Received: 3 April 2021 | Revised: 3 June 2021 | Accepted: 22 June 2021

DOI: 10.1111/liv.15004

ORIGINAL ARTICLE

Liver INTERNATIONAL WILEY

Incidence of liver-related morbidity and mortality in a population cohort of non-alcoholic fatty liver disease

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Funding
VM received a grant from the Sigrid Jusélius Foundation and The Finnish Medical Foundation; VS was supported by the Finnish Foundation for Cardiovascular Research; and FÅ reports research grants from the Mary and Georg Ehrnrooth Foundation, Medicinska Understödsföreningen Liv och Hälsa, and Finska Läkaresällskapet.

Handling Editor: Michelle Long

Abstract

Background & Aims: Non-alcoholic fatty liver disease (NAFLD) increases morbidity and mortality. However, patients in biopsy-based cohorts are highly selected and the absolute risks of liver- and non-liver outcomes in NAFLD in population remains undefined. We analysed both liver-related and non-liver-related outcomes in Finnish population cohorts of NAFLD.

Methods: We included 10 993 individuals (6707 men, mean age 53.3 ± 12.6 years) with NAFLD (fatty liver index ≥60) from the Finnish population-based FINRISK and Health 2000 studies. Liver fibrosis was assessed by the dAAR score, and genetic risk by a recent polygenic risk score (PRS-5). Incident liver-related outcomes, cardiovascular disease (CVD), cancer and chronic kidney disease (CKD) were identified through linkage with national registries.

Results: Mean follow-up was 12.1 years (1128 069 person-years). The crude incidence rate of liver-related outcomes in NAFLD was 0.97/1000 person-years. The cumulative incidence increased with age, being respectively 2.4% and 1.5% at 20 years in men and women aged 60 years at baseline, while the relative risks for CVD and cancer were 9-16 times higher. The risk of CKD exceeded that of liver outcomes at a baseline age around 50 years. 20-year cumulative incidence of liver-related outcomes was 4.3% in the high, and 1.5% in the low PRS-5 group. The dAAR score associated with liver outcomes, but not with extra-hepatic outcomes.

Conclusion: The absolute risk of liver-related outcomes in NAFLD is low, with much higher risk of CVD and cancer, emphasizing the need for more individualized and holistic risk-stratification in NAFLD.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; CVD, cardiovascular disease; dAAR, dynamic AST-ALT index; FLI, fatty liver index; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; HILMO, National Hospital Discharge Register; HR, hazard ratio; *HSD17B13*, hydroxysteroid 17-beta dehydrogenase; MAFLD, metabolic associated fatty liver disease; MONICA, monitoring trends and determinants in cardiovascular disease; NAFLD, non-alcoholic fatty liver disease; *PNPLA3*, patatin-like phospholipase domain-containing protein 3; PRS-5, polygenic risk score of five risk variants; *TM6SF2*, transmembrane 6 superfamily member 2.

KEYWORDS
advanced liver disease, cirrhosis, non-alcoholic fatty liver disease

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), also known as metabolic (dysfunction) associated fatty liver disease (MAFLD), is the most common liver disease, with a global prevalence of 25%.[1] NAFLD is closely associated with obesity, insulin resistance and type 2 diabetes.[2] NAFLD covers a spectrum of liver diseases, from simple steatosis to steatohepatitis, as well as liver fibrosis and cirrhosis.[3] NAFLD places an increasing burden on primary and secondary healthcare, but only a few patients with liver steatosis eventually progress to cirrhosis.[4] The disease also increases the risk of hepatocellular carcinoma (HCC), even before progression to liver cirrhosis and NAFLD is rapidly becoming the leading cause of HCC.[5]

Simple steatosis is generally thought to be a quite benign condition, and the stage of fibrosis is the strongest predictor of morbidity and mortality. Most individuals with NAFLD die from cardiovascular disease (CVD) or cancer before developing end-stage liver disease.[6] NAFLD is also associated with the development of chronic kidney disease (CKD).[7] Genetics is an important factor modifying the risk and progression of NAFLD. Recently, a polygenic risk score was introduced based on the five strongest and best validated risk variants,[8] including variants such as patatin-like phospholipase domain-containing 3 (*PNPLA3;* rs738409), transmembrane 6 superfamily member 2 (*TM6SF2*; rs58542926) and hydroxysteroid 17-beta dehydrogenase (*HSD17B13*; rs72613567). This genetic risk score (PRS-5) has been associated with hepatic fat content and with risk for HCC in individuals with NAFLD.[8,9]

Notably, most of the studies on the incidence of liver-related events in NAFLD come from either specialized clinics[6,10-13] or health-care registries based on ICD code registration of NAFLD.[14,15] There may be bias towards more severe forms of NAFLD in such cohorts. Registry-based case-findings are often large but typically lack comprehensive data on baseline risk factors.[16-18] Furthermore, only a few population-based health-screening studies have included longitudinal linkage to comprehensive national registries for liver-related clinical outcomes.[19]

We analysed the incidence and predictors of liver-related and extra-hepatic outcomes in a large general population sample of individuals with NAFLD, including estimates of the baseline fibrosis stage.

Key points

- This study estimated the risk of liver-related outcomes, cardiovascular disease, cancer and chronic kidney disease outcomes in Finnish population-based study cohorts with non-alcoholic fatty liver disease.
- Men with NAFLD had a higher risk of liver-related outcomes after 20 years compared with women with NAFLD; the cumulative incidence increased with age and, after 20 years of follow-up, it was 2.4% and 1.5% in men and women aged 60 years at baseline.
- The risk of cardiovascular disease or cancer was multiple times higher than the risk of liver-related outcomes.

