

Journal of Hepatology, Volume ■

Supplemental information

Longitudinal changes in fibrosis markers are associated with risk of cirrhosis and hepatocellular carcinoma in non-alcoholic fatty liver disease

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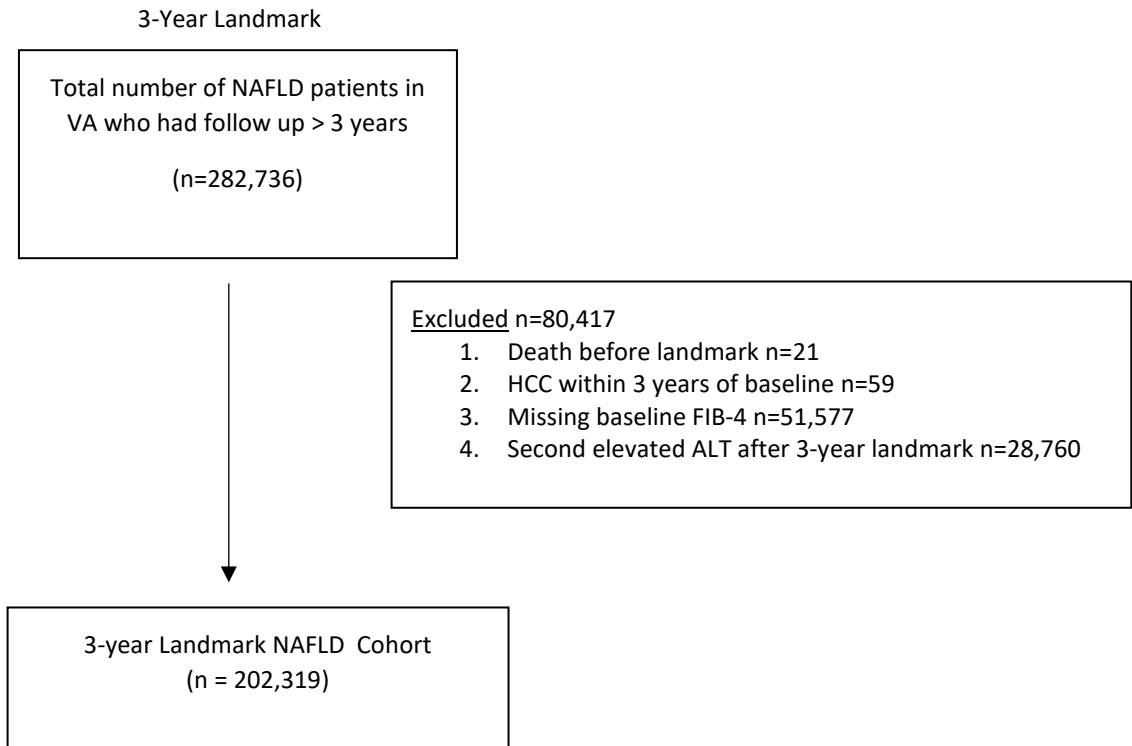
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Table of contents

Fig. S1.....	2
Fig. S2.....	4
Fig. S3.....	5
Table S1.....	6
Table S2.....	7
Table S3.....	8
Table S4.....	9
Table S5.....	10
Table S6.....	11
Table S7.....	12

