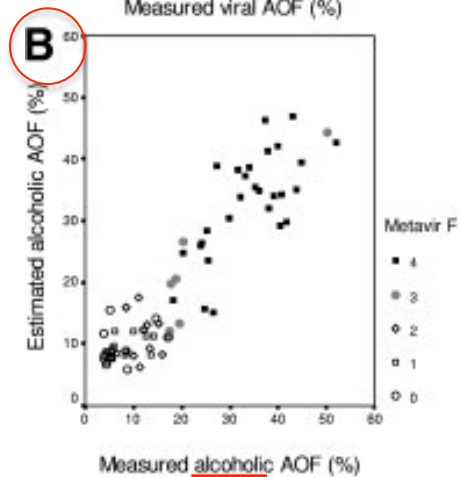
Clinical significant Fibrosis (F>2)

Fibrometer : a specific test for alcoholic liver fibrosis

Aera of Fibrosis

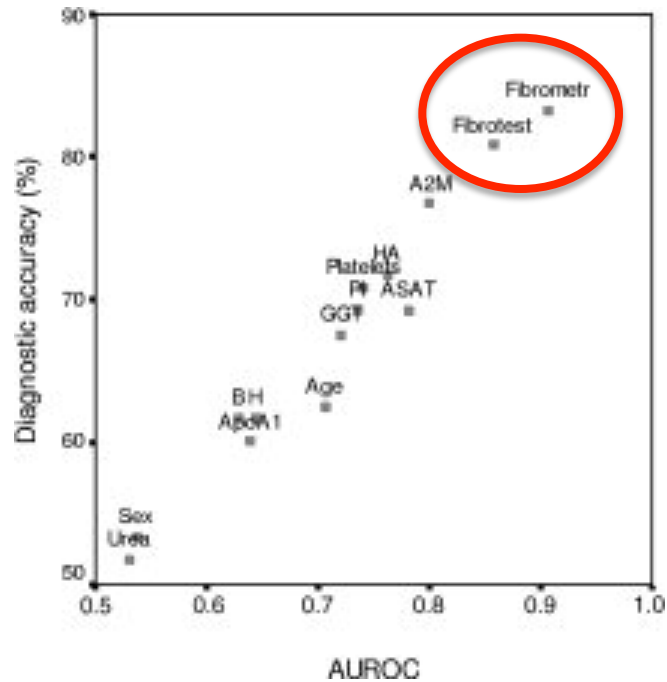


Specific regression formula

$$0.090 \text{ HA (ug/L)} + 0.028 \text{ A2M (g/L)} - 0.009 \text{ PTL (g/L)} - 0.017 \text{ HAPRI ((HA/PI) \times 100)} + 2.166$$


R=0.922

Fibrometer and Fibrotest

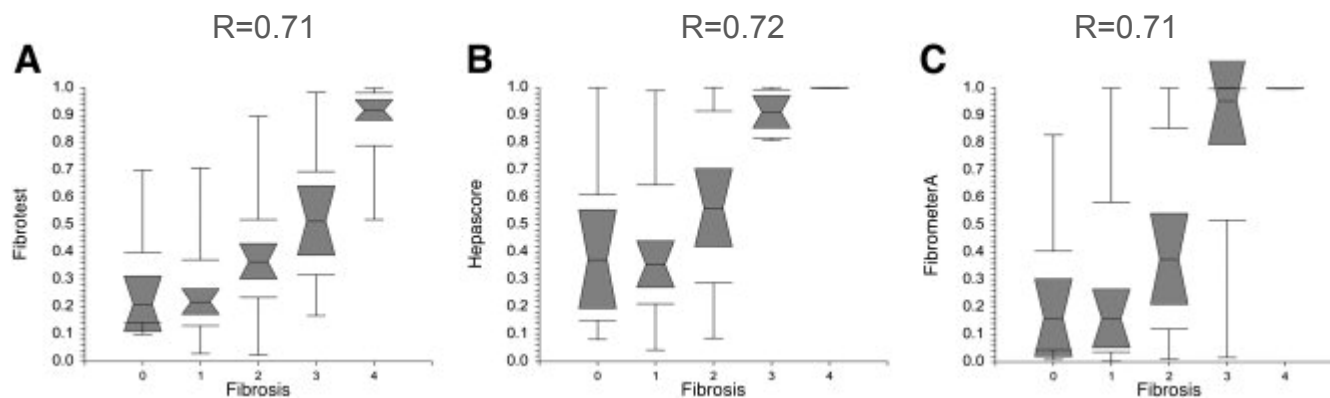


Fibrotest vs. Fibrometer

Comparable AUROCs Fibrotest vs. Fibrometer

	F \geq 1	F \geq 2	F \geq 3	F=4
Fibroscan	0.84 [0.73-0.95]	0.91 [0.85-0.98]	0.90 [0.82-0.97]	0.94 [0.87-0.98]
Fibrometer	0.72 [0.57-0.87]	0.82 [0.72-0.93]	0.88 [0.80-0.95]	0.85 [0.74-0.96]
Fibrotest	0.77 [0.63-0.90]	0.79 [0.69-0.90]	0.80 [0.70-0.91]	0.84 [0.72-0.97]
Hepascore	0.70 [0.51-0.89]	0.76 [0.64-0.88]	0.83 [0.74-0.93]	0.76 [0.63-0.90]
Hyaluronate	0.76 [0.58-0.94]	0.80 [0.70-0.92]	0.83 [0.74-0.92]	0.80 [0.68-0.92]
PGA	0.66 [0.50-0.82]	0.78 [0.68-0.89]	0.84 [0.74-0.94]	0.89 [0.82-0.97]
PGAA	0.74 [0.60-0.88]	0.81 [0.71-0.91]	0.86 [0.76-0.96]	0.83 [0.73-0.93]
APRI	0.76 [0.58-0.95]	0.54 [0.40-0.68]	0.43 [0.30-0.56]	0.56 [0.38-0.73]

Similar diagnosis performance FT vs FM vs Hepascore



Scores	Advanced Fibrosis F0F1 versus F2F3F4		Cirrhosis F0F1F2F3 versus F4	
	AUROC	95% CI	AUROC	95% CI
Patented biomarkers (n = 218)				
FibroTest [†]	0.83	0.77-0.88	0.94	0.90-0.96
FibrometerA	0.83	0.77-0.87	0.94	0.90-0.97
Hepascore	0.83	0.77-0.88	0.92	0.87-0.97
Nonpatented scores				
Forns [‡]	0.38	0.30-0.46	0.38	0.27-0.47
APRI	0.59	0.51-0.67	0.67	0.59-0.75
FIB4	0.70	0.62-0.76	0.80	0.72-0.86

Agenda

- **Non-invasive Diagnosis of Liver Fibrosis in ALD**
 - Liver Stiffness (Fibroscan)
 - Serum markers
 - Liver stiffness vs. Serum markers
- **Combinations**
 - Association of two serum markers ?
 - Liver Stiffness (Fibroscan) plus serum markers ?

