

Supplementary data:

EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease

European Association for the Study of the Liver (EASL)*, European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO)

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Supplementary Table 1. Genes

Gene (Reference)	Steatosis	NASH	Fibrosis	HCC	% Europids with allele
PNPLA3 [1-3]	Yes	Yes	Yes	Yes	40
TM6SF2 [4-6]	Yes	Yes	Yes	No	15

Supplementary info:

The *PNPLA3 I148M* gene variant is functional and impairs breakdown of intra-hepatocellular triglycerides and also possibly increases TG synthesis in the liver [7]. Approximately 40% of Europids carry at least one variant allele. The clinical utility of *PNPLA3* genotype testing has been assessed in one study where it was found to add little to the predictive ability of a score based on metabolic risk factors for detecting presence of NAFLD [8]. Genotyping might help in non-invasive diagnosis of NASH [9]. NAFLD is increasingly recognised as a common aetiology underlying many HCC cases and so there is a need to develop tools, either to target HCC surveillance to those at high-risk or to identify those at low-risk that need not enter resource-intensive surveillance programs [10]. *PNPLA3* homozygote minor allele (GG) carriage confers a 5-fold excess risk of HCC in a NAFLD cohort (independent of potential confounders including age, gender, BMI, type 2 diabetes and presence of cirrhosis), and a 12-fold

increased risk of developing HCC compared to a UK population sample [11]. Based on cohort and genotype frequency data it can be calculated that the negative predictive value of *PNPLA3* CC vs. CG/GG genotypes is 82% amongst NAFLD cases and 97% when the UK population sample is considered [11]. Although positive predictive value is low, the available evidence suggests that *PNPLA3* genotyping (combined with other clinical factors) could assist patient selection for HCC surveillance in NAFLD by helping to exclude those at least risk.

Knowledge regarding *TM6SF2* is increasing rapidly and it too appears to be a strong modifier of NAFLD fibrosis however further work is required before it is ready for clinical application [5]. Approximately 15% of Europeans carry a variant allele of *TM6SF2* at rs585422926. This gene variant impairs secretion of VLDL TGs from the liver. Like the *PNPLA3* *I148M* gene variant, this gene variant also increases the risk of steatosis as well NAFLD fibrosis [5] however the effects of *TM6SF2* appear to dissociate progression to advanced liver fibrosis from cardiovascular disease risk: individuals carrying the minor (T) allele of *TM6SF2* rs585422926 may be more prone to experience liver-related rather than cardiovascular morbidity and mortality [4, 12, 13]. Whilst NAFLD is associated with an increased risk of cardiovascular disease overall, knowledge of *TM6SF2* genotype may have value for individual risk stratification and prediction of liver-predominant morbidity and clinical outcome.

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Supplementary Table 2. Imaging

Assessment of hepatic steatosis and fibrosis by non-invasive imaging: comparison with liver biopsy

Technique, [Ref]	Outcome	Comparative histology & diagnostic cut-off	SENS % (95% CI)	SPEC % (95% CI)	AUROC (95% CI)	Strengths & Limitations
Ultrasound [1]*	Steatosis	All cases >25% of hepatocytes	Range, 73.3-90.5 85.2 (78.4-90.8)	Range, 69.6-85.2 85.2 (76.9-90.9)	----	<ul style="list-style-type: none"> • Widely available, reference for qualitative assessment • Limited by fibrosis
Computed Tomography [1]*	Steatosis	All cases >25% of hepatocytes	Range, 46.1-72.0 72.0 (59.7-81.7)	Range, 88.1-94.6 94.6 (88.1-97.7)	----	<ul style="list-style-type: none"> • Limited in the presence of variable iron
Magnetic Resonance Imaging [1]*	Steatosis	All cases >25% of hepatocytes Cut-off, 5.6% IHTG	Range, 82.0-97.4 97.4 (83.5-99.6)	Range, 76.1-95.3 76.1 (49.6-91.2)	----	<ul style="list-style-type: none"> • Limited in the presence of very high iron load
Magnetic Resonance Spectroscopy [1]*	Steatosis	All cases >25% of hepatocytes	Range, 72.7-88.5 72.7 (41.4-91.0)	Range, 92.0-95.7 95.7 (84.5-98.9)	----	<ul style="list-style-type: none"> • Reference for quantitative assessment • Limited by inhomogeneous fat distribution
Controlled Attenuation Parameter [2]	Steatosis	>10% of hepatocytes >33% of hepatocytes >66% of hepatocytes	----	----	0.79 (0.75-0.84) 0.84 (0.80-0.88) 0.84 (0.80-0.88)	<ul style="list-style-type: none"> • Limited experience • Not valid for quantitative assessment
Transient Elastography [3]^	Fibrosis	M probe threshold values: Stage ≥2 (6.7-7.8 kPa) Cut-off, 7.2 kPa [4] Stage ≥3 (>8-10.4 kPa)	Range, 67-88 NPV, 84% Range, 65-100	Range, 61-84 Range, 75-93	----	<ul style="list-style-type: none"> • Widely used and reference technique • Limitations in the