2 | MATERIALS AND METHODS

2.1 | Study population

The study population consisted of the FINRISK study cohorts from 1992, 1997, 2002, 2007 and 2012 and the Health 2000 survey cohort from 2000 to 2001. The FINRISK studies are systematic and standardized cross-sectional population-based health examination surveys carried out in Finland every 5 years since 1972 by the Finnish Institute for Health and Welfare. These studies aim to assess risk factors for chronic diseases in representative population samples of adults aged 25-74 years (FINRISK 1992:25-64 years) who are drawn from the Finnish Population Information System, stratified by sex, 10-year age groups and four to six geographic areas of Finland. The number of invitees has varied over the years, from 7927 to 13 498, and the participation rates have varied from 65% to 76%.[20]

The Health 2000 Survey was also coordinated by the Finnish Institute for Health and Welfare and originally comprised 8028 adults aged ≥30 years. The participation rate in the full examinations was 80%.[21] The cohort is considered representative of the entire Finnish population through a regional two-stage stratified cluster sampling procedure. The methods, measurements and protocols used in the FINRISK studies have been essentially the same over time and are similar to those used in the Health 2000 Survey.[20,22]

Data were collected from each participant at baseline via interviews, a questionnaire and health examination by trained physicians and/or nurses using standardized procedures from the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) and European Health Risk Monitoring projects.[23] Blood samples were collected at baseline for a broad spectrum of laboratory measurements and handled using a standardized protocol. Detailed descriptions of the study protocols have been published previously.[21,22]

The studies were approved by the Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital District and conducted according to the Declaration of Helsinki. Previously, the studies were approved by the Institutional Review Board of the National Public Health Institute. All subjects provided informed consent for the study and for future registry linkage.

2.2 | Baseline variables

At baseline, study subjects were asked to report how often they consumed alcoholic beverages during the previous year and the average amount they consumed per week during the previous month. Average daily alcohol consumption (grams of 100% ethanol per day) was calculated based on these data.

Binge drinking was defined as drinking at least five drinks 10 g ethanol per drink on one occasion and categorized as weekly, monthly or less often. The respondents were also questioned how often they drank alcohol to the level that they felt intoxicated (weekly, monthly, or less often). In addition, smoking status (never, former, current smoker), amount of smoking (number of cigarettes per day) and leisure time physical activity (frequency of moderate-intensity or high-intensity physical exercise for at least half an hour) were noted. Diabetes was defined as taking diabetes medication or a known diabetes diagnosis. Metabolic factors were defined according to the Joint Interim Statement cutoffs.[24]

Baseline NAFLD was defined by a fatty liver index (FLI) ≥60. Individuals with missing FLI data (n = 716) were excluded. Twenty-five percent of individuals in the original cohort had FLI ≥60, distribution of these individuals based on the age and sex are shown in Figure S1. We excluded individuals with prior hospitalization due to liver disease (ICD10 codes K70-K77, C22) before study baseline, missing registry linkage, those with chronic viral hepatitis (hepatitis C of B virus) at or before baseline and those with baseline alcohol risk (>140 g/week for women and >210 g/week for men). Of the original sample of 43 105 adults, the final analytical sample comprised 10 993 individuals with NAFLD (FLI ≥60 without alcohol risk). Individuals with FLI <30 and without alcohol risk use (n = 16 393) were used for comparison (non-NAFLD controls).

2.3 | Outcomes

FINRISK data were linked with the National Hospital Discharge Register (HILMO) for data on hospitalizations (data available from 1969), the Finnish Cancer Registry for malignancies (data available from 1953) and Statistics Finland for causes of death (data available from 1969).[25] Data collection in these registries is mandated by law, and the coverage and general quality are consistent and complete.[26] Linkage was performed using the unique personal identity code assigned to all Finnish residents. Follow-up for deaths and hospitalizations was until December 2015.

The primary outcome was fatal and non-fatal advanced liver disease requiring hospital admission or causing liver cancer, or liver-related death caused by non-alcoholic liver disease defined in line with a recent consensus paper[27] by the ICD codes presented in Table S1. To achieve high sensitivity, we included also ICD codes that might represent milder forms of liver disease than complicated cirrhosis. Hospital admission due to liver disease means that a liver-related complication was the main cause or 1st or 2nd side cause of hospitalization.

Secondary outcomes were fatal and non-fatal incident cardiovascular events, cancer and CKD. Cardiovascular event codes were those used in the original FINRISK cardiovascular risk studies and based on hospital admission or death.[28] The ICD codes used to define the various outcomes are presented in Table S1.

2.4 | Laboratory analysis

Subjects were asked to fast for 4 hours and to avoid heavier meals prior to blood sampling. The median fasting time was 5 hours (interquartile range 4-6 hours). Lipids and gamma-glutamyltransferase (GGT) measurements were performed using serum samples. The rest of the serum and plasma biomarkers were determined from samples stored at −70°C. The laboratory analyses were described in detail elsewhere.[20,21]

The FLI was calculated based on body mass index (BMI), waist circumference, GGT and triglycerides as described previously.[29] The recently developed dynamic aspartate aminotransferase-alanine aminotransferase ratio (dAAR) score[30] to predict liver fibrosis was calculated for the subset with available liver enzyme data, namely the FINRISK 2002-2012 cohorts.

PNPLA3 rs738409, *TM6SF2* rs58542926, *MBOAT7* rs641738, *GCKR* rs1260326 and *HSD17B13* rs72613567 were genotyped using the Illumina 610K, Omniexpress and HumanCoreExpress chips.