A



B

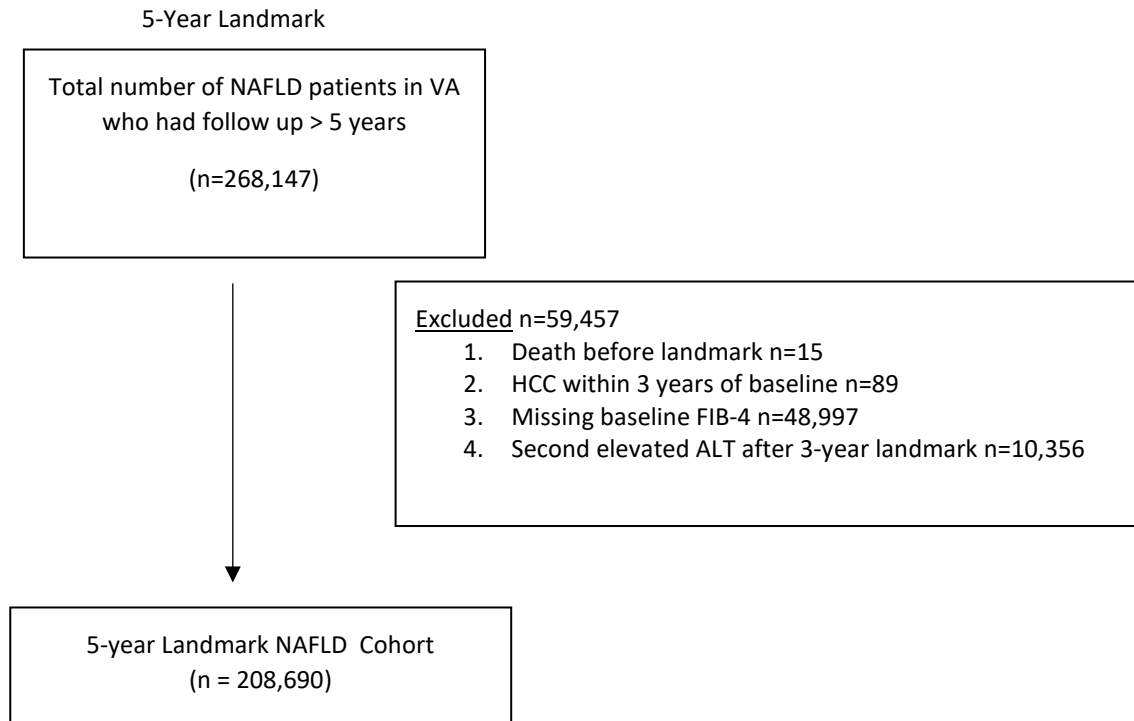
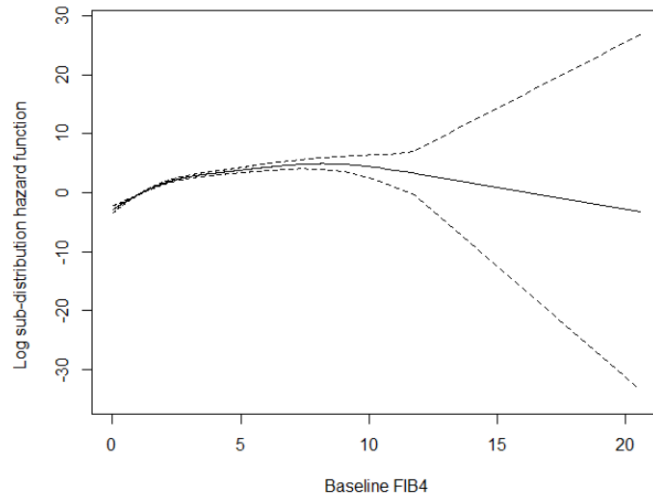


Fig. S1: Flow diagram describing inclusion and exclusion criteria to determine baseline NAFLD cohort and at 3- and 5-year landmark.

- A. 3-year landmark
- B. 5-year landmark

A



B

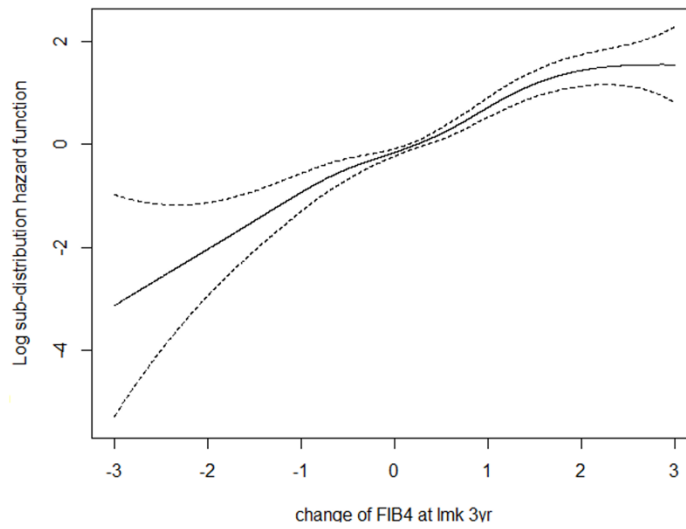


Fig. S2: Restricted cubic spline models reflecting FIB-4 score and the change in FIB-4 scores between baseline and follow-up as continuous variables and the rate of developing hepatocellular carcinoma between 2 time points.