Agenda

- **Non-invasive Diagnosis of Liver Fibrosis in ALD**
 - Liver Stiffness (Fibroscan)
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Agenda

- **Non-invasive Diagnosis of Liver Fibrosis in ALD**
 - Liver Stiffness (Fibroscan)
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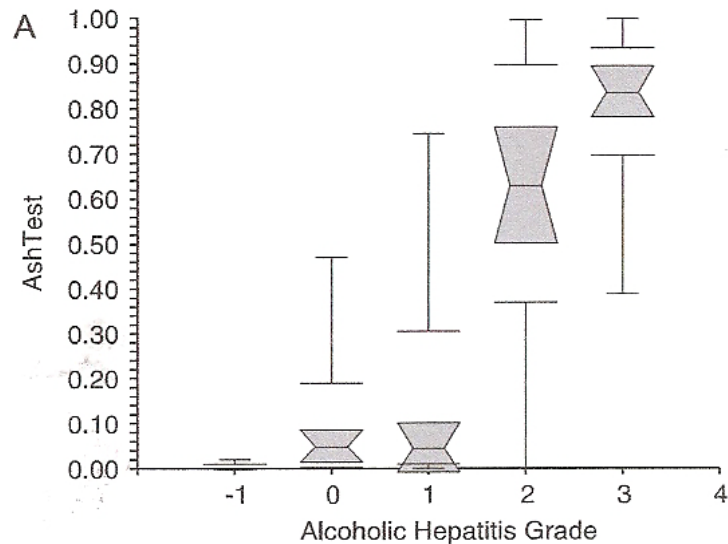
Agenda

- **Non-invasive Diagnosis of Liver Fibrosis**
 - Transient Elastography (Fibroscan)
 - Serum tests
 - Combination Fibroscan plus serum tests
- **Non-invasive Diagnosis of Alcoholic Hepatitis**
- **Prediction of Portal Hypertension**
- **Prognosis and long term survival**

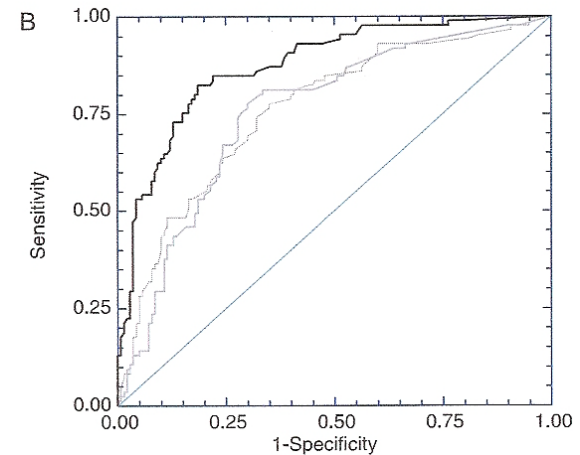
Non-invasive diagnosis of alcoholic hepatitis by Fibrotest – « Ashtest »

225 patients, 23.5% (53) AAH, DF \geq 32

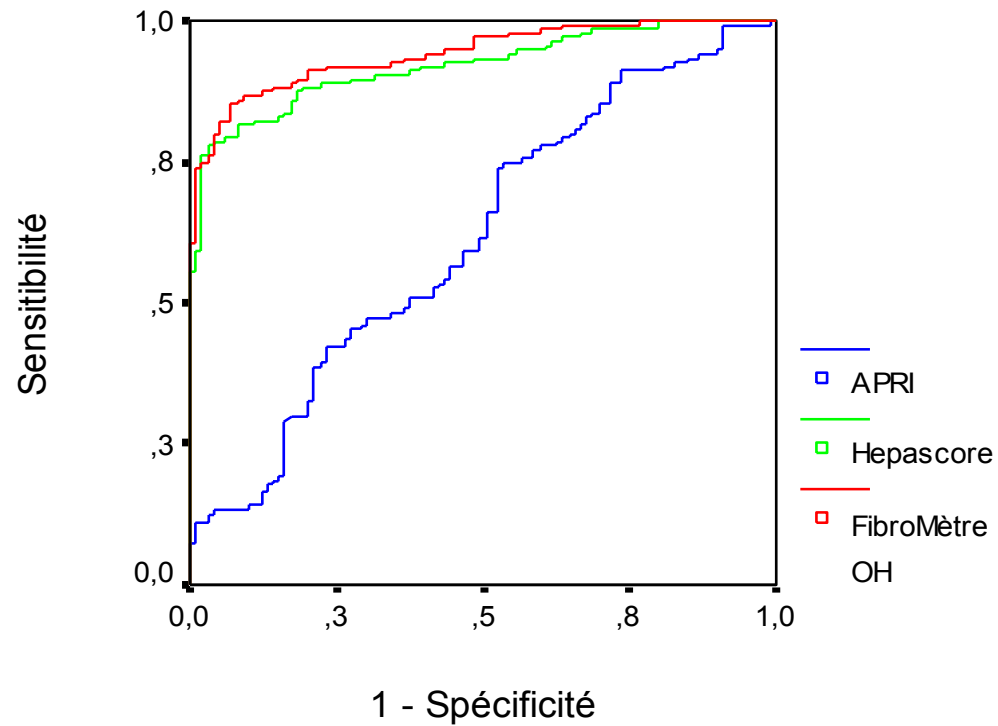
Ashtest : GGT, AST, Bilirubin,
 α 2M, apoA1, haptoglobin



	Sens.	Spec.	AUROC
Ashtest >0,50	80	84	0,88
DF \geq32	43	87	0,78
AST/ALT >2	70	74	0,78