2.5 | Statistical analysis

To compare groups, we used the chi-squared or Mann-Whitney tests as appropriate. The cumulative incidence of liver-related outcomes and incident CVD, cancer and CKD were analysed both using the nonparametric cumulative incidence function and by separate competing-risk models according to the Fine and Gray method[31] considering death without the specific outcome as a competing risk. Fine and Gray models were adjusted for age and study cohort and estimated separately for men and women. Subjects with a baseline diagnosis of CVD (n = 769), cancer (n = 444) or CKD (n = 139) were removed from that respective incidence analysis.

The association of genetic risk on liver outcomes was assessed under the additive model using the previously reported polygenic risk score of five risk variants named PRS-5. The PRS-5 was calculated by summing the number of steatosis-predisposing alleles in the *PNPLA3*, *TM6SF2*, *MBOAT7*, *GCKR* and *HSD17B13* genotype weighted using the coefficients previously described. The cohort was stratified into high- and low-genetic risk groups based on the PRS-5 cutoff of 0.495, as reported previously.[8,9]

Baseline lifestyle, socioeconomic and genetic predictors of liver-related outcomes were estimated by Fine and Gray models adjusted for age, sex, cohort, BMI, type 2 diabetes and number of metabolic factors (0-5).

For the subset with dAAR scores available to estimate liver fibrosis, we analysed age-adjusted Fine and Gray cumulative incidences

of outcomes according to the baseline dAAR score. For comparison, we present median dAAR scores by fibrosis stage according to a previous biopsy study of NAFLD patients in which the median dAAR score was 0.92 in fibrosis stage 0-1, 1.28 in stage 2, 1.79 in stage 3 and 2.43 in stage 4.[30] Data were analysed using R software version 3.6.1.

3 | RESULTS

3.1 | Clinical characteristics

The total cohort consisted of 10 993 individuals (6707 men and 4286 women) with mean age 54.3 ± 12.6 years and mean BMI 31.6 ± 4.4 kg/m^2. Baseline demographics are demonstrated in Table 1 and the numbers of missing baseline data in Table S2. Mean follow-up was 11.7 ± 6.1 years (median 12.8 years, range 0.0-23.0 years, 128 069 person-years of follow-up).

A total of 124 liver-related outcomes occurred during the follow-up. Of these 124, 52 (42%) were diagnosed as alcohol-related liver disease in the registries, with the proportion categorized as alcohol-related decreasing with increasing age from 40%-100% among those under 50 years of age to 0%-28% among those over 60 (Figure S2). Furthermore, 1606 incident CVD events (1110 men, 496 women), 1171 cancers (750 men, 421 women) and 161 CKD cases (94 men, 67 women) were diagnosed during the follow-up, along with 1832 deaths (1218 men, 614 women).

3.2 | Incidence of liver-related outcomes in NAFLD by age and gender

The crude incidence rate of liver-related outcomes in NAFLD was 0.97 per 1000 person-years. Incidence rates per sex and age group are shown in Table S3. The cumulative incidences of liver-related outcomes and non-liver death in NAFLD by age and sex are shown in Figure 1. Corresponding results for the reference population without NAFLD are shown for comparison (Figure 1B,C). Men with NAFLD had a higher risk of liver-related outcomes compared with women with NAFLD; the cumulative incidence increased with age and, after 20 years of follow-up, it was 2.4% and 1.5% in men and women aged 60 years at baseline, respectively (Figure 1B,C).

3.3 | Cumulative incidence of extra-hepatic outcomes

When analysing the cumulative incidence of extra-hepatic events in NAFLD, the highest incidence was for CVD compared with cancer and CKD (Figure 2A). Importantly, we found that the relative risk of CVD and cancer was many times higher than the risk of advanced liver disease in the NAFLD population (Figure 2B). The cumulative 20-year incidence of CVD, cancer and CKD increased with age. For

TABLE 1 Baseline characteristics of the study population

	All subjects (mean ± SD) (n = 10 993)
Age (years)	54.3 ± 12.6
Sex male/female (%)	6707 (61) / 4286 (39)
BMI	
Education (%)	
Low	4204 (39)
Average	3585 (33)
High	3045 (28)
Employment (%)	
Part-or full time employed	5610 (52)
Other	1104 (10)
Retired	4168 (38)
Marital status (%)	
Married/partnership	8050 (73)
Single	1204 (11)
Widow, separated, divorced	1722 (16)
BMI (kg/m^2)	31.6 ± 4.4
Waist circumference (cm)	104.8 ± 10.1
Type 2 diabetes (%)	1639 (15)
Systolic blood pressure (mm Hg)	142.0 ± 19.5
Diastolic blood pressure (mm Hg)	85.3 ± 11.0
Alcohol use (g/week)	45 ± 54
Alcohol user	8250 (87)
Life-time abstainer	1199 (12)
Current abstainer	715 (7)
Alcohol user	8254 (81)
Binge drinking (≥5 drinks per day) (%)	
Less often	3932 (78)
Monthly	731 (15)
Weekly	360 (7)
Frequency of Intoxication (%) episodes	
Less often	4976 (74)
Monthly	1351 (20)
Weekly or more often	377 (6)
Smoking (%)	
Current	2411 (22)
Former	3213 (30)
Never	5278 (48)
Number of cigarettes per day	3.5 ± 7.9
Exercise for 20-30 min (%)	
At least 2 times a week	4800 (53)
2-4 times a month	2479 (27)
Less often	1824 (20)
Number of the components of metabolic syndrome (%)	
0	35 (0.3)

(Continues)

TABLE 1 (Continued)

	All subjects (mean ± SD) (n = 10 993)
1	442 (4)
2	2834 (26)
3	3924 (36)
4	2787 (25)
5	971 (9)
P-Total cholesterol (mmol/L)	5.8 ± 1.1
P-LDL cholesterol (mmol/L)	3.6 ± 1.0
P-HDL cholesterol (mmol/L)	1.2 ± 0.3
P-Triglycerides (mmol/L)	2.2 ± 1.3
P-ALT (U/L)	35.6 ± 24.4
P-AST (U/L)	31.1 ± 15.5
P-GGT (U/L)	51.0 ± 55.4
FLI	80.52 ± 11.57
dAAR	0.47 ± 1.21
PRS-5	0.22 (0.19)
PRS-5	
Low	8043 (91)
High	823 (9)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase, BMI, body mass index; dAAR, dynamic ALT-AST-ratio; FLI, fatty liver index; GGT, g-glutamyltransferase; HDL, high-density lipoprotein; PRS-5, genetic risk score.