		Cut-off, 8.7 kPa [4]	NPV, 95%				
		Stage 4 (>8-10.4 kPa)	Range, 78-100	Range, 82-98	----		presence of steatosis (risk of false positive results [6])
		Cut off, 10.3 kPa [4]	NPV, 99%				
		XL probe threshold values [5]:	(NPV, 89%)	----	----		• Use XL probe when high BMI (cut-off for fibrosis stages are 1.3 kPa lower)
		Stage 3 (7.2 kPa)	(NPV, 98%)	----	----		
		Stage 4 (7.9 kPa)					
Acoustic radiation Force Impulse [7] [°]	Fibrosis	Stage ≥3; cut-off, 1.77 m/sec	100 (65.5-100)	90.9 (77.4-95.7)	0.973		
		Stage 4: cut-off, 1.90 m/sec	100 (51.7-100)	95.8 (84.6-98.4)	0.976		• Similar SENS and SPEC as TE, but higher failure rates (2.1% vs. 6.6% for TE [8])

*Meta-analysis of 46 studies; ^Meta-analysis of 9 studies; °Meta-analysis of 13 studies

Abbreviation: IHTG, intra-hepatic triglyceride content

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Supplementary Table 3. Biomarkers

Biomarkers and scores predicting NAFLD and NAFLD severity (NASH and fibrosis)

Author, year [ref]	Test/ score	Elements [^]	No. of cases	Cut-offs [^]	AUROC	SENS %	SPEC %	PPV %	NPV %	Strengths/Limitations
Steatosis										
Bedogni, 2006 [1]	FLI - Fatty Liver Index	BMI, WC, TG, GGT	496	>60	0.85	61	86	NA	NA	- External validation in the general population in Europe [2, 3], Asia [4] and North America [5]. - Predicts metabolic [6-8] and CV [9] outcomes, and hepatic [10] and CV mortality[11].
Lee, 2010 [12]	HSI - Hepatic steatosis index	ALT, AST, BMI	183	>36	0.81	45	93	86.7	NA	- Only validated in a Korean population.
Bedogni G, 2010 [13]	LAP - Lipid Accumulation Product	WC, TG	588	>4 (Males), >4.4 (Women)	0.79	NA	NA	NA	NA	- Population-based from NHANES III; predicts CV risk & DM [14, 15]
Kotronen 2009 [16]	NAFLD liver fat score	MS, DM, AST, ALT	470	-0.640	0.86	86	71	NA	NA	- Prediction not improved by PNPLA3 analysis
Hyysalo, 2014 [17]	NASH liver fat score	PNPLA3, AST, INS	296 Finnish cohort	2.12	0.734	59.5	79.7	54.0	82.8	- Validated in 2 distinct biopsy-proven cohorts
			(380) Italian cohort	2.122	0.737	92.9	32.7	52.5	85.2	
Poynard,	SteatoTest®	GGT, ALT, BG,	494	>0.69	0.80	38.4	81.4	71.0	52.7	- External validation in

2012 [18]	#	TG, CHOL°, BMI, BIL, apoA1, haptoglobin, α2macroglobulin	(range 0-1)							the general population [19] and in morbidly obese patients [20] - Predicts overall mortality [21].
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Steatohepatitis

Dixon, 2001 [22]	HAIR	HTN, ALT, IR	105	2	0.90	80	89	NA	NA	- Developed in bariatric surgery patients
Poynard, 2006 [23]	NASH Test ®	Age, sex, GGT, CHOL, AST, ALT, BIL, apoA1, haptoglobin, TG, α2macroglobulin	257	NA	0.78	33	94	66	81	- Developed and validated in a multicenter population
Campos, 2008 [24]	-----	HTN, DM, AST, ALT, OSAS, race	186	0-2 3-4 5 6-7	0.80	NA NA NA NA	NA NA NA NA	7 27 59 93	93 73 41 7	- Developed in morbidly obese patients; patients are classified in 4 risk categories (very high, high, intermediate, low)
Ulitsky, 2010 [25]	-----	DM, ALT, TG, OSAS	253	0-1 2-3 4 5	0.76	NA NA NA NA	NA NA NA NA	11 24.7 60 75	89 75.3 40 25	- Developed in morbidly obese patients; patients are classified in 4 risk categories (very high, high, intermediate, low)
Younossi, 2008 [26]	NASH diagnostics	CK18, resistin, adiponectin	101	>0.27	0.91	94.5	70.2	60	97	- Developed in a strictly selected population
Sookoian,	-----	BMI, WC, ALT,	101	>1.31	0.79	63.3	82.9	85.4	64.2	- Constructed on the