Meta analysis Alcohol



Agenda

- **Non-invasive Diagnosis of Liver Fibrosis**
 - Transient Elastography (Fibroscan)
 - Serum tests
 - Combination Fibroscan plus serum tests
- **Non-invasive Diagnosis of Alcoholic Hepatitis**
- **Prediction of Portal Hypertension**
 - **Fibroscan**
 - **Serum test**
- **Prognosis and long term survival**

Portal hypertension and Elastometry: prediction of LOV

n=183 patients with cirrhosis, (53.3% alcohol)

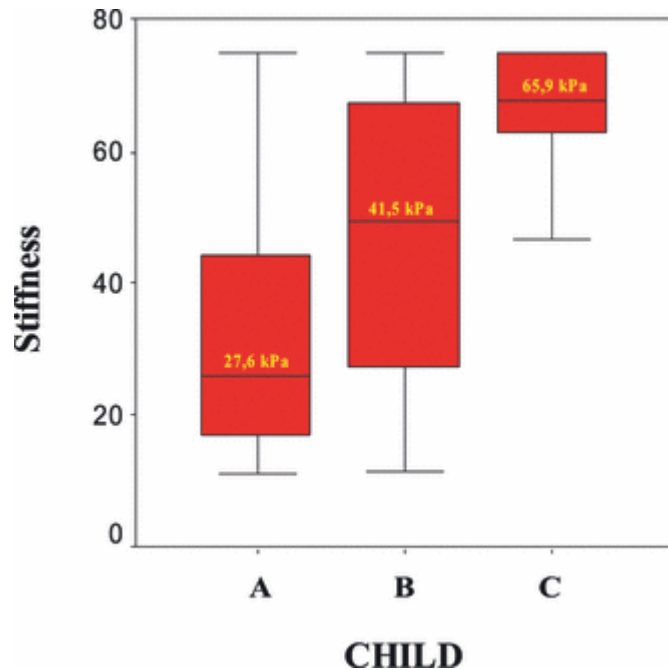


Table 4. Comparison of the Mean Stiffness (kPa) as a Function of the Complications of Portal Hypertension

	Patients		<i>p</i>
	Without	With	
Large esophageal variceal	29.81 ± 1.82	51.24 ± 1.61	<0.0001
Gastric variceal	33.19 ± 1.85	50.87 ± 1.63	0.08
Previous variceal bleeding	31.34 ± 1.84	60.28 ± 1.32	<0.0001
Previous ascites	27.94 ± 1.77	51.92 ± 1.63	<0.0001
Hepatocarcinoma	33.36 ± 1.86	37.76 ± 1.70	0.56

Portal hypertension and Elastometry: prediction of LOV

Table 5. Diagnostic Performances of the FibroScan With Respect to the Presence of Large Variceal

Population	Stiffness threshold (kPa)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUROC	95% CI
Overall population (<i>n</i> = 183)	48	73.2	73.2	44.1	90.4	0.755	0.69–0.82
Alcoholic cirrhosis (<i>n</i> = 103)	47.2	84.6	63.6	44	92.5	0.77	0.68–0.85
Viral cirrhosis (<i>n</i> = 58)	19.8	88.9	55.1	26.7	96.4	0.73	0.60–0.84

AUROC, area under receiver operating characteristic; PPV, positive predictive values; NPV, negative predictive values.

Portal hypertension and Elastometry: prediction of LOV

Strong influence of the etiology of Cirrhosis

Authors, journal	Whole population (n)	Cut Offs (kPa)	Alcohol proportion n (%)	HCV proportion n (%)
Vizzuti et al, Hepatology 2007	61	17.6 kPa	0 (0%)	61 (100%)
Kazemi et al, J Hepatol 2006	165	19 kPa	37 (22%)	98 (59.3%)
Bureau et al, Aliment Pharmacol Ther 2008	150	29.3 kPa	51 (34%)	46 (31%)
Nguyen-Khac et al, ACER 2010	183	48 kPa	109 (59%)	58 (31.7%)

Agenda

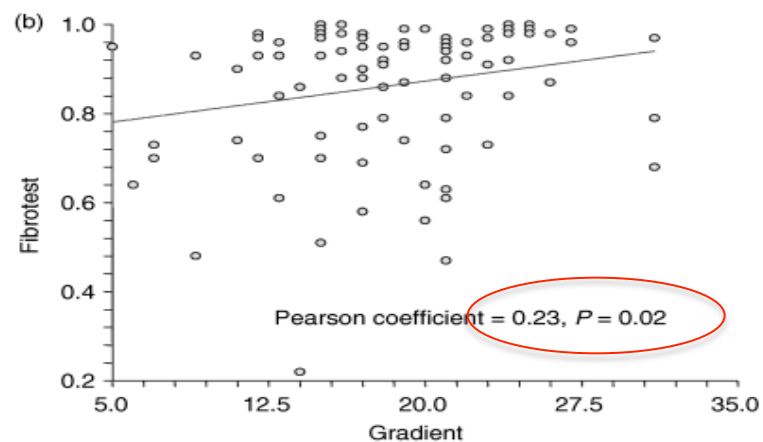
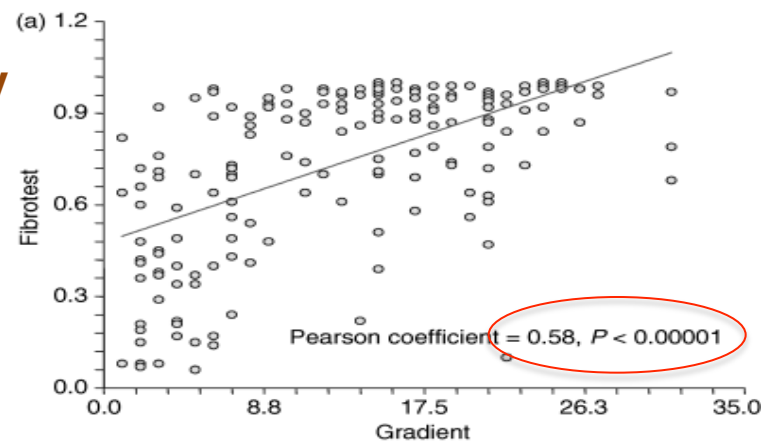
- **Non-invasive Diagnosis of Liver Fibrosis**
 - Transient Elastography (Fibroscan)
 - Serum tests
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Portal hypertension & Fibrotest

(n=130, 72% alcohol)