Components of metabolic syndrome: waist circumference >100 cm for men, >90 cm for women; triglycerides >1.7 mmol/L; HDL cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women; blood pressure ≥130/85 mm Hg; and fasting glucose ≥5.6 mmol/L.

example, among men and women aged 60 years, the 20-year cumulative incidence of CVD events was 38% and 23%, for cancer 22% and 18% and for CKD 4% and 3%, respectively. Therefore, the corresponding 20-year cumulative incidence of CVD in NAFLD was 16 and 15-times higher, for cancer 9 and 12-times higher and for CKD 1.5 and 2.1-times higher compared with the cumulative incidence of liver-related outcomes in NAFLD (Figure 1).

3.4 | Cumulative incidence of liver-related outcomes according to genetic risk

The 20-year cumulative incidence of liver-related outcomes was 4.3% in the high PRS-5 group and 1.5% in the low PRS-5 group (P <.001) (Figure 3). The corresponding 20-year incidence of non-liver death was 26.9% in the high-PRS-5 group and 25.4% in the low-PRS-5 group (P =.41). The 20-year age-adjusted cumulative incidence of liver-related outcomes modelled over the range of PRS-5 in NAFLD and non-NAFLD controls by sex is shown in Figure 3B.

3.5 | Factors associated with liver-related outcomes

Variables associated with liver-related outcomes in Fine and Gray competing-risk regression analyses adjusted for age, sex, cohort, BMI, diabetes and number of metabolic risk factors are presented in Table 2. Being unemployed (subdistribution hazard ratio [SHR] 2.24), weekly alcohol use (SHR 1.53), weekly and monthly binge drinking (SHR 3.91 and 4.56, respectively), weekly and monthly intoxication episodes (SHR 3.56 and 2.00, respectively), current smoking (SHR 2.04), smoked cigarettes per day (SHR 1.27), alanine aminotransferase (ALT) (SHR 1.22), aspartate aminotransferase (AST) (SHR 1.18), GGT (SHR 1.28) and PRS-5 (SHR 1.37) were associated with liver-related outcomes.

Regarding the competing risk of non-liver death, average and high education level (SHR 0.83 and 0.75, respectively), being unemployed or retired (SHR 1.46 and 1.67, respectively), being single or widow/separated/divorced (SHR 1.62 and 1.39, respectively), monthly intoxication episodes (SHR 1.34), former or current smoking (SHR 1.22 and 2.22, respectively), cigarettes per day (SHR 1.31), low amount of exercise (SHR 1.45) and GGT (SHR 1.08).

3.6 | Cumulative incidence of liver-related and non-liver-related outcomes based on the dAAR fibrosis score

Data were available to calculate the dAAR fibrosis score[30] in 5133 individuals with NAFLD (ie those participating in the FINRISK 2002-2012 studies). These individuals experienced 35 liver-related outcomes during the follow-up. As expected, the 20-year age-adjusted cumulative incidence of liver-related outcomes increased with higher baseline dAAR scores (SHR 2.49, 95% CI 1.82-3.42, P <.001) (Figure 4). The dAAR score had no effect on the competing risk of non-liver death SHR 1.01, 95% CI 0.91-1.12, P =.85) (Figure 4).

There were 347 incident cases of CVD, 293 cancers and 29 cases of CKD. The dAAR score did not have any significant effect on the age-adjusted risk of incident CVD (SHR 1.00, 95% CI 0.91-1.09, P =.99), cancer (SHR 0.96, 95% CI 0.86-1.07, P =.49), or CKD (SHR 1.24, 95% CI 0.84-1.84, P =.28).

4 | DISCUSSION

In this study, the incidence of clinical liver outcomes in NAFLD was ~1% during a mean 12-year follow-up, which demonstrates that the risk of end-stage liver disease in individuals with NAFLD is small at the population level. The cumulative risk of liver-related outcomes was higher in men than women, and the risk was 2.4% and 1.5% in 60-year-old men and women, respectively, during 20 years of follow-up. Importantly, our population-level findings demonstrate that the risk of CVD and cancer is many times higher than that of liver-related outcomes.