2009 [27]		AST, ALP, GGT, HOMA, CRP, ICAM-1									basis of validity of individual biomarkers - High PPV
Anty, 2010 [28]	Nice model	ALT, CK-18, MS	464	>0.14	0.83	84	86	44%	98%		- Developed in bariatric surgery patients
Sumida, 20011 [29]	NAFIC	Ferritin, sex, INS, type IV collagen	442 (Validation group)	1 2	0.782	88 60	43 87	66 85	75 64		- Parameters combined in a weighted sum, easy to calculate
Poynard, 2012 [18]	Acti-Test® #	BIL, GGT°, ApoA1, haptoglobin, α2macroglobulin	494	>0.62 (range 0-1)	0.74	28.2	90.7	38.7	85.9		- Developed in bariatric surgery patients
Shen 2012 [30]	-----	CK-18 AFABP FGF-21	146 146 146	>338 >15 >332	0.70 0.59 0.62	66 84 54	65 37 72	71 63 71	60 65 55		- FGF-21 improves the accuracy of CK-18 (NPV, 74%; PPV, 82%)
Ogawa, 2013 [31]	-----	sCD-14	113	29.5	0.75	79.6	62.9	78	72		-----
Alkouri, 2014 [32]	Ox NASH	13-HODE/LA, BMI, AST	122	54.6	0.73	77.8	67.2	72	74		- Validated in a pediatric population
Hyysalo, 2014 [17]	NASH score	PNPLA3, AST, INS	296 Finnish cohort (380) Italian cohort	-1.054 -1.054	0.77 0.76	71.6 39.1	73.5 89.1	52.5 64.6	86.4 74.2		- Validated in 2 distinct biopsy-proven cohorts
Otgonsuren, 2014 [33]	Index of NASH	WHR, TG, ALT, HOMA	861	> 50	0.77	92	60	NA	NA		- Based on NHANES III population; predicts CV & DM-related mortality
Bazick 2015 [34]	-----	Ethnicity, BMI, WC, ALT, AST, albumin, HbA1c,	346 NASH CRN cohort	≥0.77	0.80	57	90	93	48		- Based on routinely available clinical and biochemical data - Validated in the

HOMA, ferritin

presence of T2DM

Fibrosis

Ratziu, 2006 [35]	Fibro-Test®	GGT, BIL, haptoglobin, apoA1°, α2macroglobulin	267	>0.30 >0.70	0.81	92 25	71 97	33 60	92 89	- Advanced fibrosis predicts overall mortality; progression to advanced fibrosis associated with CV mortality [21]
Angulo, 2007 [36]	NFS - NAFLD fibrosis score	Age, BG, BMI, platelets, albumin, AST/ALT	733	≥0.676	0.84	43	96	82	80	- Predicts liver-related events [37, 38], incident diabetes [39], all-cause and CV mortality [40] - Changes in NFS predict mortality [38]
Guha, 2008 [41]	ELF – Enhanced Liver Fibrosis	HA, TIMP1, PIIINP	192	>0.3576	0.90	80	90	71	94	- Predicts overall and CV mortality
Harrison, 2008 [42]	BARD	BMI, AST/ALT, DM	827	2-4	0.81	NA	NA	43	96	- Predicts liver-related events [37]
Cales, 2009 [43]	Fibrometer NAFLD	BG, AST, ALT, ferritin, weight, age	235	≥0.715	0.94	79	96	88	92	- Initially developed in hepatitis C [44]
Shah, 2009 [45]	FIB-4 index	Age, ALT, AST, platelets	541	≥2.67	0.80	33	98	80	83	- Predicts all-cause and CV mortality [40] and liver-related events [37]
Cales 2009 [43]	APRI – AST/platelet ratio index	AST, platelets	235	≥0.918	0.87	66	91	73	87	- Developed in hepatitis C [46]; predicts liver-related events [37, 40]
McPherson 2010 [47]	AST/ALT ratio	AST, ALT	145	>0.8 >1	0.83 0.83	74 52	78 90	44 55	93 89	- High NPV to exclude advanced fibrosis

Sumida, 2011 [29]	NAFIC score	Ferritin, sex, INS, type IV collagen	619	0 2-4	0.83 0.83	95 84	33 74	32 52	96 93	- Multicenter validation in a Japanese population
Adams, 2011 [48]	Hepascore	Age, gender, α 2macroglobulin, HA, BIL, GGT	242	>0.37	0.81	75.5	84.1	57	92	- Initially validated for hepatitis C [49]
Poynard, 2012 [18]	Fibro-Test®	Components as above	494	>0.58 (range 0-1)	0.65	NA	NA	87	94	- Validated in morbid obesity
Alkouri, 2014 [32]	Ox NASH	13-HODE/LA, BMI, AST	122	>54.6	0.67	75	60.6	62	74	- Validated in a pediatric population
Bazick 2015 [34]	-----	Age, ethnicity, BMI, WHR, HTN, ALT/ALT, ALP, albumin, BIL, globulins, INS, HCT, INR, platelets	346 NASH CRN cohort	\geq 0.60	0.80	57	90	80	75	- Based on routinely available clinical and biochemical data - Validated in the presence of T2DM

