A Dynamic Aspartate-to-Alanine Aminotransferase Ratio Provides Valid Predictions of Incident Severe Liver Disease

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The aspartate-to-alanine aminotransferase ratio (AAR) is associated with liver fibrosis, but its predictive performance is suboptimal. We hypothesized that the association between AAR and liver disease depends on absolute transaminase levels and developed and validated a model to predict liver-related outcomes in the general population. A Cox regression model based on age, AAR, and alanine aminotransferase (ALT) level (dynamic AAR [dAAR]) using restricted cubic splines was developed in Finnish population-based health-examination surveys (FINRISK, 2002-2012; n = 18,067) with linked registry data for incident liver-related hospitalizations, hepatocellular carcinoma, or liver death. The model was externally validated for liver-related outcomes in a Swedish population cohort (Swedish Apolipoprotein Mortality Risk [AMORIS] subcohort; n = 126,941) and for predicting outcomes and/or prevalent fibrosis/cirrhosis in biopsied patients with nonalcoholic fatty liver disease (NAFLD), chronic hepatitis C, or alcohol-related liver disease (ALD). The dynamic AAR model predicted liver-related outcomes both overall (optimism-corrected C-statistic, 0.81) and in subgroup analyses of the FINRISK cohort and identified persons with >10% risk for liver-related outcomes within 10 years. In independent cohorts, the C-statistic for predicting liver-related outcomes up to a 10-year follow-up was 0.72 in the AMORIS cohort, 0.81 in NAFLD, and 0.75 in ALD. Area-under-the-curve (AUC) for detecting prevalent cirrhosis was 0.80-0.83 in NAFLD, 0.80 in hepatitis C, but only 0.71 in ALD. In ALD, model performance improved when using aspartate aminotransferase instead of ALT in the model (C-statistic, 0.84 for outcome; AUC, 0.82 for prevalent cirrhosis). *Conclusion:* A dAAR score provides prospective predictions for the risk of incident severe liver outcomes in the general population and helps detect advanced liver fibrosis/cirrhosis. The dAAR score could potentially be used for screening the unselected general population and as a trigger for further liver evaluations. (*Hepatology Communications* 2021;5:1021-1035).

Liver disease represents a rapidly increasing health care burden. As a consequence, liver biochemistry testing is increasing, and liver tests are now the third most common type of biochemical test.[1] The transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), reflect

Abbreviations: AAR, aspartate-to-alanine aminotransferase ratio; AIC, Akaike Information Criterion; ALD, alcohol-related liver disease; ALT, alanine aminotransferase; AMORIS, Apolipoprotein Mortality Risk; APRI, aspartate aminotransferase/platelet ratio index; AST, aspartate aminotransferase; AUC, area under the curve; CI, confidence interval; dAAR, dynamic aspartate-to-alanine aminotransferase ratio; FIB-4, fibrosis-4; HCV, hepatitis C virus; HR, hazard ratio; ICD, International Classification of Disease; IQR, interquartile range; NAFLD, nonalcoholic fatty liver disease.

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hepatocellular damage and are the most common liver tests used in clinical practice in attempts to identify and exclude liver injury in a wide variety of situations.

Although the magnitude of elevation in ALT and AST has traditionally been used to guide the need for further liver investigations, recent United Kingdom guidelines concluded that this strategy is not supported by evidence.[2] Elevated transaminases are a common finding affecting around 10% of the general population,[3,4] while only a minority of these persons will develop liver-related events.[4,5]

The stage of liver fibrosis on biopsy is currently considered the best prognosticator in chronic liver disease across different etiologies.[6] Transaminases do not correlate directly with liver fibrosis stage.[7] In fact, the majority of individuals with asymptomatic advanced liver fibrosis or cirrhosis have transaminases within current reference limits.[8] In contrast, the AST to ALT ratio (AAR or "the De Ritis ratio"[9]) seems to correlate with the severity of liver fibrosis,[10] even when absolute transaminase values are within reference limits.[11,12] AAR has been associated with liver fibrosis stage in several cohort studies, including nonalcoholic fatty liver disease (NAFLD),[13,14] chronic hepatitis C (HCV) and B,[10,15,16] and alcohol-related liver disease (ALD).[17-19] In addition, the AAR is incorporated in many noninvasive fibrosis scores.[13,20]

On the other hand, the performance of the AAR in discriminating advanced fibrosis or cirrhosis is inconsistent between studies,[10,21] and discrimination performance in NAFLD and ALD is at best suboptimal.[17,22] Adding to the controversy, an AAR >1 was observed in 36%-77% of apparently healthy adult volunteers lacking any signs of liver disease, which was dependent on weight and alcohol intake.[23] There is a paucity of studies on the role of the AAR for predicting incident liver-related events.

We hypothesized that the predictive performance of the AAR for detecting prevalent advanced fibrosis or cirrhosis and incident liver-related events due to cirrhosis may be dependent on absolute transaminase levels. We tested this hypothesis by developing such a prediction model in a Finnish general population cohort with linked outcome data for incident clinical

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liver disease. We then performed external validation for both incident liver-related events and histologic cirrhosis and advanced fibrosis.

Materials and Methods

FINNISH FINRISK POPULATION DATA (DERIVATION COHORT)

Data were from the FINRISK studies from 2002, 2007, and 2012. The FINRISK studies were cross-sectional population surveys carried out in Finland in a systematic and standardized fashion every 5 years since 1972 by the Finnish Institute for Health and Welfare (previously National Public Health Institute). The objective was to assess risk factors for chronic diseases in representative population samples of adults aged 25-74 years drawn from the Finnish Population Information System, stratified by sex, 10-year age groups, and five to six geographic areas of Finland.[24] The FINRISK 2002-2012 cohorts together comprised 20,540 participants with available registry linkage. Detailed descriptions of study protocols have been published.[24] Definitions of baseline variables are described in the Supporting Materials. All participants provided signed informed consent, and the studies were approved by the Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital District.

FINRISK data were linked with the National Hospital Discharge Register for data on hospitalizations (data available from 1969), with the Finnish Cancer Registry for malignancies (data available from 1953), and with Statistics Finland for death (data available from 1969). Data collection to all these registries is mandatory by law, and coverage and general quality are consistent and complete.[25] Linkage was performed using the unique personal identity code assigned to all Finnish residents. Follow-up for deaths and hospitalizations were until December 2015 or until emigration. Study endpoints ascertained from these registries were those representing fatal and nonfatal severe liver disease requiring hospital admission or causing hepatocellular carcinoma or liver-related death. The International Classification of Disease (ICD) codes used to define the severe liver-related outcomes are listed in Supporting Table S1. We excluded individuals with a history of chronic liver disease in the outcome registries at baseline (ICD tenth revision: K70-K77, C22.0; ICD eighth/ninth revisions: 570-573, 155.0) and those with chronic viral hepatitis at baseline or during follow-up.