Significant correlation HVPG and Elastometry

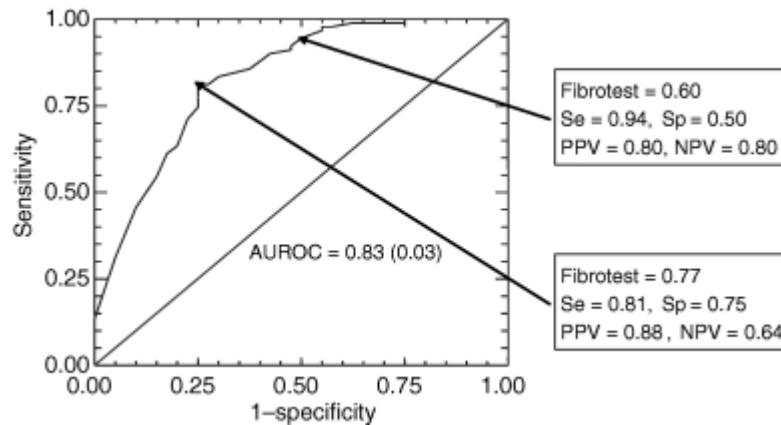
PHT, % (n)	HVPG	FT value	p
No 14% (18)	<5	0.33 ± 0.25	<0.001
Moderate 17% (22)	5 - 11	0.72 ± 0.24	
Severe 69% (90)	>11	0.88 ± 0.14	



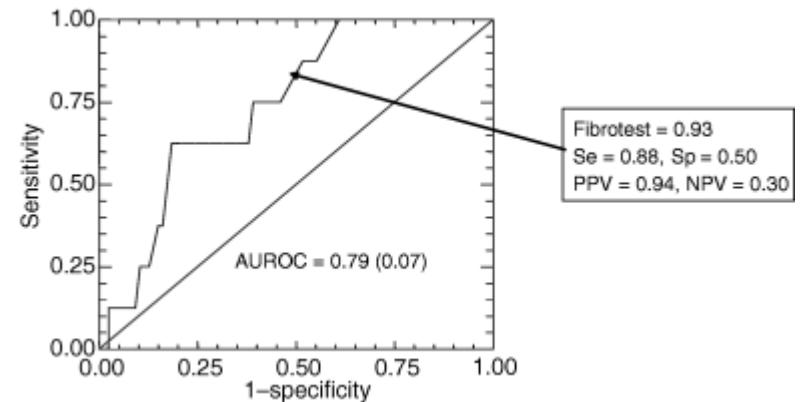
Portal hypertension & Fibrotest

(n=130, 72% alcohol)

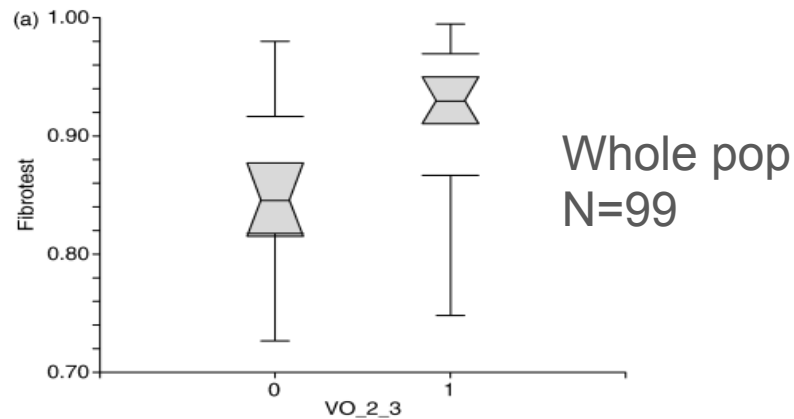
Whole population
(n=130)



Cirrhotic patients
(n=92)
72% alcohol



Non-invasive diagnosis of large oesophageal varices with FibroTest in patients with cirrhosis: a preliminary retrospective study

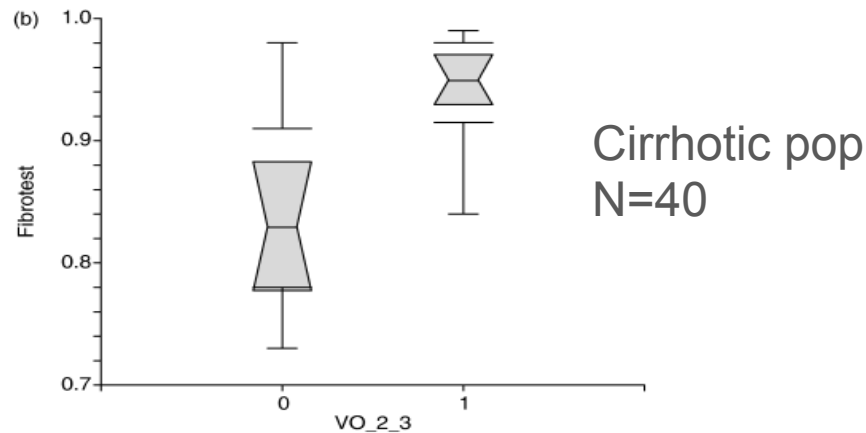


FT value

LOV 0.89 ± 0.11

Small, no OV 0.82 ± 0.14

(P=0.02)



FT value

LOV 0.93 ± 0.11

Small, no OV 0.83 ± 0.14

(P=0.002)

Non-invasive diagnosis of large oesophageal varices with FibroTest in patients with cirrhosis: a preliminary retrospective study

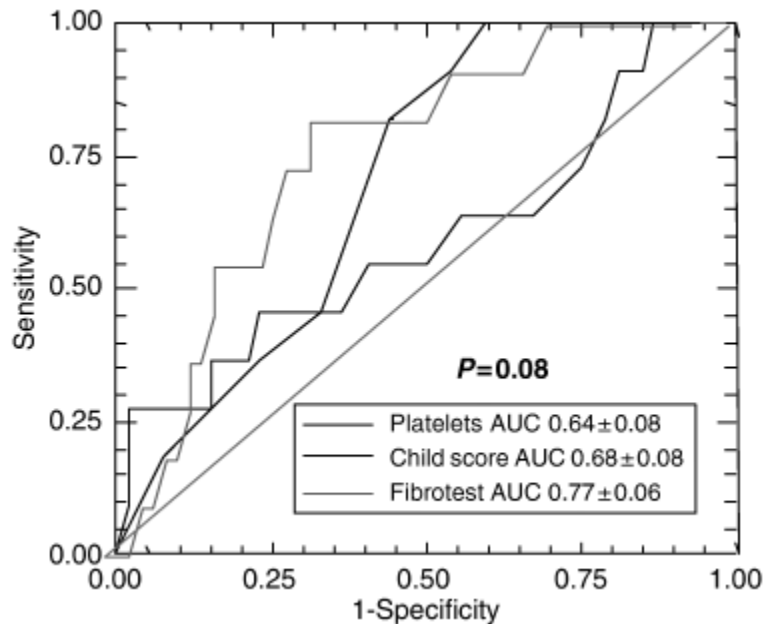


Table 2. Diagnostic values of FibroTest for predicting large oesophageal varices in our study population (prevalence of LOV = 72%)