[^] Refer to original articles for specific formulae; [°]Adjusted for age and sex

AUROC, area under the receiver operator characteristic curve; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; 13-HODE, 13-OH-octadecadienoic acid; AFABP, adipocyte fatty acid binding protein; ALT, alanine aminotransferase; AP, alkaline phosphatase; ApoA1, apoprotein A1; AST, aspartate aminotransferase; BG, fasting glucose; BIL, total bilirubin; BMI, body mass index; ALP, alkaline phosphatase; CHOL, fasting cholesterol; CK-18, cytokeratin-18; CRN, Clinical Research Network; CRP, c-reactive protein; CV, cardiovascular; DM, type 2 diabetes mellitus; FGF-21, fibroblast growth factor-21;

GGT, γ -glutamyl-transpeptidase; HA, hyaluronic acid; HbA1c, glycosylated haemoglobin; HCT, haematocrit; HOMA, homeostasis model assessment; HTN, hypertension; ICAM-1, intercellular adhesion molecule-1; INR, international normalized ratio; INS, fasting insulin; IR, insulin resistance; LA, linoleic acid; MS, metabolic syndrome; NA, not available; OSAS, obstructive sleep apnoea syndrome; PIIINP, amino-terminal propeptide of type III collagen; PNPLA3, PNPLA3 genotype at rs738409; sCD-14, soluble cluster of differentiation-14; TG, fasting triglycerides; TIMP1, Tissue inhibitor of metalloproteinase 1; WC, waist circumference; WHR, waist-to-hip ratio.

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Supplementary Table 4. Cardiovascular diseases

Pivotal studies associating NAFLD with the presence of cardiovascular disease and cardiovascular outcomes in adults and adolescents.

Author, year [ref]	Population	Measurement	Results	Comments
Pathophysiology				
Lautamaki, 2006 [1]	55 T2DM with CHD divided on the basis of their median (8%) liver fat content.	Myocardial perfusion and glucose uptake measured by PET during euglycemic clamp. LF content determined by MRS.	The high-LF group had lower insulin-stimulated myocardial glucose uptake. LF was the most significant explanatory variable for myocardial IR. The high-LF group had higher prevalence of CV risk biomarkers and lower coronary flow reserve	In patients with T2DM and CHD, LF content is an independent indicator of myocardial IR and reduced coronary functional capacity
Targer, 2008 [2]	45 biopsy-proven NASH, 45 BMI-matched with no steatosis at US, 45 healthy volunteers	Nontraditional CV risk biomarkers measured in all participants.	hs-CRP, fibrinogen, PAI-1, and adiponectin were markedly different between overweight and NASH patients, and the differences were only slightly weakened after adjustment. NASH and visceral adiposity independently predicted CV risk biomarkers	NASH can predict a more atherogenic risk profile, partly independent of visceral adiposity
Perseghin, 2008 [3]	Young men w/wo FL (n = 21+21), matched for anthropometric features	IHF measured by ¹ H-MRS, cardiac fat by MRI, myocardial energy metabolism by cardiac ³¹ P- MRS. IR measured by clamp	No differences in LV morphology and functions detected between NAFLD and non-NAFLD cases. The intrapericardial and extrapericardial fat was increased in NAFLD and PCr/ATP, <i>in vivo</i> marker of myocardial energy metabolism, was reduced in NAFLD	NAFLD is associated with fat accumulation in the epicardial area; despite normal LV morphology and functions, energy metabolism is defective
Verrijken, 2014 [4]	273 biopsy-proven NAFLD (age, 44 yrs;	An extensive panel of coagulation factors	Coagulation factors levels associated with metabolic features, not with liver histology. PAI-1 higher in NASH, and associated with steatosis,	PAI-1 might partly explain the increased CV risk associated with NAFLD

BMI, 39.6 kg/m²).

lobular inflammation, ballooning and fibrosis.