SWEDISH APOLIPOPROTEIN MORTALITY RISK COHORT (VALIDATION COHORT)

For purposes of external validation, we used the Swedish Apolipoprotein Mortality Risk (AMORIS) cohort, a general population cohort that underwent health examinations with blood sampling between 1985 and 1996 (baseline period).[26] The cohort consists of 812,073 individuals who were either taking part in yearly routine health checkups through occupational health screening or outpatients in primary care referred for laboratory testing. All individuals of the AMORIS cohort were residents of Sweden and predominantly living in Stockholm County (67%), together constituting approximately 35% of the total population of Stockholm County during this period. A detailed cohort description is available elsewhere.[26]

Herein, we used the same subpopulation of the cohort as in a recent study,[27] comprising 126,941 adults aged 35-79 years at baseline with available data to calculate both our risk score and the fibrosis-4 (FIB-4) score[28] for comparison. Persons with a diagnosis of chronic liver disease at baseline or any diagnosis of drug or alcohol abuse at or before baseline were excluded by use of ICD codes. Study baseline was defined as the date of blood sampling. AST and ALT were determined using an enzymatic ultraviolet test by a Technicon DAX 96 Multichannel Analyzer with a total imprecision <6.0% of coefficient of variation. Subjects were linked using the unique Swedish personal identification number with Swedish national registers for hospitalizations, specialized outpatient care, incident cancers, causes of deaths, and migration or continued residency in Sweden until December 31, 2011. The liver outcomes were ICD codes corresponding to a diagnosis of cirrhosis, liver failure, hepatocellular carcinoma, liver transplantation, or decompensated liver disease. The registers used and the linkage procedure are described in detail in the Supporting Materials.

SWEDISH NAFLD DATA (VALIDATION COHORT)

Further validation was performed in a cohort comprising all patients with biopsy-proven NAFLD at Karolinska University Hospital, Huddinge, and Linköping University Hospital from 1985 to 2009 (n = 646).[29] Subjects enrolled before 1985 (n = 167, 26%) were excluded due to uncertainty regarding the standardization of transaminase measurements at that time. Steatosis had been identified through the systemized nomenclature of medicine (SNOMED), and the diagnosis of NAFLD was further ascertained through medical chart review, as reported.[29] Exclusions were daily alcohol use of >30 g for men or >20 g for women at baseline or during follow-up, binge drinking (≥5 drinks for men or ≥4 drinks for women on the same occasion), any concurrent liver disease, use of medications associated with steatosis, and baseline hepatocellular carcinoma or baseline decompensated liver disease. Histopathologic evaluation was centralized and post hoc using current classifications.[29] Subjects were linked with Swedish national registers in the same way and for the same incident liver-related outcomes as in the AMORIS cohort.

BOSTON NAFLD AND HCV DATA (VALIDATION COHORTS)

The Boston NAFLD cohort was derived from a prospective NAFLD registry of 182 patients with biopsy-proven NAFLD at Beth Israel Deaconess Medical Center started in 2009. Patients with other forms of chronic liver diseases, alternative causes for fatty liver, or daily alcohol use of >20 g were excluded. Laboratory tests, blood collection, as well as medical history were performed at enrollment. Liver biopsy was performed within 3 months of the index visit.

The Boston HCV cohort comprised a total of 124 patients enrolled for a transient elastography registry study between 2004 and 2017. All subjects had a diagnosis of HCV by HCV-RNA polymerase chain reaction. Liver biopsies were performed as part of the routine clinical care for staging of liver disease at the time. Coexisting chronic liver diseases were reasonably excluded by history and standard clinical care, except for coexisting hepatitis B or human immunodeficiency virus infections, as reported.[30]

DANISH ALD DATA (VALIDATION COHORT)

Finally, we included for validation purposes a prospective cohort of 444 adult subjects (18-75 years) with a history of excessive alcohol use (>24 g/day for women and >36 g/day for men) for ≥1 year recruited from either primary care (two municipal alcohol rehabilitation centers and community call) or secondary care (three outpatient hospital liver clinics) between 2013 and 2017, as described.[17,31] Main exclusions were baseline decompensated liver disease, concurrent liver disease, and cholestasis.[17,31] Histologic liver fibrosis was staged from 0 to 4 according to Kleiner.[17,31] Liver-related outcomes comprising alcoholic hepatitis, varices needing treatment, upper gastrointestinal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular carcinoma, hepatorenal syndrome, or jaundice were ascertained from medical records.

STATISTICAL ANALYSES

For comparing groups, we used chi square or Mann-Whitney tests, as appropriate. Pairwise correlations between continuous variables were assessed by the Spearman test. In the FINRISK cohort, baseline predictors of liver outcomes were estimated by Cox proportional hazards models with time to first event as the outcome variable. The proportional hazards assumption of the Cox model was checked using Schoenfeld residuals, and no violations were detected. Covariates were assessed for a possible nonlinear relationship with the outcome using restricted cubic splines with degrees of freedom selected using the Akaike Information Criterion (AIC). Models were compared using the log-likelihood test.

The best model was chosen by comparing the Harrell's C-statistic, AIC, net reclassification improvement (categories were 1%, 5%, and 10% for 10-year follow-up), and by the likelihood ratio test to examine model fit of the nested models. We assessed internal validation of the final model to correct the C-statistic for optimism (overfitting) by bootstrapping 200 samples of the derivation data. Subgroup analyses of the FINRISK cohorts were performed separately by sex, age (<50 years or ≥50 years), body mass index ≥30 kg/m^2, and presence at baseline of diabetes or metabolic syndrome or NAFLD or alcohol risk use.

In the FINRISK cohort, we calculated the 10-year cumulative incidence of liver-related events in quantiles of the linear predictor of the final Cox model, each quantile including a minimum of five outcome events; quantiles were then combined to yield groups with 10-year cumulative incidence of liver events <1%, 1%-4%, 5%-9%, and ≥10%. Cumulative incidence was analyzed by the Aalen-Johansen method, considering death without severe liver disease as a competing risk.