Cut-off	SNS (%)	SPC (%)	PPV (%)	NPV (%)	+LR
0.80	92	21	79	44	1.17
0.85	84	53	86	50	1.78
0.90	69	74	90	41	2.61
0.95	41	89	93	31	3.86

FT 0.75

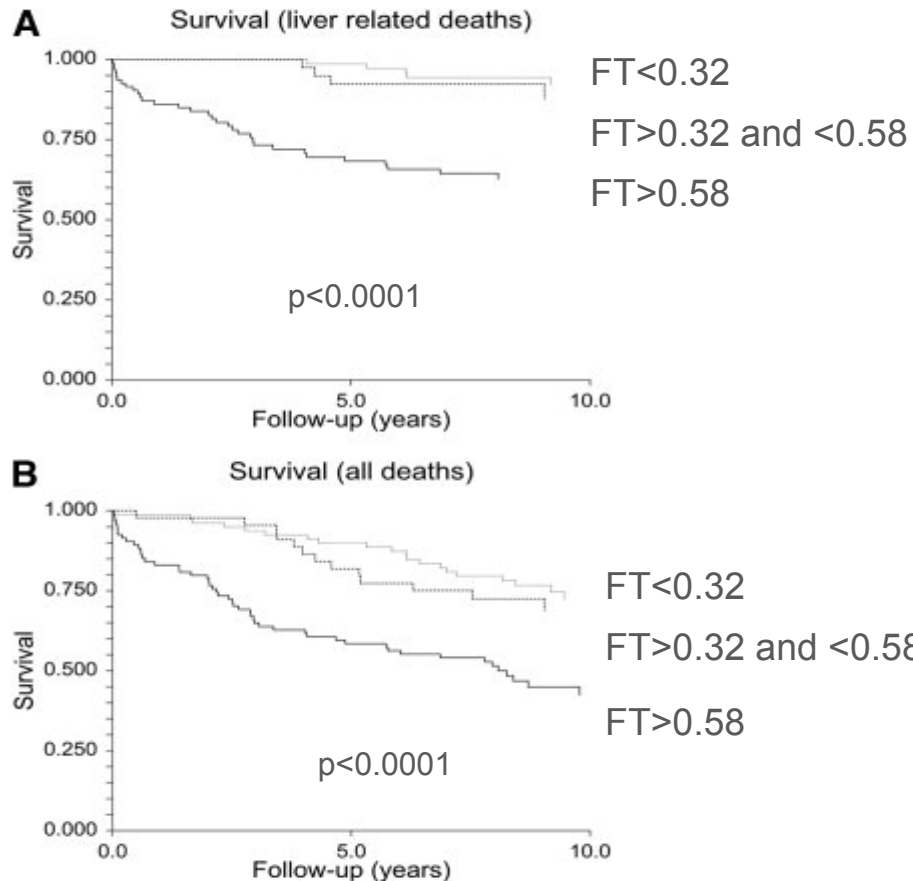
SNS 100%

NPV 100%

Agenda

- **Non-invasive Diagnosis of Liver Fibrosis**
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Prognostic value of Fibrotest in 218 patients with ALD



Markers	Survival or Nonliver-Related Death		Overall Death	
	AUROC	95% CI	AUROC _s	95% CI
FibroTest [†]	0.79	0.68-0.86	0.69	0.61-0.76
FibrometerA [‡]	0.80	0.71-0.87	0.69	0.61-0.76
Hepascore [‡]	0.77	0.69-0.85	0.69	0.62-0.76
Fibrosis staging at biopsy	0.77	0.70-0.83	0.69	0.61-0.76
Pugh	0.69	0.58-0.77	0.62	0.54-0.68
FIB4	0.65	0.54-0.74	0.64	0.55-0.71
APRI	0.60	0.50-0.69	0.56	0.48-0.64
Forns	0.40	0.30-0.49	0.43	0.35-0.51

Conclusions

I - Diagnosis of Liver Fibrosis (1)

- Transient Elastography

- Use Specific Stiffness cut offs, especially 19.6 kPa for F4

- **Cautions if :**

- Alcohol withdrawal, especially in F2
- Alcoholic hepatitis symptomatic

Conclusions

I - Diagnosis of Liver Fibrosis (2)

- Serum tests
 - Fibrotest and Fibrometer, also Hepascore
 - are accurate for diagnosis of liver fibrosis in ALD
 - Same diagnosis performances
 - FT and FM Are licenced serum tests
 - **Caution** : APRI Should not be to use in ALD

Conclusions

I - Diagnosis of Liver Fibrosis (3)

- **Comparison Fibroscan vs. Serum tests**
 - Fibroscan seems better than serum tests only for F2 stage
- **Combination Serum tests**
 - Fibrotest *plus* Fibrometer : not superior to one test alone
- **Combination Fibroscan plus Serum test**
 - Fibroscan *plus* one serum test : not superior to Fibroscan alone

Conclusions

take home messages

II - Diagnosis of portal Hypertension

- **Fibroscan** and serum tests
 - Promising, but data are scarce
 - LS cut off for large esophageal varices diagnosis is higher than in viral liver disease.
 - Validation studies are still lacking

Conclusions

take home messages

III - Diagnosis of alcoholic hepatitis

- *Ash test* a licensed serum test derived from Fibrotest is promising
- But independant validations are lacking