- A. FIB-4 score as continuous variable
- B. Change in FIB-4 score

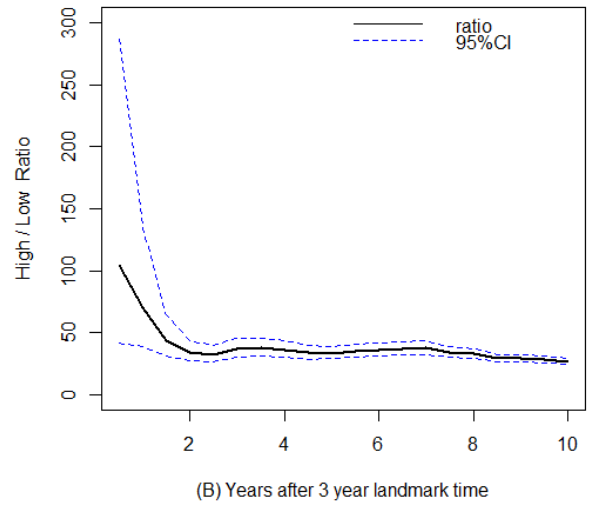
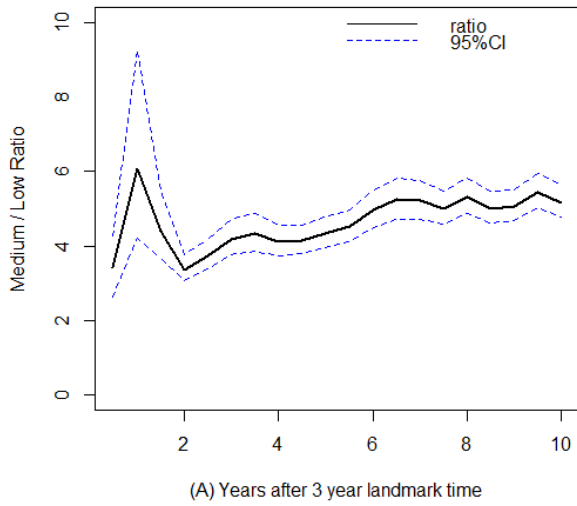


Fig. S3: Graphical check of the proportional hazard assumption

A. Indeterminate FIB-4 risk group compared to low (reference) FIB-4 risk group

B. High FIB-4 risk group compared to low (reference) FIB-4 risk group

Table S1. C-index at the landmark time of 3 and 5 years, with a prediction horizon of 2 and 5 years respectively

c-index	2-yr	5-yr
3- year landmark cohort (N=202319)	87.5%	86.3%
5-year landmark cohort (N=208690)	89.9%	86.8%

Table S2. Association between fibrosis risk groups, defined based on FIB-4 values at baseline and 5-year landmark time, and subsequent risk for developing HCC in NAFLD patients.

Baseline	5-year landmark time	N (%) ^a	HCC Events	HCC Incidence per 1,000 PY (95% C.I.) ^b	Baseline ^c	5-year landmark time	N (%) ^a	Cirrhosis or HCC Events ^c	Cirrhosis or HCC Incidence per 1,000 PY (95% C.I.) ^b
Overall cohort		208690	403	0.29 (0.26, 0.32)	Overall Cohort		207381	1660	1.21 (1.15, 1.27)
Low Risk N=158089	Low Risk	115802 (73.3)	45	0.06 (0.04, 0.08)	Low Risk N=157684	Low Risk	115632 (73.3)	259	0.32 (0.29, 0.37)
	Indeterminate Risk	38613 (24.4)	45	0.17 (0.13, 0.23)		Indeterminate Risk	38465 (24.4)	294	1.14 (1.02, 1.28)
	High Risk	3674 (2.3)	19	0.88 (0.53, 1.37)		High Risk	3587 (2.3)	155	7.43 (6.31, 8.70)
Indeterminate Risk N=43420	Low Risk	8362 (19.3)	13	0.25 (0.13, 0.43)	Indeterminate Risk N=4295	Low Risk	8316 (19.4)	52	1.01 (0.75, 1.32)
	Indeterminate Risk	26019 (59.9)	75	0.46 (0.36, 0.57)		Indeterminate Risk	25842 (60.2)	286	1.76 (1.56, 1.97)
	High Risk	9039 (20.8)	106	2.05 (1.67, 2.47)		High Risk	8793 (20.5)	364	7.31 (6.58, 8.10)
High Risk^d N=7181	Low/ Indeterminate Risk	3232 (45.0)	13	0.70 (0.37, 1.19)	High Risk N=6746	Low/ Indeterminate Risk	3159 (456.8)	34	1.87 (1.30, 2.62)
	High Risk	3949 (55.0)	87	4.29 (3.44, 5.30)		High Risk	3587 (53.2)	216	11.76 (10.24, 13.43)

^a Percentage (%) calculated with number within FIB-4 group at baseline as the denominator

^b Incidence rate calculated using multiple imputed data

^c Excluded patients who developed composite outcome (cirrhosis or HCC) prior to 3-year landmark time

^d Data from high to low and indeterminate risk were combined due low number of patients and events in high to low risk group.