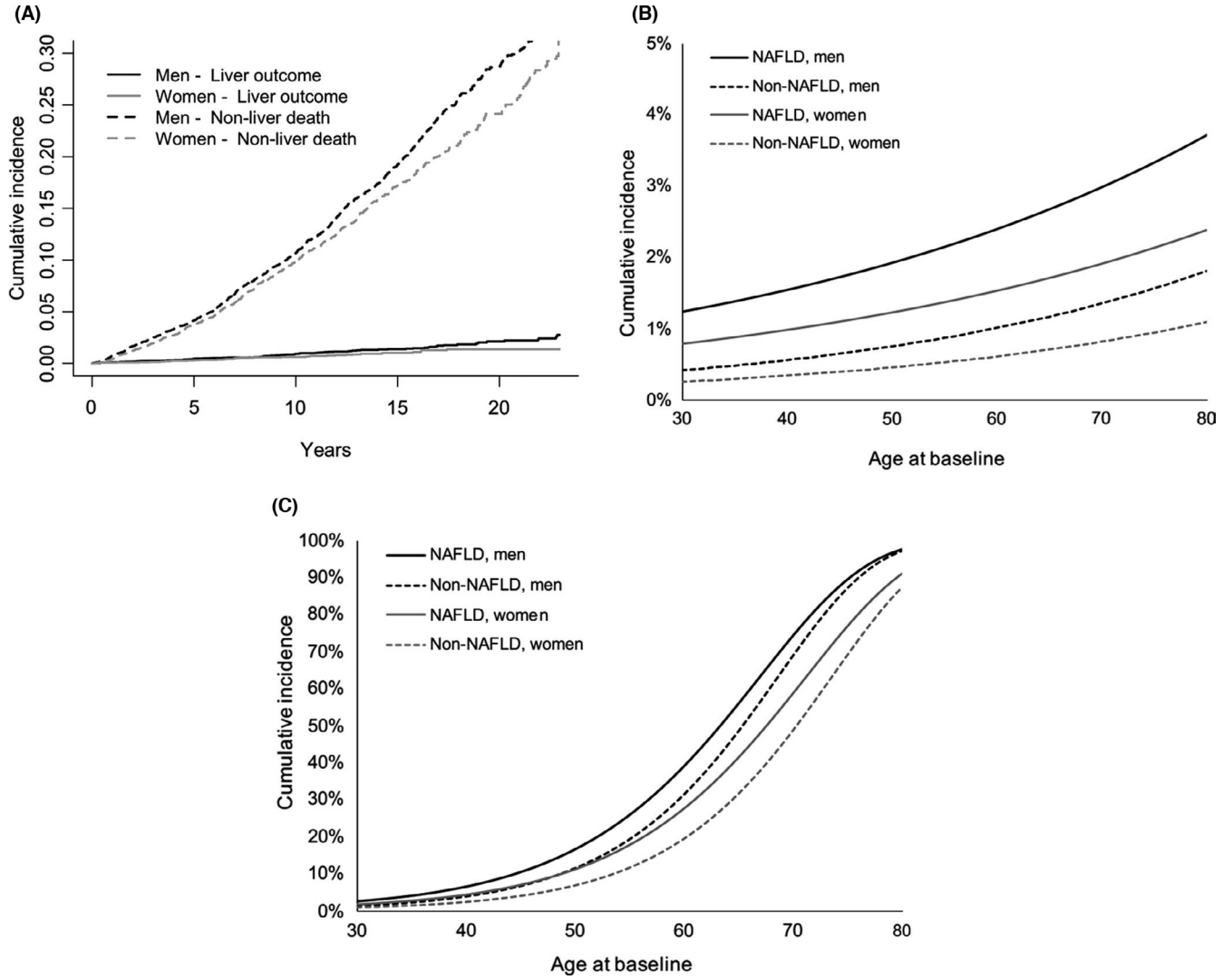

FIGURE 1 Cumulative incidence of liver-related outcomes and non-liver death in non-alcoholic fatty liver disease (NAFLD) by age and sex. (A) Cumulative incidence of liver-related outcomes and non-liver death in NAFLD (cumulative incidence function); (B) 20-year cumulative incidence of liver-related outcomes in NAFLD and non-NAFLD controls modelled over age, separately by sex and adjusted for cohort using the Fine and Gray competing-risk method; (C) 20-year cumulative incidence of non-liver deaths in NAFLD and non-NAFLD controls modelled over age, separately by sex and adjusted for cohort using the Fine and Gray competing-risk method

Previous research has shown that CVD and cancer are the most common causes of death in individuals with NAFLD,[6] and only a small number of individuals with NAFLD will die from liver disease. The incidence of severe liver disease, defined as cirrhosis, liver decompensation/failure or HCC in those with biopsy-proven NAFLD has been reported to be 12%.[32] Angulo et al have presented two international multicentre biopsy studies on the prevalence and risk of advanced NAFLD. In the first study, they analysed 320 NAFLD patients with a median follow-up of 8.7 years. In this cohort, 14% developed liver-related events and 13% died or underwent liver transplantation.[33] Their other study comprised 619 NAFLD patients with a median follow-up of 12.6 years; only 4.2% developed liver-related events.[12] These were hospital-based cohorts of strictly selected patients, which probably partly explains the higher incidence of advanced NAFLD compared with our findings including also less severe outcomes at the population level. However, though our study population is representative of the general population, there is some selection regarding older individuals, as persons with greater illness are more likely to not attend a health survey. Thus, the actual risk of liver-related (and non-liver-related) outcomes may be slightly higher at the population level.

The prognosis of NAFLD has also been evaluated in population-based and registry studies, which have associated NAFLD with advanced fibrosis as an independent predictor of mortality.[15] In the NHANES III cohort of 13 298 individuals with a mean age of 49 years, the incidence of death from liver disease during a median follow-up of 23 years was 1.1%, and both an intermediate to high NAFLD liver fat score and high NAFLD fibrosis score were associated with liver mortality.[34] Recently, a large cohort study (271 906 patients, mean age 55 years) of NAFLD patients from the Veterans Healthcare Administration in the USA reported that 8.4% developed cirrhosis and 0.09% HCC during mean 9 years of follow-up. In this study, the NAFLD diagnosis was primarily based on two or more elevated ALT