Conclusions

take home messages

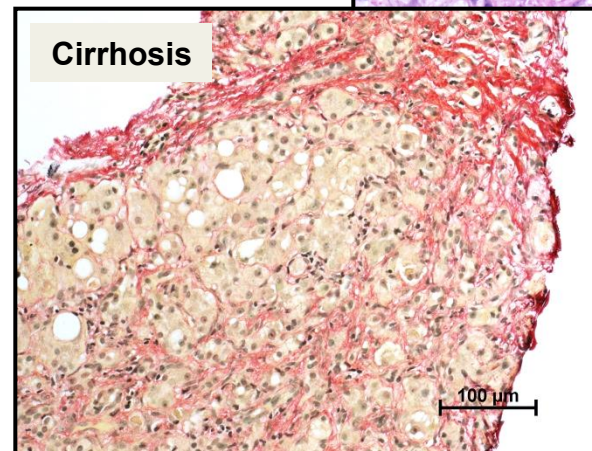
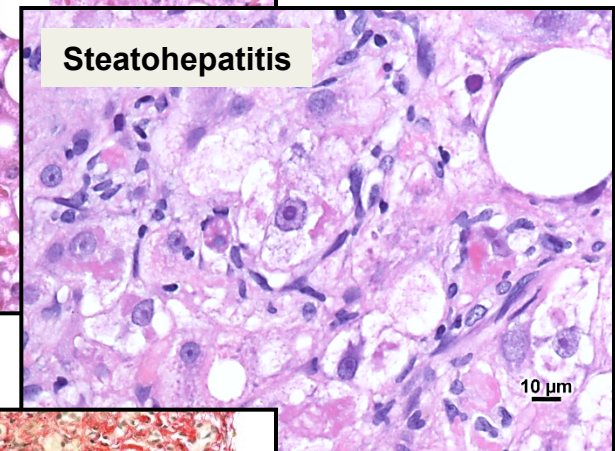
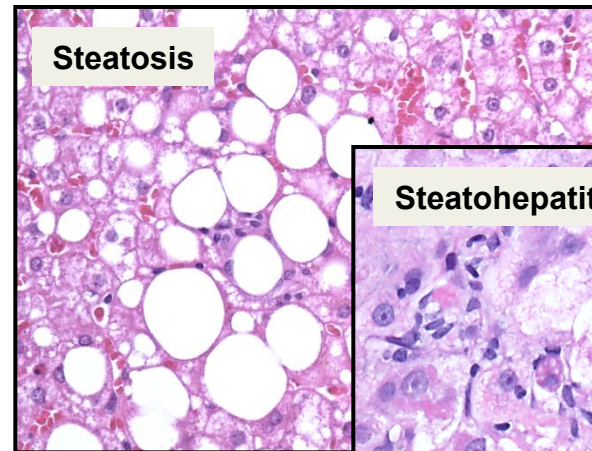
IV – Prognostic use in ALD

- Fibrotest has a strong prognostic value both for predicting liver related death and all cause death, at 5 and 10 years.
- Fibroscan: no data

Histological features of ALD

- Lesions predominate in centrilobular regions (in pre-cirrhotic stages)
 - **Alcoholic steatosis**
 - Macro and eventually variable blend of macro- and microvesicles
 - **Alcoholic steatohepatitis (ASH)**
 - Variable degree of macrovesicular steatosis
 - Hepatocellular injury with ballooning, potentially necrosis
 - Lobular inflammation
 - **Alcoholic fibrosis/cirrhosis**
 - Pericellular fibrosis (PCF) and/or septal F in precirrhotic stage
 - Micronodular cirrhosis ± PCF
- A single lesion or any combination may be found in a given individual

Main histological diagnoses:



Diagnostic tests in the management of ALD: Liver biopsy: Indication and performance

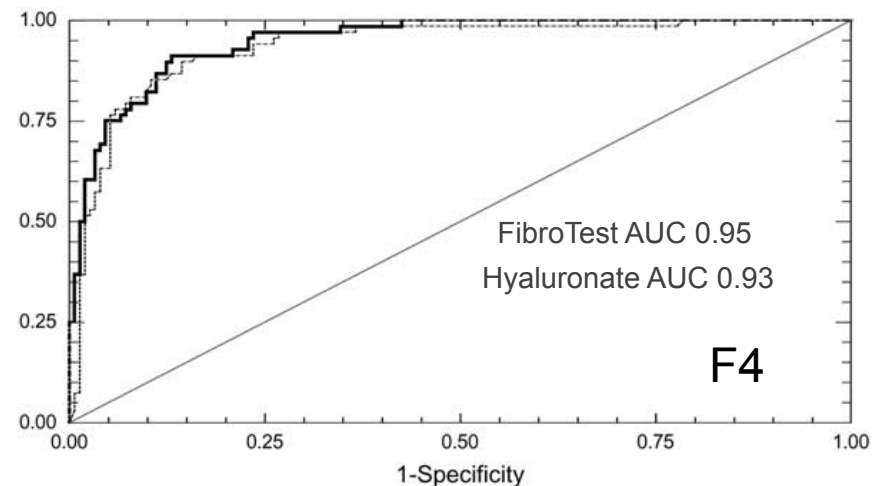
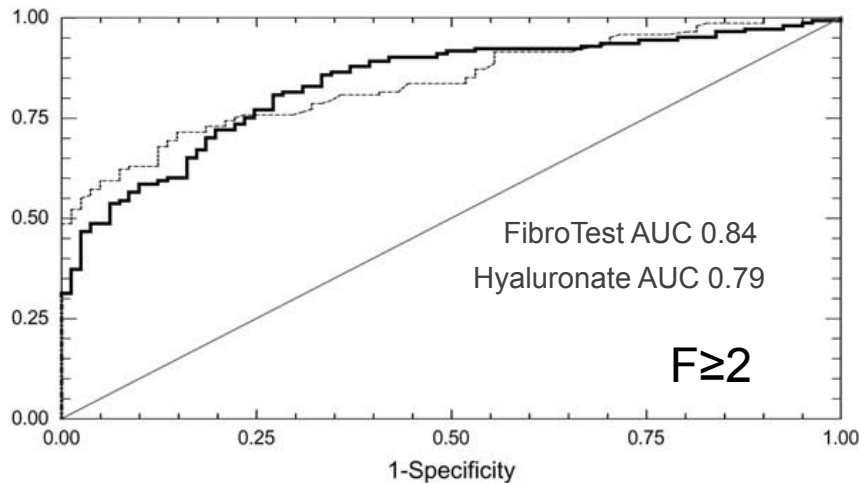
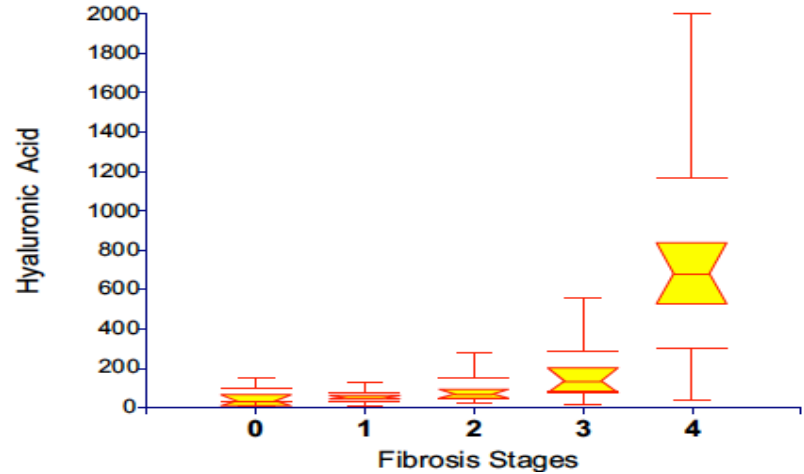
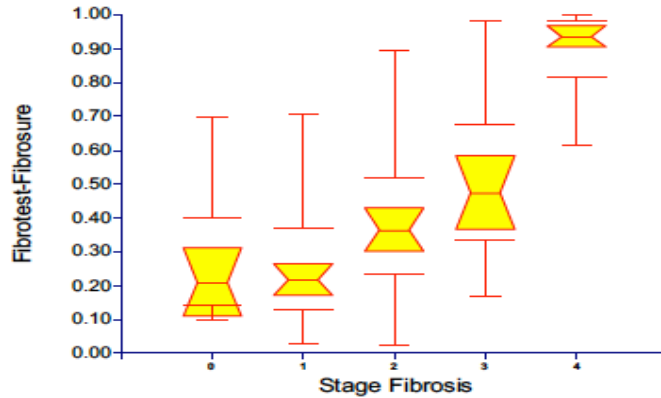
- Liver biopsy may be used to:
 - Establish the definite diagnosis of ALD
 - Assess the exact stage and prognosis of liver disease
 - Exclude alternative or additional causes of liver injury
- Liver biopsy is recommended:
 - Within Phase 2 clinical trials, and should be considered in larger scale Phase 3 clinical trials
 - In case of inconclusive non-invasive test results
 - In case of any suspicion of a competing liver disease
- Liver biopsy is not generally recommended for all patients with suspected ALD
 - Risks should be carefully weighed against the clinical benefits and therapeutic consequences