Clinical – Cross-sectional

Targher, 2004 [5]	85 male volunteers (age, 42 years)	Carotid artery IMT and liver fat by US; intra-abdominal fat by CT scan	Greater IMT in subjects with steatosis, but also higher BMI, visceral fat and CV risk profile. Differences in IMT were scarcely affected by metabolic data, but were abolished after controlling for visceral fat	Enlarged IMT in the presence of steatosis is associated with visceral fat accumulation.
Brea, 2005 [6]	40 patients US-detected NAFLD and 40 controls	Carotid artery IMT, MS components, CRP	NAFLD patients showed an enlarged IMT and a higher prevalence of carotid plaques (50% vs. 25%). NAFLD was associated with increased IMT	NAFLD patients have a cluster of risk factors and more severe carotid atherosclerosis
Villanova, 2005 [7]	52 biopsy-proven NAFLD and 28 age- and sex-matched controls	Brachial artery FMV, CV risk measured by Framingham, PROCAM and ATPIII scores)	FMV were halved in NAFLD vs. controls, and higher in NAFL vs. NASH. Low FMV was associated with NASH (aOR, 6.8). The 10-year probability of CV events was increased in NAFLD (mainly in NASH)	First evidence of endothelial dysfunction in NAFLD, associated with CV risk profile
Lin, 2005 [8]	2088 male aircraft-maintenance workers (22-65 yrs, mean 40.5)	Annual health examination (visit, routine biochemistry, abdominal US and digital ECG	Rates for conventional IHD risk factors increased with the severity of steatosis. The prevalence of ischemic ECG for NAFLD subjects w/wo overweight was 30.1% and 19.1%, while that of non-NAFLD overweight subjects was 14.4%.	US-detected steatosis and its severity is an independent risk factor for IHD. Fatty liver and overweight interact to generate a risk for IHD.
Targher, 2006 [9]	400 + 400 T2DM patients w/wo NAFLD, matched for age and sex	CVD assessed by ECG and echo-Doppler of carotid and lower limb arteries, steatosis by US, MS defined by ATPIII	The prevalences of coronary, cerebrovascular and peripheral vascular disease were all higher in NAFLD vs. non-NAFLD. MS and its components were more prevalent in NAFLD. In regression analysis, NAFLD was not independently related to CVD	CVD prevalence is increased in T2DM and NAFLD in association with an increased prevalence of MS
Targher, 2006 [10]	85 biopsy-proven NAFLD	Carotid artery IMT and the classical CV risk factors,	NAFLD patients had enlarged carotid IMT. MS and its individual features were more prevalent in	The severity of liver histopathology among

	and 160 age-, sex-, and BMI-matched controls	HOMA-IR, and MS features (ATPIII criteria)	NAFLD. Carotid IMT was strongly associated with the degree of steatosis, necroinflammation, and fibrosis, and the severity of liver histology predicted carotid IMT	NAFLD patients is strongly associated with carotid atherosclerosis
Fracanzani, 2008 [11]	125 NAFLD and 250 matched healthy controls,	B-mode US for carotid IMT and the presence of small plaques.	NAFLD cases were characterized by enlarged IMT and a higher prevalence of plaques. An IMT >0.64 mm (median value in controls) was predicted by the presence of steatosis, age, and systolic blood pressure	Patients with NAFLD, even with no or mild alterations of liver tests, are at high risk for cardiovascular complications
Gastaldelli, 2009 [12]	1,307 European cases (RISC study, age 30-60 yrs, no T2DM)	FL estimated by FLI; IMT, IR (clamp) and CHD risk (Framingham score)	IMT increased with FLI. FLI was associated with high CHD risk, liver enzymes, metabolic risk factors, physical activity, IMT, and IR. The correlations were maintained in adjusted multivariate analysis	Fatty liver is associated early atherosclerosis and IR in middle-age nondiabetic subjects.

Clinical – Longitudinal

Eckstedt, 2006 [13]	129 biopsy-proven NAFLD (F-UP, 13.7 yrs)	Survival and causes of death compared with a matched reference population.	Mortality was not increased in patients with pure FL. Survival was reduced in NASH, and higher rates of both CV and liver-related deaths were recorded	Survival is lower in NASH, not in FL, mainly driven by . higher CV mortality
Hamaguchi, 2007 [14]	Prospective study of 1637 healthy Japanese subjects, in a health check-up program	NAFLD diagnosed by US; MS was defined by NCEP-ATPIII. In 1221, incident CVD assessed by a questionnaire 5 years later	CVD incidence higher in 231 with NAFLD at baseline than in 990 without NAFLD. NAFLD predicted CVD independently of conventional risk factors. In a multivariate model, NAFLD (not MS) retained a significant correlation with CVD	NAFLD is a strong predictor of CVD and plays a central role in the CV risk associated with MS.
Fraser, 2007 [15]	2,961 records from the British Women's Heart and Health study; meta-	Associations of GGT and ALT with incident CHD, stroke, and a combined outcome of CHD or stroke	A change of 1 U/L of GGT is associated with 20% increased risk CHD, 54% for stroke, 34% for CHD or stroke. Data confirmed in nondrinkers. Meta analyses of 2 studies that examined the association of ALT with incident CV events did not	GGT levels are associated with incident vascular events, independently of alcohol intake.