External validation was performed by calculating a risk score for each person in the validation cohort using the predictors and the respective beta coefficients as estimated in the derivation cohort. We then examined the performance of the model by the C-statistic (incident severe liver disease) or area under the curve (AUC) (fibrosis stage) and stratified subjects in risk groups by the risk-score cutoffs derived from the derivation cohort. We calculated sensitivity, specificity, and positive and negative predictive value (PPV and NPV) for advanced fibrosis (stage 3-4) and cirrhosis (stage 4) by risk strata. In the clinical biopsy cohorts, a possible increasing trend between the risk score and fibrosis stage was tested by the Jonckheere-Terpstra trend test. In the two largest biopsy cohorts, we studied which histologic features contributed independently to the risk model by a multivariable linear regression with histologic fibrosis stage (0-4), lobular inflammation (0-3), ballooning (0-2), and steatosis score (1-3) as independent covariates and the risk score (dynamic AAR [dAAR] score) as the dependent variable. We compared the C-statistic and AUC of the model to the following published fibrosis scores: FIB-4,[28] AST/platelet ratio index (APRI),[32] and NAFLD fibrosis score.[20] Data were analyzed with R software version 3.6.1 and STATA version 14.2.

between ALT and AST was moderate (r = 0.67, P < 0.001; Supporting Fig. S1) but stronger among alcohol risk drinkers (r = 0.74, P < 0.001) than non-risk drinkers (r = 0.65, P < 0.001). There was a strong inverse correlation between AAR and ALT (r = −0.82, P < 0.001; Supporting Fig. S2) showing higher AARs at lower levels of ALT.

We observed 89 incident events of severe liver disease during a mean follow-up of 8.2 years (SD, 4.1; range, 0-13.0 years; 148,149 person-years at risk). ALT, AST, and AAR exhibited significantly nonlinear age-adjusted associations with incident liver-related outcomes in both men and women (P < 0.001; Supporting Figs. S3-S5).

The C-statistic for a Cox model considering only the AAR with or without age was 0.71 and 0.64, respectively (Supporting Table S2). Performance characteristics for all the candidate models are shown in Supporting Table S2. The final model consisted of age (continuous variable), AAR, and ALT; this model was chosen as it exhibited the best AIC and significantly better model fit compared to the other candidate models (P < 0.005, likelihood ratio test). In addition, this model yielded a net reclassification improvement of 15%-50% compared to the other candidate models (Supporting Table S2). ALT and AAR remained significantly nonlinear in the model (P < 0.001; Fig. 1). The bootstrap optimism-corrected C-statistic for the final age-adjusted model was 0.81. The hazards ratio of the model's linear predictor for liver outcomes was 2.72 (95% confidence interval [CI], 2.37-3.11). The equation for the final model in R language is:

$$\begin{aligned}\text{dAAR} < {} & -10.129915 + 0.039811813 * \text{dataset\$AGE} + 0.25387407 * \text{dataset\$ALT} - 0.0023607234 * \text{pmax}(\text{dataset\$ALT} - 11, 0)^3 + \\ & 0.0079492072 * \text{pmax}(\text{dataset\$ALT} - 17, 0)^3 - 0.0076811579 * \text{pmax}(\text{dataset\$ALT} - 22, 0)^3 + 0.0021985068 * \\ & \text{pmax}(\text{dataset\$ALT} - 30, 0)^3 - 0.00010583268 * \text{pmax}(\text{dataset\$ALT} - 58, 0)^3 + 3.5333535 * \text{dataset\$AAR} - 7.3473709 * \\ & \text{pmax}(\text{dataset\$AAR} - 0.63, 0)^3 + 32.911587 * \text{pmax}(\text{dataset\$AAR} - 0.92, 0)^3 - 44.937707 * \text{pmax}(\text{dataset\$AAR} - 1.14, 0)^3 + 21.786619 * \\ & \text{pmax}(\text{dataset\$AAR} - 1.41, 0)^3 - 2.4131284 * \text{pmax}(\text{dataset\$astalt} - 2.13, 0)^3.\end{aligned}$$

Results

FINNISH DERIVATION COHORT (FINRISK)

The FINRISK cohort comprised 18,067 adult subjects from the general population with available ALT and AST measurements. Demographics of the FINRISK cohort are shown in Table 1. Correlation

The equation in other software languages and a calculator are in the Supporting Material.

In sensitivity analyses, the C-statistic of the final model remained at or above 0.8 in both sexes and age groups and in subjects with obesity, diabetes, metabolic syndrome, NAFLD, or alcohol risk use (Table 2). The best discrimination was observed in subjects with alcohol risk drinking (C-statistic, 0.87), obesity (C-statistic, 0.85), or diabetes (C-statistic, 0.85).

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY COHORTS

	Population Cohorts		Liver Biopsy Cohorts			
Cohort	Population (FINRISK)	Population (AMORIS)	NAFLD patients	NAFLD patients	HCV patients	ALD patients
Country	Finland	Sweden	Sweden	USA	USA	Denmark
Cohort profile	Populationbased	Populationbased	Biopsy	Biopsy	Biopsy	Biopsy
Persons, n	18,067	126,941	479	182	124	444
Age (years), mean (SD)	49.7 (4.8)	53.2 (11.8)	48.4 (14.0)	55.7 (12.6)	48.1 (9.23)	56.5 (10.5)
Women, n (%)	9,787 (54)	70,893 (56)	183 (38)	74 (41)	33 (27)	108 (24)
Alcohol use (g/week), mean (SD)	84 (35)		<140 (women) <210 (men)	<140		185 (310)†
Active smokers, n (%)	3,976 (22)		101 (21)	19 (10)		247 (56)
Body mass index (kg/m^2), mean (SD)	26.9 (4.8)	24.5 (4.0)*	28.4 (4.2)	34 (6.5)	26.7 (4.4)	27.5 (5.3)
Waist circumference (cm), mean (SD)	91.0 (13.8)				94 (14.7)	104.4 (15.6)
Diabetes, n (%)	1,456 (8)	5,091 (4)	65 (14)	55 (30)	11 (10)	62 (14)
ALT (U/L), mean (SD)	26.9 (18.5)	27.5 (37.8)	87.2 (53.0)	74.4 (50)	72.5 (59.1)	39.9 (33.2)
AST(U/L), mean (SD)	28.3 (19.0)	22.7 (20.1)	51.0 (33.6)	50.7 (32.1)	60 (47)	46.2 (37.7)
Histologic fibrosis stage, n (%)						
0			119 (25)	57 (31)	14 (12)	36 (10)‡
1			187 (39)	40 (22)	50 (44)	122 (35)
2			115 (24)	51 (28)	13 (11)	104 (29)
3			40 (8)	20 (11)	13 (11)	26 (7)
4			18 (4)	14 (8)	24 (21)	66 (19)

*Available for 11,646 persons.
†Median 48 g/week (IQR, 0-276) and 187 (42%) were abstaining from alcohol at the time of inclusion.
‡Since 2016, the ALD cohort included 90 patients with a liver stiffness <6.0 kPa (FibroScan) and therefore not biopsied; these are considered not to have fibrosis stage 3 or 4 in the analyses.
Abbreviation: USA, United States of America.