Diagnostic tests in the management of ALD: Histological features and diagnosis of ALD types

- Types of ALD differ with respect to prognosis
- About 90% of heavy drinkers have hepatocellular steatosis
 - Prognosis is debated; may be associated with progression to cirrhosis (particularly mixed steatosis pattern)
- ASH is considered a progressive lesion
 - Increases the risk of cirrhosis and HCC
- Morphological lesions of ALD and metabolic syndrome-associated NAFLD show broad overlap:
 - Hepatocellular injury and fibrosis are often more severe in ALD
 - Some lesions of ALD are very rare or have not been described in patients with pure NAFLD:
 - Sclerosing hyaline necrosis, alcoholic foamy degeneration (i.e., large portions of the parenchyma affected by microvesicular steatosis), fibro-obliterative changes in hepatic veins, portal acute inflammation, cholestasis

Comparison Head to Head

Fibrotest vs. Hyaluronate (p=ns)

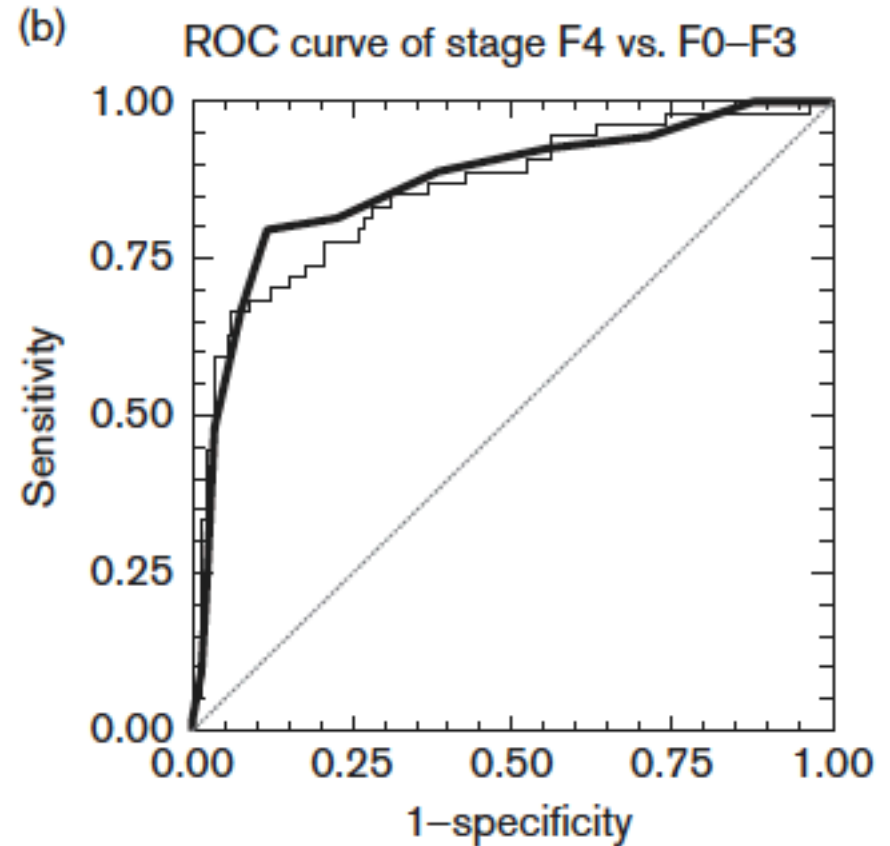
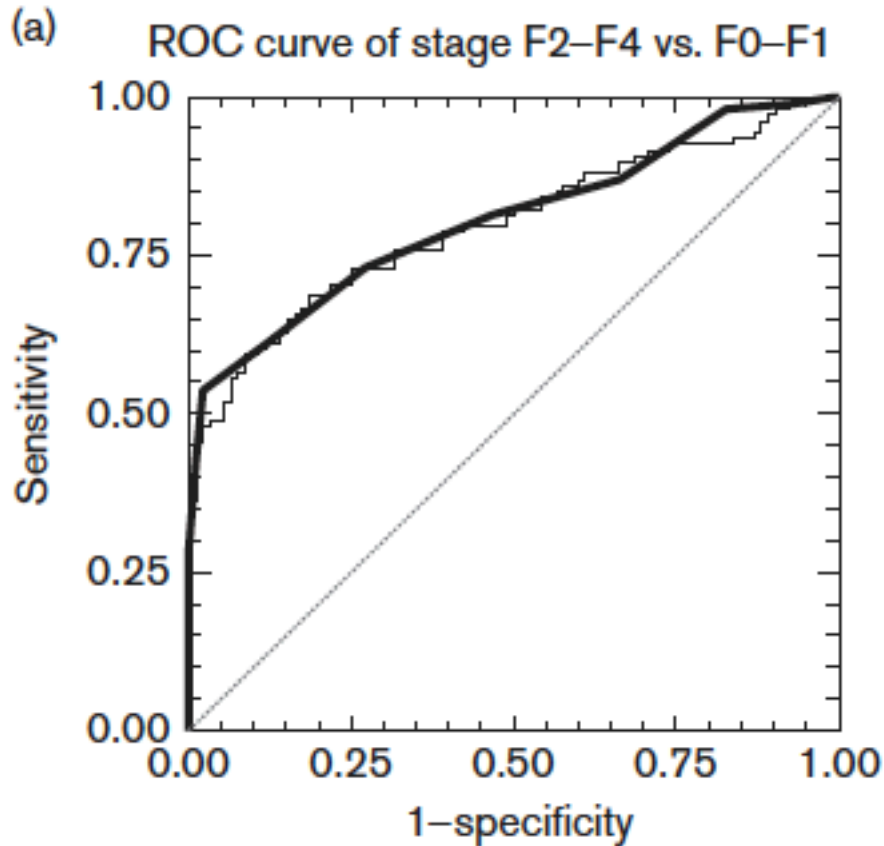