	analysis of 10 population-based studies		show any association	
Soderberg, 2010 [16]	256 Swedish subjects with high ALT; 118 biopsy-proven NAFLD (51 NASH); F-UP, 28 yrs	Mortality among NAFLD assessed in comparison with the general Swedish population (source: National Swedish Cause of Death Registry)	During F-UP, 113 (44%) of total cases and 47 (40%) of the 118 subjects with NAFLD died (37 for CVD). Compared with the total Swedish population, NAFLD was associated with increased mortality (SMR, 1.69), with CVD as the most common death cause	Patients with NASH are at increased risk of death (mainly CVD death) compared with the general population
Wong, 2011 [17]	612 patients at CV disease risk; F-UP, 87 weeks	Coronary angiogram to detect CHD ($\geq 50\%$ stenosis in at least one coronary artery); US-assessed NAFLD.	356 (58.2%) had US-detected NAFLD, 318 high liver enzymes, and 465 had significant CHD. After adjusting for confounders, FL was associated with CHD, but at F-UP, FL did not predict events	FL is associated with CHD, independent of CV risk factors, but cannot predict CV mortality and morbidity
Calori, 2011 [18]	2,074 Italian subjects (Cremona study); F-UP, 15 yrs	FLI; all-cause, hepatic-, CV-, and cancer-related deaths were registered in 2,011 (Regional Health Registry)	FLI independently associated with all-cause, hepatic-, CV-, and cancer-related deaths, after adjustment for confounders	The presence of NAFLD, independent of its severity, is associated with outcome in the general population.
Targher, 2014 [19]	400 T2DM cases, free from AF at baseline; F-UP, 10 yrs.	NAFLD diagnosed by US at baseline; incident AF by annual ECG (confirmed by a cardiologist)	42 (10.5%) cases of incident AF. NAFLD associated with an increased risk of incident AF, independent of confounders.	US-diagnosed NAFLD is associated with an increased incidence of AF in T2DM
Zelber-Sagi, 2014 [20]	213 subjects, without liver disease; F-UP, 7 yrs	US-diagnosed NAFLD at baseline and at F-UP	Non-HDL-CHOL higher in 28/147 (19%) subjects who did not have NAFLD at baseline and developed NAFLD at F-UP in subjects who developed NAFLD. Non-HDL CHOL predicted new onset NAFLD	The association of non-HDL-C with NAFLD may explain the increased CV risk
<hr/>				
Pediatrics				
Sert, 2013	180 obese	LV function evaluated by	NAFLD had normal LV systolic function, impaired	IR independently impacts

[21]	adolescents (w/wo NAFLD), 68 controls	echocardiography and PW tissue Doppler imaging; carotid IMT by doppler	diastolic function, and altered global systolic and diastolic myocardial performance. In NAFLD, ALT, IR, and LV mass were associated with enlarged IMT	on IMT and LV remodeling in NAFLD. LV dysfunction at an earlier stage in NAFLD
Huang, 2013 [22]	Australian cohort; 2-step cluster analysis on 964 17-year-olds	NAFLD detected by US; arterial stiffness (PWV and AI) by applanation tonometry	Overall NAFLD prevalence, 13.3%. The "high risk" cluster participants (16% males and 19% females) had greater WC and CV risk factors. NAFLD cases had greater PWV and AI (only in males)	Also in adolescents, NAFLD is associated with increased arterial stiffness in the "high risk" metabolic cluster
Lawlor, 2014 [23]	1,874 cases from a UK birth cohort (age, 17.9 yrs)	US-assessed liver stiffness (shear velocity) and metabolic CV risk factors	NAFLD prevalence, 2.5%. NAFLD subjects had larger liver volume, greater shear velocity, different levels of cardiometabolic risk factors	European adolescents with NAFLD have more liver fibrosis and CV risk factors

AI, augmentation index; AF, atrial fibrillation; ALT, alanine transaminase; ATPIII, Adult Treatment Panel III; CHD, coronary heart disease; CHOL, cholesterol; CRP, C-reactive protein; CV cardiovascular; CVD, cardiovascular disease; FL, fatty liver; FLI, fatty liver index; F-UP, follow-up; HDL, high-density lipoprotein; FMV, flow-mediated vasodilation; GGT, gamma-glutamyltransferase; HOMA, homeostasis model assessment; IHD, ischemic heart disease; IHF, intra-hepatic fat; IMT, intima-media thickness; IR, insulin resistance; LV, left ventricular; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MS, metabolic syndrome; PCr, phosphocreatine; PAI-1, plasminogen activator inhibitor-1; PROCAM, Prospective Cardiovascular Munster; PWV, pulse wave velocity; T2DM, type 2 diabetes mellitus; US, ultrasonography; WC, waist circumference

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Supplementary Table 5. Hepatocellular carcinoma