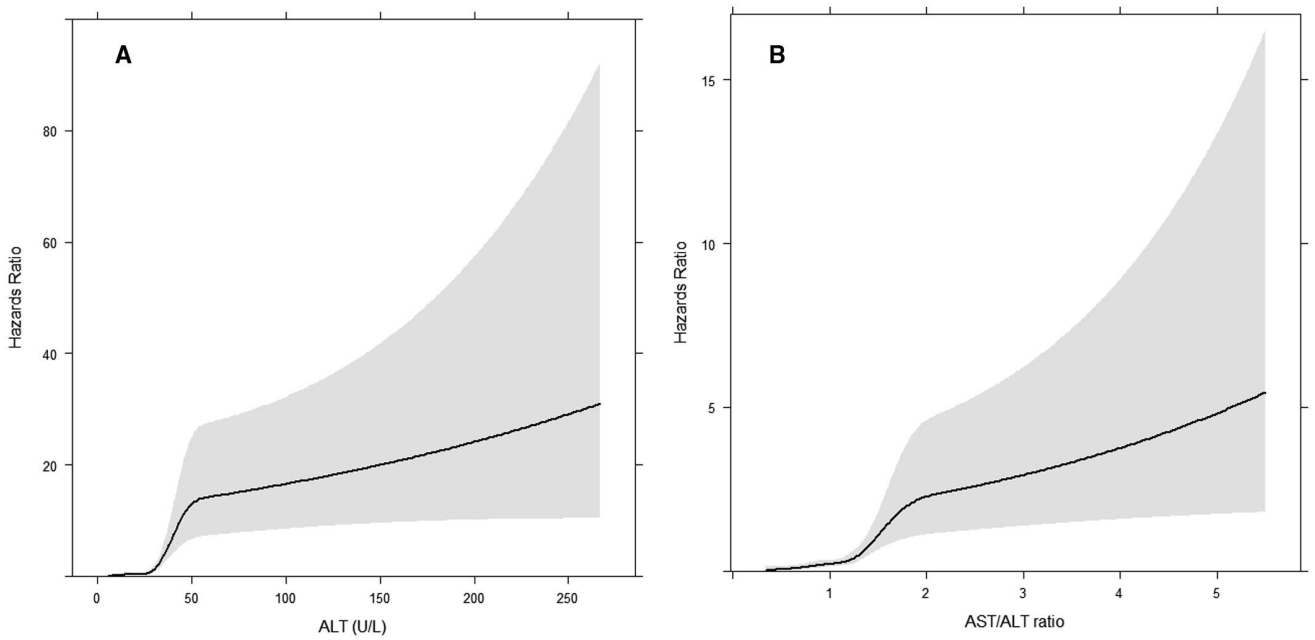


FIG. 1. Association between transaminases and incident liver disease. (A) ALT and incident liver disease and (B) AST/ALT ratio and incident liver disease in the final prediction model in the FINRISK cohort.

TABLE 2. SENSITIVITY ANALYSES FOR THE dAAR RISK MODEL DISCRIMINATION OF INCIDENT SEVERE LIVER DISEASE IN THE FINRISK COHOSRT

Subgroup	Persons	Events	C-statistic	95% CI
Men	8,280	59	0.808	0.749-0.867
Women	9,787	30	0.816	0.724-0.908
Age <50 years	9,148	31	0.821	0.743-0.899
Age ≥50 years	8,919	58	0.804	0.735-0.873
Obesity (body mass index ≥30 kg/m^2)	3,964	32	0.849	0.775-0.923
Diabetes	1,456	14	0.851	0.724-0.978
Metabolic syndrome	5,504	48	0.836	0.775-0.897
Alcohol risk drinker*	1,992	36	0.868	0.811-0.925
Nonrisk drinker	14,399	45	0.786	0.713-0.859
NAFLD†	7,707	34	0.800	0.718-0.882

*Average alcohol intake ≥30 grams of ethanol per day for men and ≥20 grams per day for women.
†Nonrisk drinking and fatty liver index ≥30.

SWEDISH VALIDATION COHORT (AMORIS)

The AMORIS cohort comprised 126,941 subjects with 1,738 incident liver-related events (1.4%) during a median 18-year follow-up (interquartile range [IQR], 15-21; 2,146,700 person-years at risk), 717 liver-related events within 10 years, and 343 within 5 years from baseline (Table 1). For follow-up restricted to 5 years, the C-statistic for the age-adjusted model was 0.74; for follow-up restricted to 10 years, 0.72; and for the entire follow-up period of 27 years, 0.68 (Table 3). For men, the corresponding values for C-statistic were somewhat higher, 0.73, 0.76, and 0.76, respectively, compared to women (Table 3). The hazard ratio (HR) of the model's linear predictor for liver-related outcomes was 1.54 (95% CI, 1.51-1.57).

SWEDISH NAFLD VALIDATION COHORT

In the Swedish NAFLD biopsy cohort of 479 patients with a median follow-up of 18 years (IQR, 12-24), there were 53 (11%) incident severe liver-related clinical events. Of these events, 27 (51%) occurred within 10 years from baseline. The C-statistic of the risk model for follow-up restricted to 10 years was 0.81 for the entire cohort, 0.88 for men, and 0.73 for women (Table 3).

The risk model was significantly associated with prevalent histologic fibrosis stage (Fig. 2). The AUC was 0.80 (95% CI, 0.67-0.93) for detection of prevalent cirrhosis and 0.75 (95% CI, 0.68-0.83) for prevalent advanced fibrosis stage 3-4 (Fig. 3). By multivariable linear regression analysis considering fibrosis stage, lobular inflammation, ballooning, and steatosis score as dependent covariates, fibrosis stage was the only factor significantly associated with the risk score.

BOSTON NAFLD AND HCV VALIDATION COHORTS

In both the U.S. biopsy cohorts of 182 patients with NAFLD and 124 patients with HCV from a tertiary referral hospital in Boston, MA (Table 1), the risk model was significantly associated with histologic fibrosis stage (Fig. 2). In the NAFLD cohort, the AUC was 0.83 (95% CI, 0.73-0.93) for detection of cirrhosis and 0.80 (95% CI, 0.71-0.88) for advanced fibrosis stage 3-4 (Fig. 3). In the HCV cohort, the AUC was 0.82 (95% CI, 0.72-0.92) for detection of cirrhosis and 0.77 (95% CI, 0.67-0.87) for advanced fibrosis (Fig. 3).

DANISH ALD VALIDATION COHORT

The Danish ALD cohort comprised 444 patients (Table 1). During a median follow-up of 3.1 years (IQR, 1.5-4.7) of 409 patients with available follow-up data, there were 71 (17%) incident liver-related clinical events. The C-statistic of the risk model was 0.75 (95% CI, 0.69-0.80) and was 0.77 for men and 0.68 for women.