Naveau et al. Clin Gastroenterol Hepatol 2005

Comparison Head to Head

Fibrotest vs. PGAA (p=ns)

Same diagnosis performances



— PGAA — Fibrotest

Non-invasive biological tests - *in ALD*

Indirect markers (simple biochemicals)

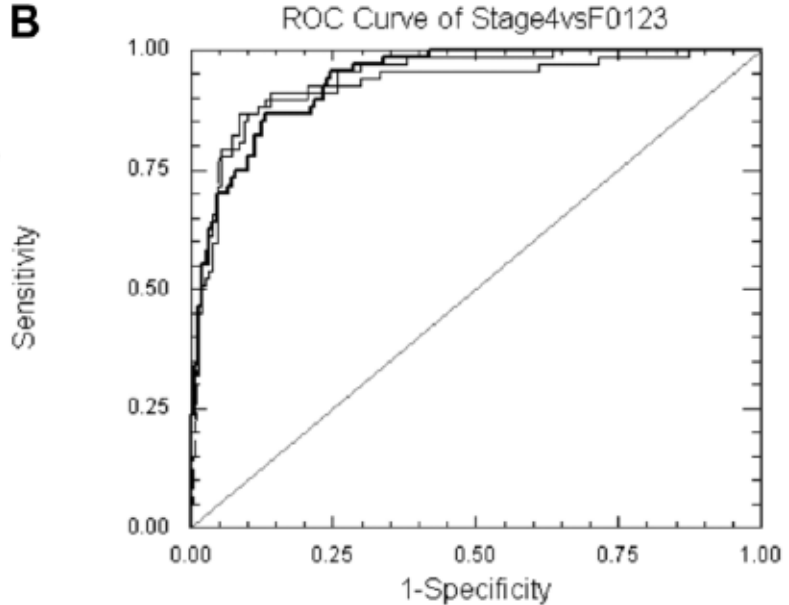
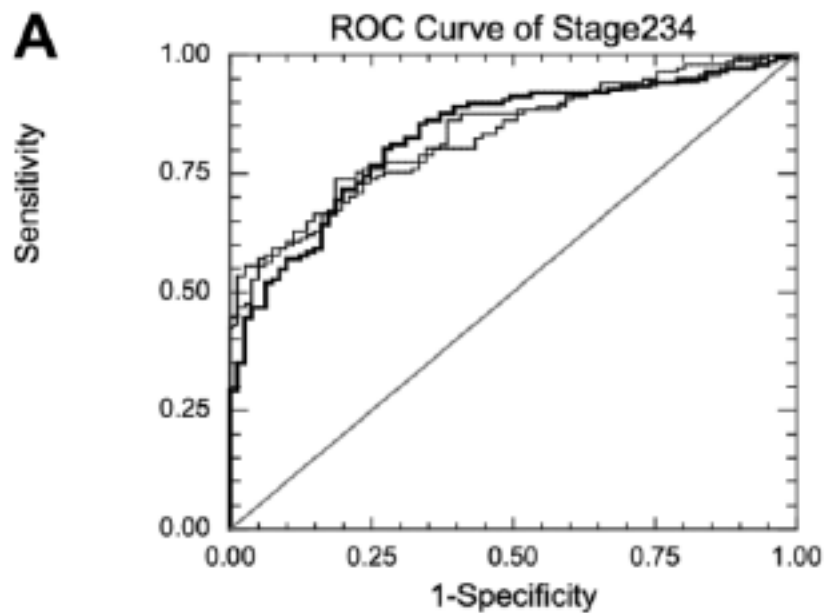
Tests		Sexe	Age	Transa	Platelet	PT	GGT	Bilirubin	A2M	Apo A1	Haptoglob	Cholesterol
Simple tests	PGAA					X	X		X	X		
	APRI			X	X							
	FIB-4		X	X	X							

1991

LICENSED

Comparison Head to Head : Fibrotest vs Fibrometer vs Hepascore

Same diagnosis performances



Scores	Advanced Fibrosis F0F1 versus F2F3F4		Cirrhosis F0F1F2F3 versus F4	
	AUROC	95% CI	AUROC	95% CI
Patented biomarkers (n = 218)				
FibroTest*	0.83	0.77-0.88	0.94	0.90-0.96
FibrometerA	0.83	0.77-0.87	0.94	0.90-0.97
Hepascore	0.83	0.77-0.88	0.92	0.87-0.97

Hyaluronate for Alcoholic liver fibrosis diagnosis