Most relevant study on the association of NAFLD with hepatocellular carcinoma

Author, year [Ref]	Population & Methods	Results	Comments
Bugianesi, 2002 [1]	Case control study. 44 CC of 641 cirrhosis associated HCC compared for family and history data and biochemistry with viral- and alcohol-associated HCC	CC-related HCC had a higher prevalence of T2DM and obesity. Liver function was similar, but CC cases had higher glucose, cholesterol and TG, insulin resistance, and lower liver enzymes. Regression analysis identified high TG, T2DM, and normal liver enzymes as independent factors for CC-associated HCC.	NAFLD-related features are more frequent in HCC arising in CC than in age- and sex-matched HCC patients of viral or alcoholic origin.
Marrero, 2002 [2]	105 HCC patients in a single U. S. centre. Mean age, 59, 67% men, 76% non-Hispanic white. HCV (51%) and CC (29%) were the most common aetiologies. 50% of CC-related HCC resembled NAFLD	53 cases (50%) were detected during surveillance programs. These cases had smaller tumours (P =0.01), were more likely eligible for surgery (P =0.005), and had a better median survival (P =0.001). CC-related HCC were less likely to have undergone HCC surveillance and had larger tumours at diagnosis. NAFLD accounted for at least 13% of the cases.	HCV and CC account for the majority of HCC cases. Surveillance is mandatory to detect HCC amenable by surgery, but is rarely done in CC-related cases
Regimbeau, 2004 [3]	210 patients with chronic liver disease who underwent resection for HCC. The prevalence of obesity, T2DM, and histological features of the tumour were compared between cases with different aetiology	18 (8.6%) had no identifiable aetiology (CC). They were compared with matched patients with alcohol- and chronic-viral-hepatitis-related HCC. The prevalence of obesity (50% vs. 17% vs. 14%), diabetes (56% vs. 17% vs. 11%), AST/ALT ratio<1 (50% vs. 19% vs. 17%), and steatosis>20% (61% vs. 17% vs. 19%) was higher in CC-related cases than in alcohol- or virus-related patients. CC-cases were more likely to have well-differentiated tumours	The study is consistent with the hypothesis that obesity and T2DM may be important risk factors for HCC, via NAFLD and CC
Hashimoto, 2009 [4]	34 NASH patients with HCC and 348 NASH patients without HCC. Data supported by a cohort study of the outcomes of	In total, 88% of patients with HCC (median age, 70 years) had advanced fibrosis. Older age, low level of AST, low grade of histological activity, and advanced stage of fibrosis were risk factors for HCC. Within the prospective cohort study, the	Older age and advanced fibrosis are important risk factors for HCC. HCC is the major cause of mortality in

	137 NASH with advanced fibrosis, started in 1990.	5-year cumulative incidence of HCC was 7.6%, and the 5-year survival rate was 82.8%. HCC was the leading cause of death.	NASH patients with advanced fibrosis.
Ascha, 2010 [5]	Adult patients with cirrhosis secondary to chronic HCV (n = 315) or NASH (n = 195) between 2003 and 2007 were evaluated	All patients were monitored by serial CT and serum alpha-fetoprotein (median follow-up, 3.2 years). 25/195 (12.8%) of NASH-cirrhotic and 64/315 (20.3 %) of HCV-cirrhotic patients developed HCC (yearly cumulative incidence, 2.6% vs. 4.0%;). Older age and alcohol consumption were independent risk factors for HCC in NASH-cirrhosis (hazard ratio in patients who reported any regular alcohol consumption vs. non-drinkers, 3.6)	Patients with NASH cirrhosis have an increased risk of liver cancer. Alcohol consumption, a modifiable risk factor, is the most significant risk factor for HCC development
Yasui, 2011 [6]	Multicentre Japanese study in 87 HCC cases on histologically proven NASH (median age, 72; 62% male). Clinical data collected at HCC diagnosis	Obesity (body mass index ≥ 25 kg/m ²), diabetes, dyslipidemia, and hypertension were present in 54 (62%), 51 (59%), 24 (28%), and 47 (55%) patients, respectively. In non-tumour liver tissues, the degree of fibrosis was stage 3-4 in 72% of cases. The prevalence of cirrhosis was lower in male patients (39% vs. 70% in women).	Most patients with NASH who develop HCC are men, with features of MetS, who develop HCC at a less advanced stage of liver fibrosis than women
Kawamura, 2012 [7]	Retrospective cohort study in a public Japanese hospital (6508 patients with NAFLD diagnosed by US). Median F-UP, 5.6 years.	16 (0.25%) new cases with HCC were diagnosed. The annual rate of HCC was 0.043%. The independent risk factors for HCC were raised AST level, platelet count $< 150 \times 10^3$ /mL, age ≥ 60 years, and diabetes. The cumulative rate of HCC was higher subjects with APRI score indicative of fibrosis stage 3-4.	Low annual incidence of HCC among Japanese NAFLD Elderly NAFLD cases with T2DM, high AST, and low platelets are at higher risk
Valenti, 2013 [8]	460 consecutive HCC patients referred to tertiary care centres in Northern Italy, 353 with F-UP data.	Homozygosity for PNPLA3 148M was enriched in HCC cases with both ALD and NAFLD; its presence was associated with younger age, shorter history and less advanced cirrhosis, but a higher number of HCC lesions. Homozygosity for PNPLA3 148M was the only negative predictor of survival in both ALD and NAFLD	PNPLA3 148M is over-represented in both ALD and NAFLD HCC patients, and is associated with a less advanced severity of disease
Liu, 2014 [9]	100 European Caucasians with NAFLD-related HCC and 275 controls with biopsy-proven NAFLD. Allele frequency were	Genotype frequencies were significantly different between NAFLD-HCC cases and NAFLD-controls (P=0.0001), with enrichment of the PNPLA3 rs738409 minor (G) allele. Carriage of each copy of the allele increased the risk, with	Carriage of the PNPLA3 polymorphism is not only associated with greater risk of progressive NASH and