The risk model was significantly associated with histologic fibrosis stage (Fig. 2). However, the AUC was suboptimal with 0.71 (95% CI, 0.66-0.77) for detection of cirrhosis and 0.71 (95% CI, 0.66-0.76) for advanced fibrosis stage 3-4 (Fig. 3).

By multivariable linear regression analysis considering fibrosis stage, lobular inflammation, ballooning, and steatosis score as dependent covariates, all except ballooning were significantly independently associated with the risk score ($P < 0.01$; $P = 0.079$ for ballooning). Steatosis had the strongest correlation with the risk score (correlation coefficient 0.64; 95% CI, 0.48-0.80; compared to correlation coefficients of 0.25 for fibrosis and ballooning and 0.38 for lobular inflammation).

Replacing absolute ALT level with absolute AST level in the risk model improved the AUC to 0.82

TABLE 3. DAAR MODEL DISCRIMINATION OF INCIDENT SEVERE LIVER DISEASE IN THE AMORIS COHORT AND SWEDISH NAFLD COHORT

			5-Year Follow-Up	10-Year Follow-Up	Maximal Follow-Up
AMORIS cohort	Persons	Events	C-statistic (95% CI)	C-statistic (95% CI)	C-statistic (95% CI)
All	126,941	1,738	0.740 (0.711-0.769)	0.720 (0.698-0.742)	0.684 (0.670-0.698)
Men	56,048	771	0.762 (0.721-0.803)	0.759 (0.732-0.786)	0.731 (0.711-0.751)
Women	70,893	967	0.727 (0.688-0.766)	0.687 (0.658-0.716)	0.650 (0.630-0.670)
NAFLD biopsy cohort (Swedish)					
All	479	53	0.843 (0.731-0.954)	0.807 (0.731-0.883)	0.774 (0.713-0.835)
Men	296	25	0.853 (0.730-0.976)	0.880 (0.811-0.949)	0.815 (0.742-0.888)
Women	183	28	0.833 (0.635-1.000)	0.726 (0.595-0.857)	0.705 (0.597-0.813)

(95% CI, 0.77-0.87) for prevalent cirrhosis and 0.80 (95% CI, 0.75-0.85) for advanced fibrosis. Similarly, the C-statistic for predicting liver-related events improved to 0.84 (95% CI, 0.80-0.88) with 0.86 for men and 0.77 for women.

RISK STRATIFICATION

The cumulative incidence of liver outcomes by risk group was similar in the AMORIS and FINRISK cohorts (Fig. 4). Cumulative incidences of death without liver disease are shown in Supporting Fig. S6. Corresponding incidence figures by sex are shown in Supporting Fig. S7. A color-coded scoring sheet for easy application of the prediction model with risk estimates for both incident liver-related events and PPVs for the presence of advanced liver fibrosis and cirrhosis in these risk groups is provided in Fig. 5. Diagnostic measures of the risk score using cutoffs by sensitivity, specificity, or Youden index to detect advanced fibrosis and cirrhosis in the Swedish NAFLD cohort are shown in Supporting Table S3.

COMPARISON TO PUBLISHED FIBROSIS SCORES

Compared to published fibrosis scores, the present risk model exhibited a similar 10-year C-statistic for predicting incident severe liver disease in the AMORIS cohort (0.72 [present risk model] versus 0.71 for FIB-4, 0.67 for APRI, and 0.63 for NAFLD fibrosis score). In the Swedish NAFLD cohort, the 10-year C-statistic was comparable to the other scores (0.81 vs. 0.80-0.82; Supporting Table S4) and identical to that for histologic fibrosis stage (C-statistic 0.81). In the Danish ALD cohort, however, all the other fibrosis scores outperformed the present one (Supporting Table S4). On the other hand, performance of the risk score with absolute AST level instead of absolute ALT level was better than all the other fibrosis scores in the Danish ALD cohort.

For detection of advanced histologic fibrosis (stage 3-4) or cirrhosis (stage 4), performance of our risk model was comparable to the other fibrosis scores in NAFLD and HCV, varying by cohort (Supporting Fig. S8; Supporting Table S5). In ALD, however, performance was poorer but again improved by using AST instead of ALT in the model.

In the Swedish NAFLD cohort, our risk model predicted a 10-year risk of severe liver events independently of baseline histologic fibrosis stage (HR, 1.88; 95% CI, 1.30-2.72; $P < 0.001$). A similar effect was not seen for FIB-4 ($P = 0.87$) or APRI ($P = 0.62$). Even among the 58 patients with baseline fibrosis stage 3-4, our risk model predicted incident severe liver events (HR, 2.22; 95% CI, 1.76-2.80; $P < 0.001$).

Discussion

This study confirms our clinical hypothesis that the predictive performance for incident liver-related outcomes of a specific AAR level depends on the absolute ALT level. We show that a risk model built on this concept can be used for prediction of future liver-related outcomes and helps in detection of prevalent advanced chronic liver disease. The model, which we call the dAAR score, was validated