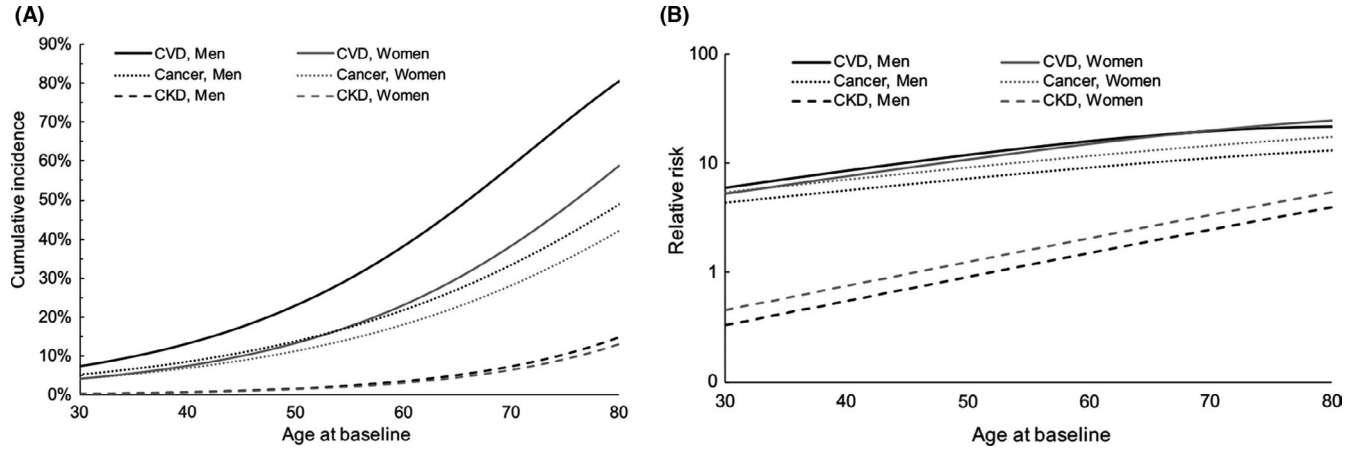

FIGURE 2 Cumulative incidence of cardiovascular events (CVD), cancer and chronic kidney disease (CKD). (A) 20-year cumulative incidence modelled over age, separately by sex and adjusted for cohort using the Fine and Gray competing-risk method. (B) Relative 20-year risk of the various outcomes compared with the 20-year risk of liver-related outcome

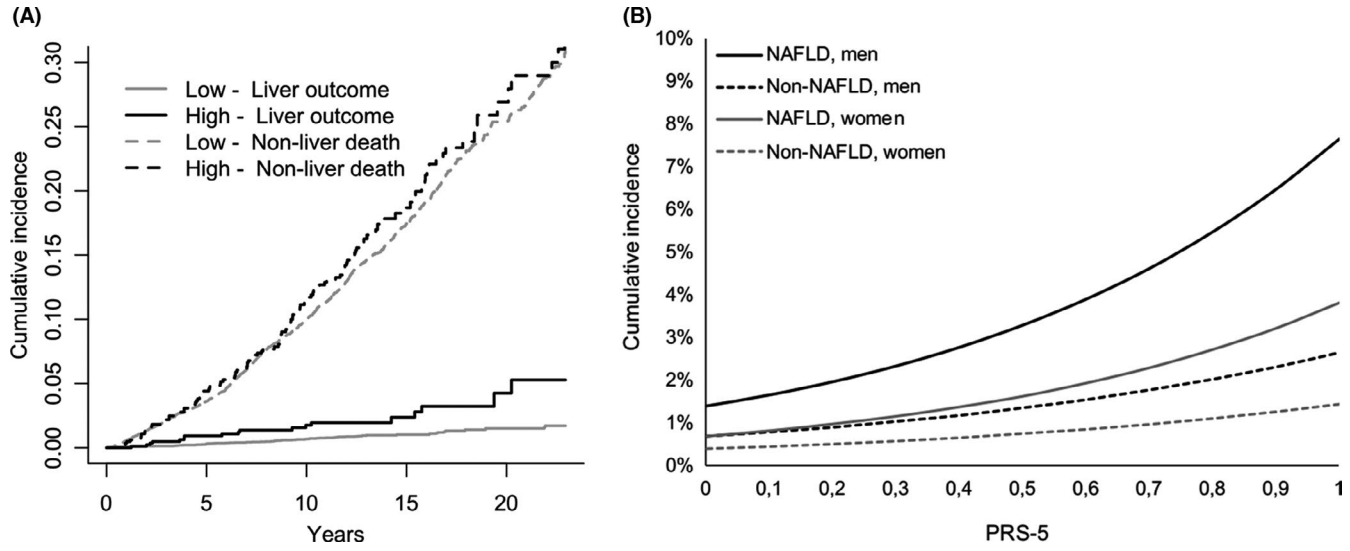

FIGURE 3 Cumulative incidence of liver-related outcomes and non-liver death in NAFLD according to the polygenic risk score (PRS-5). (A) Cumulative incidence in NAFLD according to high and low PRS-5 (cutoff 0.495) by the competing-risk cumulative incidence function; (B) 20-year cumulative incidence of liver-related outcomes in NAFLD and non-NAFLD controls modelled over the spectrum of genetic risk (PRS-5), separately by sex and adjusted for age and cohort using the Fine and Gray competing-risk method

values and excluding other liver diseases.[18] The incidence of 8% for liver cirrhosis with only 9 years of follow-up is high compared with our results. However, there was no information about smoking status or baseline fibrosis available in the cohort of veterans.[35] Thus, the results of that study cannot be directly compared with our results. In addition, Kanwal et al defined NAFLD as elevated transaminases measured at least twice.[18] However, the majority of those with NAFLD have normal transaminases. Thus, individuals in Kanwal et al's study possibly had more disease activity than our study population.