	compared to the UK general population (1958 British Birth Cohort, n=1476)	GG homozygotes exhibiting a 5-fold increased risk. When compared to the UK general population, the risk-effect was more pronounced (OR, 12.19).	fibrosis but also of HCC.
Tateishi, 2015 [10]	33782 cases with HCC in 53 tertiary care centres in Japan from 1991 to 2010 (5326 non-virus-related; 596 NAFLD-related, median age, 72 years)	The proportion of non-virus-related HCC cases increased from 1991 to 2010 (10.0% to 24.1%) in 2010. BMI \geq 25 kg/m ² was present in 39%; T2DM was present in 63% and the proportion of cirrhosis was lower in NAFLD-related cases, compared with ALD, AIH, PBC, others.	Most cases of non-B, non-C HCC are related to lifestyle factors, including obesity and T2DM
Mittal, 2015 [11]	1500 patients who developed HCC from 2005 through 2010 from VA hospitals. The annual prevalence for the main risk factors (NAFLD, ALD, HCV) was registered	NAFLD accounted for 8% of total cases. Cirrhosis was less common in NAFLD-related cases (58.3%) compared to ALD- or HCV-related HCC. A higher percentage of NAFLD-related HCC were not diagnosed during surveillance programs, and a lower proportion received HCC-specific treatments (61.5%), but 1-year survival was not different	NAFLD is the third most common risk factor for HCC in VA hospitals.
Wong, 2014 [12]	A retrospective analysis of 10061 adult LT recipients in the U.S. (2002 to 2012) for HCC (UNOS registry)	NAFLD is the second leading aetiology of HCC-related LT (8.3% in 2002, 10.3% in 2007, 13.5% in 2012). From 2002 to 2012, the number of patients undergoing LT for NASH-related HCC increased 4 folds, much more than any other HCC aetiology	NAFLD is the most rapidly growing indication for LT in HCC cases in the U.S.
Wong, 2014 [13]	2002-2012 UNOS registry data Survival outcomes were stratified by disease aetiology	NAFLD-related HCC more likely to be women, with a higher prevalence of obesity, T2DM and CVD. However, the long-term survival of ALD- and NAFLD-related cases was higher than HCV patients (P < 0.001), and the risk of graft failure was lower	LT for NAFLD-related disease are not at higher risk of mortality or graft failure
Wang, 2014 [14]	Meta-analysis of 9 publications on outcomes of 717 patients with NASH and 3520 without who underwent liver transplantation	Survival at 1, 3, and 5 years was similar between NASH and non-NASH aetiology. NASH patients had a greater risk of death from CV complications (OR, 1.65) and from sepsis (OR, 1.71), but lower risk of graft failure compared with non-NASH (OR, 0.21).	NASH patients after LT require more aggressive treatment for CV disease and sepsis
Wong, 2015 [15]	Data from UNOS/OPTN registries from 2004 through 2013 on liver transplant waitlist	New waitlist registrants with NASH increased by 170% between 2004 and 2013, with NASH becoming the second-leading disease after HCV. ALD cases had more severe liver	Patients with NASH are less likely to survive for 90 days on the waitlist than patients

registrants with disease of different aetiologies

disease. However, compared with NASH patients, patients with HCV, ALD, or HCV+ALD had higher odds for 90-day survival on the waitlist.

with HCV, ALD, or HCV+ALD

AIH, acute autoimmune hepatitis; ALD, alcoholic liver disease; AST, aspartate aminotransferase; CC, cryptogenic cirrhosis; CV, cardiovascular; F-UP, follow-up; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LT, liver transplantation; OPTN, Organ Procurement and Transplantation Network; OR, odds ratio; PBC, primary biliary cirrhosis; T2DM, type 2 diabetes mellitus; UNOS, United Network for Organ Sharing; VA, Veterans Administration

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