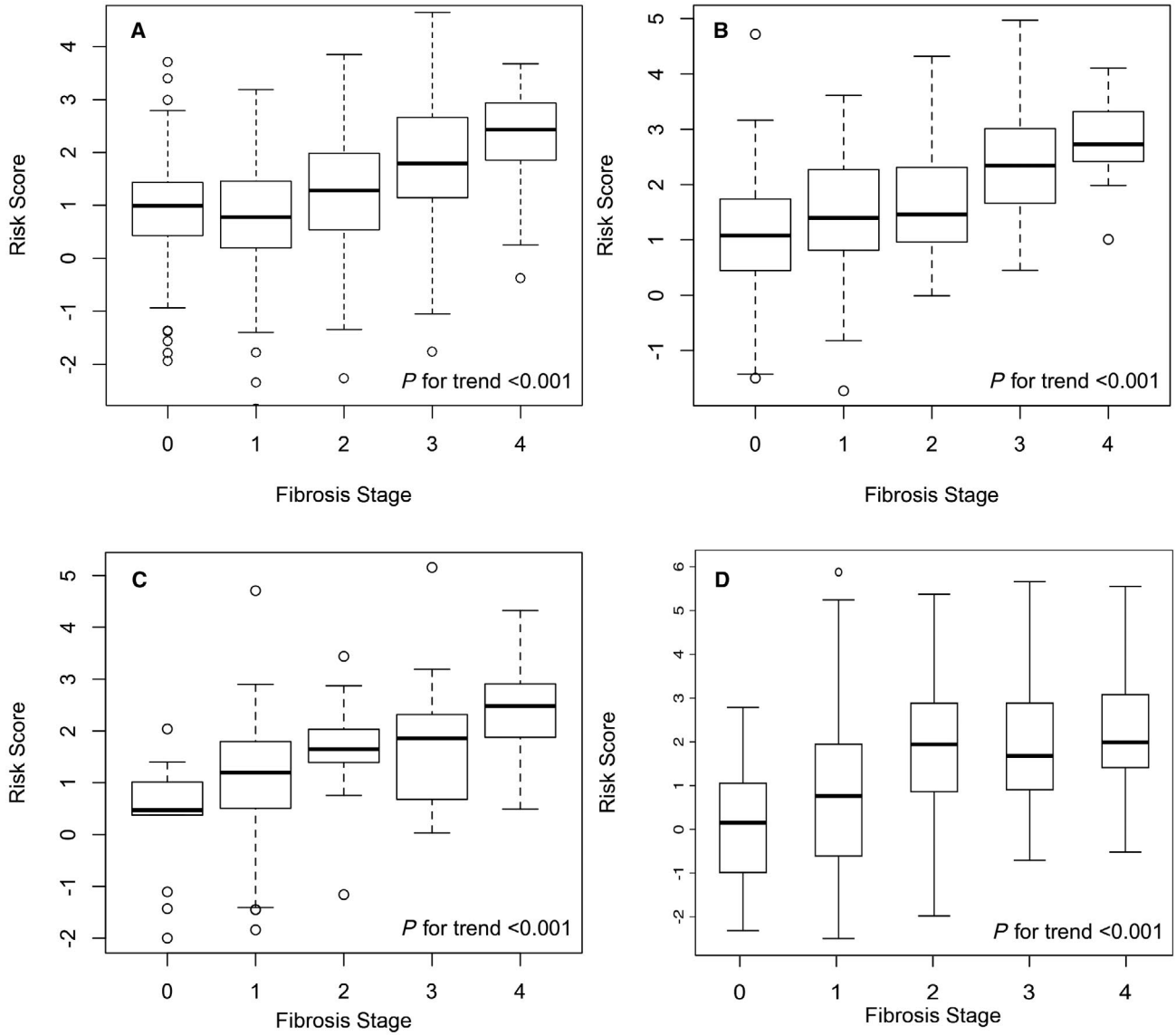


FIG. 2. Box plots showing the distribution of the dAAR score by liver fibrosis stage in the different cohorts. (A) Swedish NAFLD, (B) Boston NAFLD, (C) hepatitis C, and (D) ALD cohorts. Graphs show interquartile range (box), median (horizontal line), and outliers (whiskers).

in several independent cohorts. The dAAR score had reasonable discrimination with an optimism-corrected C-statistic of 0.81 for incident liver disease in the Finnish general population cohort. In the Swedish AMORIS cohort, model discrimination decreased with longer follow-up times, with the C-statistic being 0.74 at 5 years and 0.68 at a median follow-up of 18 years; this indicated better performance for liver events occurring early during follow-up. In the Swedish NAFLD cohort, the 10-year C-statistic of the dAAR score (0.81) for predicting incident severe liver outcomes was identical to that of the histologic fibrosis stage. In the Danish ALD cohort, the C-statistic was 0.75, which is reasonable but inferior to previous fibrosis scores. A different case mix regarding, for example, age, diabetes, and alcohol use, and therefore different ALT and AST levels, may contribute to the differences in model performance measures among the cohorts.

Our model is built on the AAR, which was first associated with liver disease stage by de Ritis et al. in 1957.[9] The underlying mechanisms for a rising

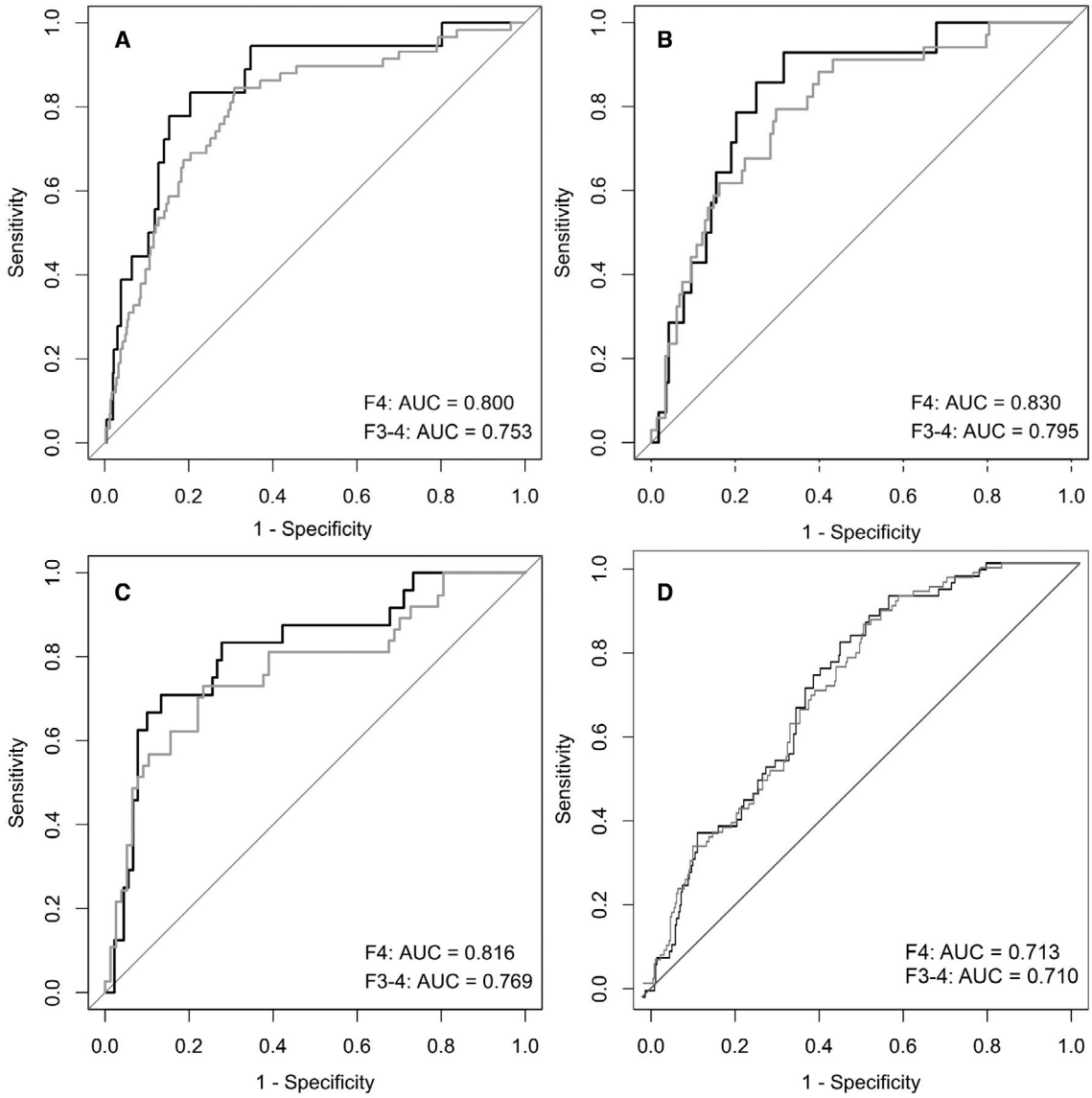


FIG. 3. Receiver operating characteristic plots for cirrhosis (fibrosis stage 4) and advanced fibrosis (stages 3-4) in the different cohorts. (A) Swedish NAFLD, (B) Boston NAFLD, (C) hepatitis C, and (D) ALD cohorts.