Importantly, we were able to estimate liver fibrosis at baseline using the dAAR score[30] in a subpopulation of the study cohort. As expected, the risk of liver-related endpoints was higher in those with more fibrosis in the liver (Figure 4).[13]

CVD is the most frequent outcome of NAFLD,[6] and it was the most common endpoint in the present study. In 60-year-old men and women, the risk of CVD was 15-16-fold higher than the risk of liver-related outcomes, highlighting that CVD is the major cause of morbidity and mortality in individuals with NAFLD.

The other major outcome in individuals with NAFLD is cancer,[32] and the risk is much higher than in the general population.[36] We found that the risk of cancer for men and women with NAFLD is 9-12-fold higher than the risk of liver-related outcomes. A major part of the excessive risk of extra-hepatic cancers in NAFLD is explained by metabolic syndrome. However, this is not the case for breast cancer, in which NAFLD is thought to have an equal effect as obesity on cancer development.[37] The risk of CKD in this population was lower than previously described in the general population,[38] though NAFLD is an independent risk factor for CKD.[7] However, we included only individuals who had been hospitalized for CKD, which limits the outcome to those with severe CKD.

TABLE 2 Predictors of liver-related outcome and non-liver death in subjects with baseline NAFLD by Fine and Gray competing-risk regression. All subdistribution hazard ratios (SHRs) are adjusted for age, sex, cohort, body mass index, type 2 diabetes and number of metabolic risk factors (0-5). For continuous variables SHRs are shown per 1 SD

	Liver-related outcomes			Non-liver death		
	SHR	95% CI	*P* value	SHR	95% CI	*P* value
Education						
Low						
Average	1.15	0.76-1.73	.510	.83	.74-0.93	<.001
High	0.79	0.49-1.26	.320	.75	.66-0.85	<.001
Employment						
Employed						
Other	2.24	1.29-3.91	.004	1.46	1.17-1.83	<.001
Retired	1.57	0.97-2.56	.069	1.67	1.44-1.94	<.001
Marital status						
Married/partnership						
Single	1.74	1.03-2.95	.040	1.62	1.38-1.90	<.001
Widow, separated, divorced	1.87	1.14-3.05	.013	1.39	1.23-1.57	<.001
Alcohol use						
Current user						
Current abstainer	1.83	0.77-4.32	.170	1.19	.98-1.45	.080
Lifetime abstainer	1.28	0.67-2.43	.460	0.92	.80-1.06	.250
Alcohol use (grams per week)	1.53	1.34-1.75	<.001	0.99	.93-1.05	.690
Binge drinking (≥5 drinks/day)						
Less often						
Monthly	4.56	2.19-9.51	<.001	1.29	.98-1.71	.070
Weekly	3.91	1.52-10.05	<.001	1.39	.96-2.00	.081
Intoxication episodes						
Less often						
Monthly	2.00	1.20-3.34	.008	1.34	1.15-1.57	<.001
Weekly	3.56	1.82-6.98	<.001	1.26	.94-1.68	.120
Smoking						
Never						
Current	2.04	1.30-3.21	.002	2.22	1.95-2.54	<.001
Former smoker	0.70	0.70-1.80	.630	1.22	1.09-1.80	<.001
Cigarettes per day	1.27	1.14-1.43	<.001	1.31	1.26-1.37	<.001
Exercise for 20-30 min						
At least 2 times/week						
2-4 times/month	1.10	0.72-1.68	.670	1.00	.88-1.13	.990
Less often	0.90	0.55-1.47	.660	1.45	1.29-1.62	<.001
Alanine aminotransferase (U/L)	1.22	1.12-1.32	<.001	1.00	.87-1.14	.970
Aspartate aminotransferase (U/L)	1.18	1.11-1.26	<.001	1.00	.87-1.15	.960
Gamma-glutamyltransferase (U/L)	1.28	1.22-1.33	<.001	1.08	1.05-1.12	<.001
PRS-5	1.37	1.14-1.66	<.001	1.01	.96-1.07	.610

Abbreviation: PRS-5, polygenic risk score.

Liver fibrosis at baseline did not affect non-liver outcomes during follow-up in the present study. In biopsy cohorts, even mild fibrosis has been linked to overall death, and significant fibrosis with CVD.[13] In addition, liver fibrosis evaluated by transient elastography has been reported as an independent risk factor for stroke,[39] and heart failure, atrial fibrillation and coronary artery disease have

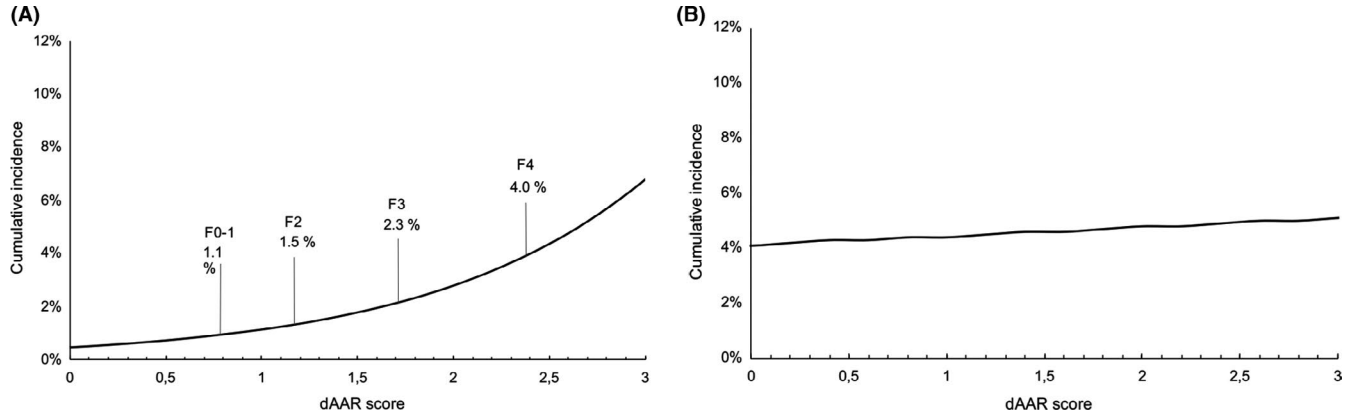

FIGURE 4 Age-adjusted 20-year cumulative incidence of (A) liver-related outcomes and (B) non-liver death based on the dynamic ALT-AST-ratio (dAAR) score