Table S3. Association between changes in FIB-4 and risk of HCC and composite outcome after 5-year landmark in NAFLD patients.

	HCC outcome		Cirrhosis or HCC Outcome ^a	
	Unadjusted HR	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Change in FIB-4				
Low-Low	Reference	Reference	Reference	Reference
Low-indeterminate	3.43 (2.19, 5.38)	3.05 (1.94, 4.79)	3.60 (3.04, 4.27)	3.21 (2.71, 3.81)
Low-High	16.66 (9.43, 29.42)	14.04 (7.92, 24.89)	22.65 (18.53, 27.68)	19.24 (15.71, 23.56)
Indeterminate-low	4.10 (2.07, 8.15)	3.59 (1.80, 7.14)	2.85 (2.07, 3.93)	2.49 (1.81, 3.44)
Indeterminate-Indeterminate	8.08 (5.42, 12.06)	6.88 (4.59, 10.32)	5.29 (4.44, 6.29)	4.54 (3.81, 5.42)
Indeterminate-High	34.03 (23.13, 50.07)	28.27 (19.12, 41.78)	22.48 (19.03, 26.54)	19.01 (16.07, 22.47)
High-Lower	9.58 (4.49, 20.45)	8.83 (4.13, 18.88)	5.74 (3.9, 8.47)	5.29 (3.60, 7.78)
High-High	61.09 (40.35, 92.48)	51.13 (33.54, 77.93)	34.36 (28.58, 41.3)	29.64 (24.60, 35.70)
Race/Ethnicity				
White	Reference	Reference	Reference	Reference
African-American	0.34 (0.20, 0.58)	0.42 (0.24, 0.72)	0.52 (0.43, 0.62)	0.63 (0.52, 0.76)
Hispanic	1.07 (0.70, 1.65)	1.27 (0.83, 1.97)	1.12 (0.93, 1.35)	1.35 (1.12, 1.63)
Other	0.99 (0.52, 1.89)	1.20 (0.63, 2.30)	0.69 (0.49, 0.99)	0.84 (0.60, 1.19)
Missing	-	-	-	-
Diabetes	3.90 (3.08, 4.93)	2.67 (2.08, 3.44)	3.74 (3.37, 4.15)	2.60 (2.32, 2.91)
Obesity, BMI > 30	1.62 (1.30, 2.03)	1.52 (1.20, 1.91)	1.73 (1.56, 1.92)	1.60 (1.44, 1.77)
Hypertension	4.48 (2.71, 7.38)	1.74 (1.03, 2.96)	3.62 (2.95, 4.44)	1.39 (1.12, 1.73)
Dyslipidemia	1.26 (0.90, 1.77)	0.48 (0.34, 0.69)	1.52 (1.29, 1.78)	0.63 (0.53, 0.74)

^a Composite outcome of cirrhosis or HCC analyzed in NAFLD patients only without evidence of cirrhosis prior to landmark time

Table S4. Association between fibrosis risk groups, defined based on FIB-4 values at baseline and 3-year landmark time, and subsequent risk for developing HCC in NAFLD patients with and without cirrhosis.

Without Cirrhosis N=199306	3-year Landmark^a	N (%)	HCC Events (Total PY)	HCC Incidence per 1,000 PY (95% C.I.)
Low Risk N=150139	Low Risk	119027 (79.3)	42 (1024746.9)	0.04 (0.03, 0.06)
	Indeterminate/High Risk	31112 (20.7)	39 (249490.6)	0.16 (0.11, 0.21)
Indeterminate Risk N=42215	Low Risk	9594 (22.7)	18 (76805.3)	0.23 (0.14, 0.37)
	Indeterminate Risk	26080 (61.8)	77 (202512.2)	0.38 (0.30, 0.48)
	High Risk	6541 (15.5)	31 (44474.4)	0.70 (0.47, 0.99)
High Risk N=6952	Low/Indeterminate Risk	3341 (48.1)	7 (23581.5)	0.30 (0.12, 0.61)
	High Risk	3611 (51.9)	30 (22223.6)	1.35 (0.91, 1.93)
With Cirrhosis N= 3013	3-year landmark	N (%)	HCC Events (Person Years)	HCC Incidence per 1,000 PY (95% C.I.)
Low Risk N=1055	Low Risk	520 (49.3)	14 (4076.0)	3.43 (1.88, 5.76)
	Indeterminate/High Risk	535 (50.7)	22 (3705.0)	5.94 (3.72, 8.99)
Indeterminate Risk N=1126	Low/Indeterminate Risk	636 (56.5)	34 (4515.5)	7.53 (5.21, 10.52)
	High Risk	490 (43.5)	59 (2991.3)	19.72 (15.01, 25.44)
High Risk N=832	Low/Indeterminate Risk	142 (17.2)	14 (967.1)	14.48 (21.29, 32.87)
	High Risk	690 (82.9)	86 (3230.9)	26.62 (21.29, 32.87)

^a Risk groups at landmark time were combined (low/indeterminate) due to low number of patients and events.