AAR with increasing liver fibrosis are unclear but may involve both an increased hepatocyte mitochondrial release and prolonged clearance by liver sinusoidal endothelial cells of especially mitochondrial AST.[10,33-35] Our finding that a clinically relevant cutoff for AAR decreases with increasing ALT is well in line with a study showing that healthy adults without liver disease often have an AAR above 1 when their ALT is low.[23] In contrast, according to the dAAR score, at high ALT levels, an AAR as low as 0.5 may indicate advanced liver fibrosis.

In the FINRISK cohort, the dAAR score yielded a net reclassification improvement of 20%-21% for prediction of incident severe liver outcomes compared to ALT or AST alone. In the AMORIS population cohort and the clinical cohorts, the dAAR score performed well compared to other noninvasive fibrosis scores, while performance was poorer in the Danish ALD cohort. Advantages of the dAAR score over existing noninvasive scores include simplicity to use with the color-coded sheet (Fig. 5) and the fact that it was specifically designed to predict clinical

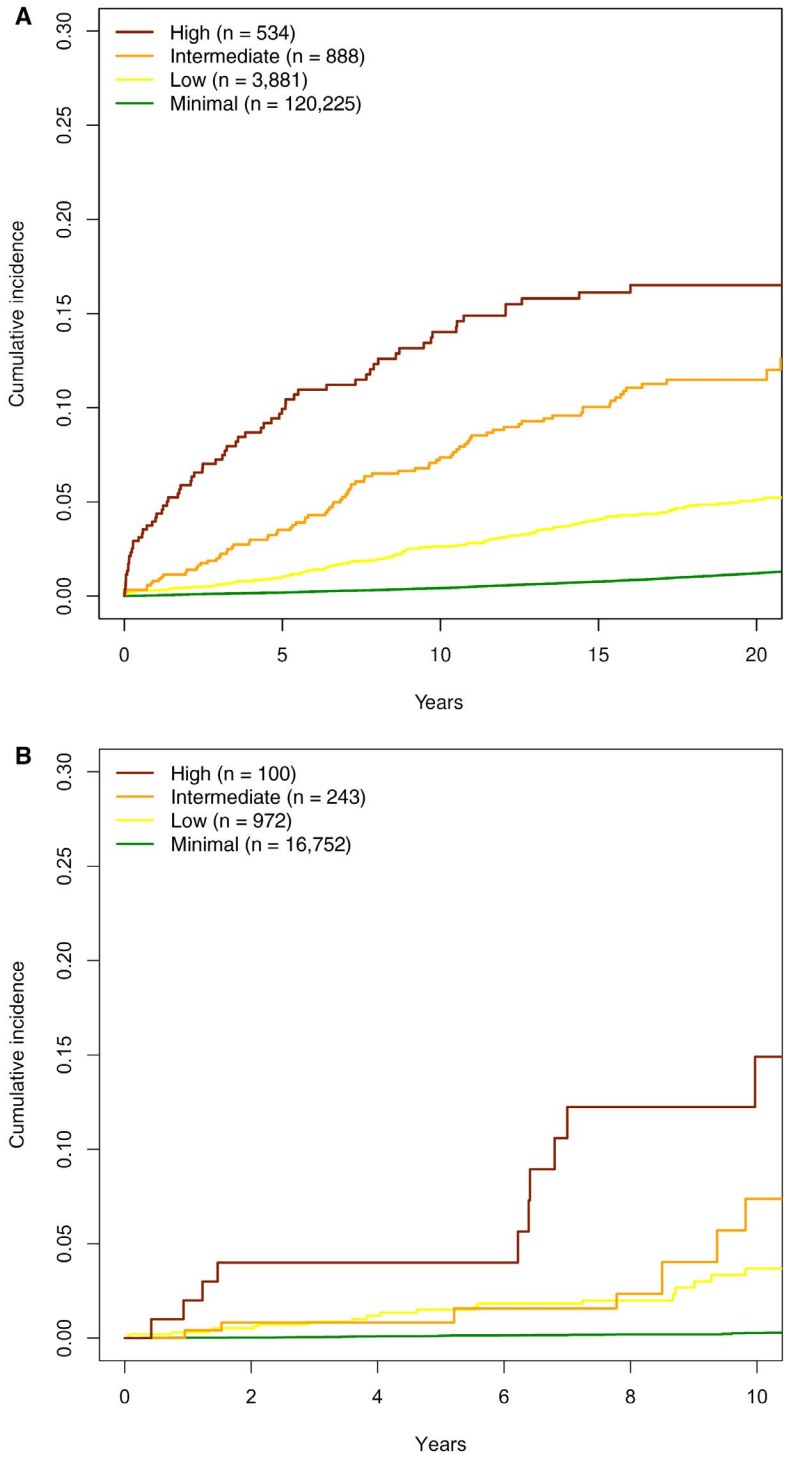
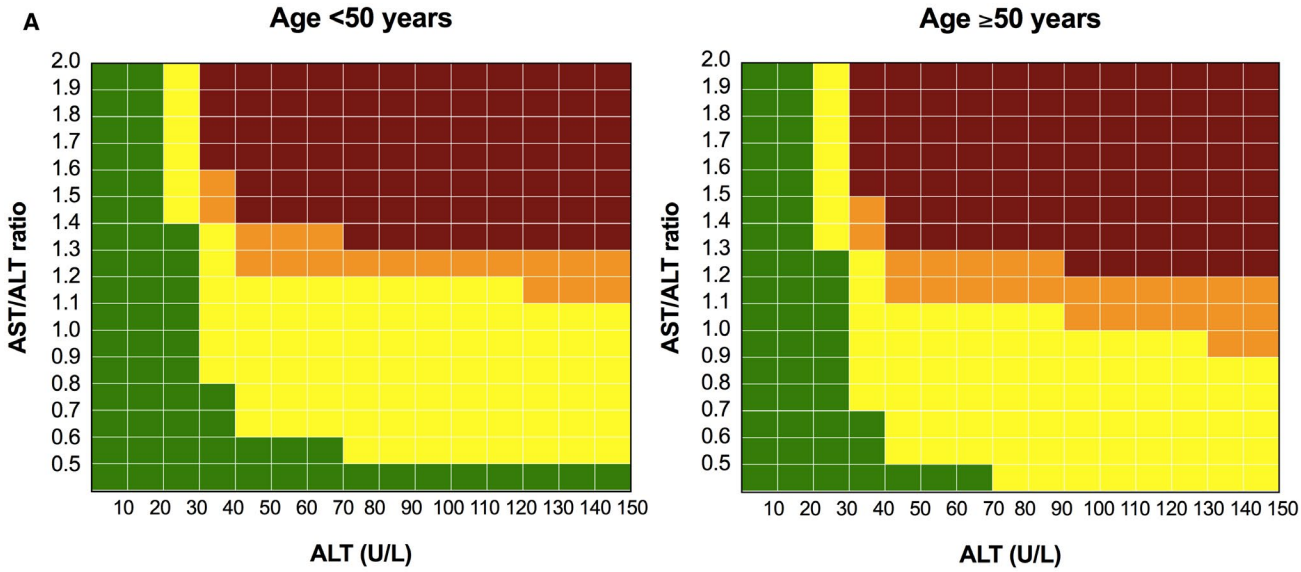