been associated with fibrosis on magnetic resonance imaging.[40] In this study, the majority of patients had only mild fibrosis at baseline, which may be the reason that we could not find the association between liver fibrosis and the endpoints.

4.1 | Risk factors for liver-related outcomes

In competing-risk regression analysis unemployment, marital status, alcohol use, smoking, liver enzymes and PRS-5 were associated with liver-related outcomes. Unemployment and marital status have been associated with many diseases including cardiovascular disease and type 2 diabetes. Many unhealthy habits such as diet might explain that finding, although the causality has not been convincingly proven.[41,42]

Alcohol use as a weekly amount and binge drinking, even once per month, and intoxication episodes were associated with liver-related outcomes, although the alcohol use was within the limits fulfilling current NAFLD criteria. This is not surprising as most people with NAFLD also consume at least some amount of alcohol,[25] and obesity and alcohol are known to have synergistic effects on the risk of liver disease. Thus, even light alcohol use in patients with obesity/NAFLD can significantly increase the risk of advanced liver disease.[43] Interestingly, in most young individuals (age <50 years) in our study, the liver outcome was recorded as alcohol-related. This underlines the fact that NAFLD is usually a slowly progressing disease,[44] but alcohol use may considerably aggravate disease progression.[19,43]

Former and current cigarette smoking were associated with liver-related outcomes in line with previous findings that smoking is associated with liver fibrosis in those with NAFLD.[45] Liver enzymes were linked to liver-related outcomes suggesting that these enzymes may reflect disease activity and risk of disease progression. Similar associations have previously been reported especially with GGT.[46]

PRS-5 associated positively with liver-related outcomes in this study. Genetic risk factors play an important role as risk factors for NAFLD and disease progression. The PRS-5 used in this study is a genetic risk score based on the five most common genetic risk factors of NAFLD. It has been shown that PRS (without *HSD17B13*) strongly associated with hepatic fat content and that the association of genetic risk variants on fibrosis was proportional to hepatic fat content.[9] Recently, an updated PRS-5 was associated with the risk of HCC in individuals with NAFLD.[8] We confirm that the PRS-5 predicts clinical liver outcomes in metabolic NAFLD, and our results further support the use of genetic risk factors in prediction of liver outcomes in NAFLD at the population level.

Factors associated with non-liver death were quite similar to those associated with liver-related outcomes. However, ALT and AST were not associated with non-liver death, while GGT was. GGT has been reported to reflect overall oxidative stress in the body, and thus the association with non-liver death is not surprising.[47] In addition, education level, unemployment and marital status and amount of exercise were associated with non-liver death. These have also previously been linked to mortality.[42,48]

4.2 | Strengths and limitations

A major strength of this study is that we used large, national, population-based study cohorts with a follow-up of 128 069 person-years. In addition, we reliably collected liver- and non-liver-related endpoints from national registries, which have virtually 100% coverage and negligible losses to follow-up. In addition, we strengthened our study of fibrosis by using the recently developed dAAR score. Although, the number of endpoints was rather low in this sub-cohort, this index has been reported to increase with increasing histological fibrosis stage and it has been shown to perform equally as well or better than the FIB-4, APRI and NAFLD fibrosis score for detecting advanced fibrosis in NAFLD.[30]

A limitation of this study is that we used FLI as a surrogate marker of NAFLD and the dAAR score as a surrogate for fibrosis. The FLI does not have perfect accuracy to distinguish those with NAFLD. However, FLI is a widely used index for NAFLD in population studies, and the positive predictive value of FLI for hepatic steatosis is 99%.[49] In addition, based on the variables in the FLI, we

can specifically determine which individuals have possible metabolic liver disease, which is the most common form of fatty liver disease.[50]

5 | CONCLUSION

The risk of liver-related outcomes is low even in the presence of mild fibrosis in individuals with NAFLD in the general population.

ACKNOWLEDGEMENT

FINRISK and Health 2000 data used for the research were obtained from THL Biobank. We thank all study participants for their generous participation at THL Biobank and the FINRISK and Health 2000 studies.

AUTHOR CONTRIBUTIONS

VM: study design, interpretation of results, writing the manuscript; VS: study design, data collection, interpretation of results, critical revision of manuscript; MF: interpretation of results, critical revision of the manuscript; AJ, SM, IE, JS, AL, MP: study design, data collection, interpretation of results, critical revision of manuscript; FÅ: study design, statistical analyses, interpretation of results, writing the manuscript. All authors revised and approved the manuscript.

DATA AVAILABILITY STATEMENT

FINRISK and Health 2000 data are available from the THL biobank based on a written application as explained on the website of the THL biobank (https://thl.fi/en/web/thl-biobank/for-researchers).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Männistö VT, Salomaa V, Färkkilä M, et al. Incidence of liver-related morbidity and mortality in a population cohort of non-alcoholic fatty liver disease. *Liver Int*. 2021;41:2590–2600. https://doi.org/10.1111/liv.15004