Table S5. Association between fibrosis risk groups, defined based on FIB-4 values at baseline and 3-year landmark time, and subsequent risk for developing HCC in NAFLD patients with normal ALT value and radiologic evidence of steatosis.

Baseline	3-year landmark time	N (%)	Cirrhosis/HCC	Total PY	Cirrhosis/HCC Incidence per 1,000 PY (95% C.I.)
Low Risk^a N= 1504	Low Risk	1197 (79.6)	7	9467.8	0.74 (0.30, 1.52)
	Indeterminate Risk	293 (19.5)	3	2209.7	1.36 (0.28, 3.97)
Indeterminate Risk N=499	Low Risk	71	1	542	1.85 (0.05, 10.28)
	Indeterminate Risk	356	5	2658	1.88 (0.61, 4.39)
	High Risk	72	2	489	4.09 (0.5, 14.77)
High Risk N= 67	Low-Indeterminate Risk	2	1	144	6.93 (0.18, 38.62)
	High Risk	65	7	432	16.21 (6.52, 33.4)

^aData for a low to high risk group not presented because of limited number of patients in this group

Table S6. Association between fibrosis risk groups in NAFLD patients with non-missing FIB-4, at baseline and 3-year landmark time, and subsequent risk for developing HCC.

Baseline	3-year landmark time	N (%)	HCC Events	HCC Incidence per 1,000 PY (95% C.I.)
Overall cohort		147270	389	0.32 (0.29, 0.35)
Low Risk	Low Risk	83853 (78.1)	47	0.06 (0.05, 0.09)
N=107415	Indeterminate/High Risk	23562 (21.9)	48	0.25 (0.19, 0.34)
Indeterminate Risk	Low Risk	7084 (20.9)	15	0.27 (0.15, 0.44)
N=33814	Indeterminate Risk	21193 (62.7)	94	0.57 (0.46, 0.70)
High Risk	High Risk	5537 (16.4)	78	2.08 (1.64, 2.59)
N=6041	Low/Indeterminate Risk	2665 (44.1)	13	0.69 (0.36, 1.17)
	High Risk	3376 (55.9)	94	4.61 (3.73, 5.65)

Table S7. Comparison of clinical characteristics of patients with and without FIB-4 measurements at baseline, for 3-year and 5-year landmark cohort.

	3-year Landmark			5-year Landmark		
	With FIB-4 N=147270	Missing any FIB-4 N=55049	<i>P</i>	With FIB-4 N= 149954	Missing any FIB-4 N= 58736	<i>P</i>
N new HCC	389 (0.3)	84 (0.2)	<0.001	346 (0.2)	57 (0.1)	<0.001
Age in years, SD	59.3 (12.3)	55.2 (13.9)	<0.001	60.7 (12.2)	57.0 (14.1)	<0.001
Gender			<0.001			<0.001
Female	9105 (6.2)	3183 (5.8)		9071 (6.1)	3373 (5.7)	
Male	138165 (93.8)	51866 (94.2)		140883 (93.9)	55363 (94.3)	
Ethnicity/Race			<0.001			<0.001
White	104101 (70.7)	36484 (66.3)		106088 (70.8)	38953 (66.3)	
African-American	16625 (11.30)	6422 (11.7)		17619 (11.8)	7020 (11.9)	
Hispanic	8427 (5.7)	3238 (5.9)		8768 (5.9)	3305 (5.6)	
Other	3831 (2.6)	1661 (3.0)		3952 (2.6)	1774 (3.0)	
Missing	14286 (9.7)	7244 (13.2)		13527 (9.0)	7684 (13.1)	
Cirrhosis	939 (0.6)	156 (0.3)	<0.001	1125 (0.8)	184 (0.3)	<0.001
Diabetes	58115 (39.5)	15477 (28.1)	<0.001	64562 (43.1)	18550 (31.6)	<0.001
Obesity, BMI > 30	84299 (57.2)	21358 (38.8)	<0.001	85952 (57.3)	20936 (35.6)	<0.001
Hypertension	121647 (82.6)	38581 (70.1)	<0.001	128166 (85.5)	43277 (73.3)	<0.001
Dyslipidemia	125489 (85.2)	40986 (74.5)	<0.001	133358 (88.9)	46132 (78.5)	<0.001