FIG. 4. Cumulative incidence of severe liver disease by risk group. (A) AMORIS cohort and (B) FINRISK cohort. Analysis was performed using the Aalen-Johansen method considering death without liver disease as a competing risk event. Follow-up was 20 years for the AMORIS cohort and 10 years for the FINRISK cohort.

A



B

		<1%		1%-4%		5%-9%		≥10%	
		10-year risk		10-year risk		10-year risk		10-year risk	
FINRISK		0.3%		3.7%		7.4%		14.9%	
AMORIS		0.4%		2.6%		7.4%		14.0%	
		% F3-4	% F4	% F3-4	% F4	% F3-4	% F4	% F3-4	% F4
NAFLD		7%	1%	24%	11%	44%	18%	53%	24%
HCV		16%	7%	50%	34%	75%	63%	67%	50%
ALD	ALT	15%	10%	22%	16%	18%	16%	43%	29%
	AST	7%	3%	23%	13%	8%	8%	56%	46%

FIG. 5. Use of the dAAR score for prediction of liver outcomes and advanced fibrosis. (A) Interaction between AST/ALT ratio and ALT levels by age in the dAAR score. Risk is depicted by different colors, with green representing the lowest risk and red the highest risk. (B) The 10-year cumulative incidence estimates from the FINRISK and AMORIS cohorts and likelihood of advanced liver fibrosis (%F3-F4) or cirrhosis (%F4) in biopsied patient cohorts of NAFLD (Swedish and Boston cohorts combined), HCV, and ALD. The percentages for %F3-F4 and %F4 depict the number of individuals with F3-F4 or F4 on liver histology divided by the number of individuals in that specific risk category. For ALD, results are given both for the dAAR score with absolute ALT level and for the dAAR score with absolute AST level because performance of the AST model was substantially better in this cohort.

liver outcomes in an unselected general population. Previous fibrosis models have been developed to detect fibrosis in highly selected patient cohorts from specialized clinics, but their accuracy for fibrosis detection in unselected population cohorts is poorer (AUC 0.6-0.7).[4] In contrast to other fibrosis scores, the dAAR score is not reliant on platelet counts, and this may be beneficial in specific clinical situations when there are competing causes for thrombocytopenia and in epidemiologic studies when platelet counts are unavailable.

The AAR was traditionally regarded as a marker of alcohol abuse.[36] However, a recent study showed

that the AAR was useful in distinguishing patients with alcoholic cirrhosis from subjects with similar alcohol use without cirrhosis (AUC, 0.77).[18] In that study, the AAR decreased somewhat with abstinence but still remained higher in abstinent previous heavy drinkers with cirrhosis than in abstinent previous heavy drinkers without cirrhosis.[18] In our study, the dAAR score showed excellent performance for predicting incident liver-related outcomes in the subgroup of risk drinkers in the Finnish cohort while performance was poorer in the Danish ALD cohort. The reason for this difference is unclear. Interestingly, performance of the dAAR score improved substantially when using absolute AST level instead of ALT in the score. Further study is needed to clarify when to possibly substitute ALT for AST in risk prediction using the dAAR score.

Strengths of our study include the large and representative cohorts. External validation was undertaken in five different cohorts from four different countries, showing reasonable performance for the prediction of incident liver-related events. Validation in several countries also reduces potential bias from laboratory- or assay-specific issues. Both the Finnish and Swedish national registries used for outcome data are considered to be of very high quality.[25,37-41] The clinical cohorts used histology, the gold standard, as reference.

Study limitations include the absence of a reference measure for baseline liver fibrosis in the general population cohorts. We also had only one measurement of liver tests at baseline, while repeat measurements over time might have increased model performance. The Nordic populations are fairly homogeneous, and we therefore validated the model also in independent U.S. cohorts but only for fibrosis stage. More external validation in diverse ethnic populations are welcomed.

Despite the existence of several noninvasive liver fibrosis tests, these have so far been poorly adopted by primary care physicians.[42,43] The dAAR score based on only two standard, widely-used, and inexpensive liver tests can help risk stratify persons in the community with regard to both advanced liver fibrosis and risk for incident clinical liver events with minimal added resource burden. Whereas most noninvasive fibrosis tests were developed for use in at-risk populations, such as those with confirmed steatosis or elevated ALT, to detect subclinical fibrosis,[44,45] the dAAR score was specifically developed for an unselected general population and prediction of liver-related outcomes. This implies that one can apply our model as a screening tool for the population without the need for preceding "suspicion-triggers" for liver disease. This information could then be used to guide referral practices in primary health care. An example of how the dAAR score can be applied in clinical practice is provided in Supporting Fig. S9.

The dAAR score is intended for use in primary care outpatient settings where transaminase levels are stable and not in situations with acute liver injury. It must also be kept in mind that AST elevations may be due to nonhepatic causes, which should be excluded before use.

In conclusion, the dAAR score provides a means to easily stratify the general population with regard to risk for incident severe liver disease. The predictive performance of the dAAR score is largely based on the ALT-dependent ability of the AAR to detect subclinical cirrhosis. The dAAR score is intended as a screening tool for the unselected general population and as a trigger for further liver evaluations.

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Supporting Information

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