Surrogate scores of advanced fibrosis in NAFLD/NASH do not predict mortality in patients with medium-to-high cardiovascular risk

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Abstract

Background: Untreated NAFLD may have significant consequences including an increase in mortality and cardiovascular injury. Thus, early detection of NAFLD is currently believed not only to prevent liver related but also cardiovascular mortality. However, almost nothing is known about co-existing NAFLD in patients with coronary artery disease (CAD).

Aims: We investigated the impact of surrogates scores of fibrosis in NAFLD in a large cohort of patients referred to coronary angiography.

Results: Modelling the common NALFD and fibrosis scores FIB-4 and NFS as splines revealed significant associations with all-cause and cardiovascular mortality when Cox regression models were only adjusted for cardiovascular risk factors that were not already included in the calculation of the scores. Stratifying the scores into quartiles yielded hazard ratios (95% CI) for all-cause and cardiovascular mortality for the 4^{th} quartile vs the 1^{st} quartile of 2.28 (1.90-2.75) and 2.11 (1.67-2.67) for FIB-4 and of 3.21 (2.61-3.94) and 3.12 (2.41-4.04) for NFS.

However, we did not observe an independent association of FIB-4 or NFS with overall or cardiovascular mortality in our prospective CAD cohort after full adjustment for all cardiovascular risk factors (all-cause mortality HR 1.13 (0.904-1.41) and 1.17 (0.903-1.52); cardiovascular mortality HR 1.06 (0.8-1.41) and 1.02 (0.738-1.41). Thus, neither FIB-4 nor NFS, as surrogate markers for NAFLD/NASH, were independent risk factors for overall or cardiovascular mortality in patients with CAD.

Conclusion: Our data shows that surrogate risk scores for NAFLD-related fibrosis do not add information in assessing the CVD events in patients with CAD proven by angiography.

New & Noteworthy

We investigated the impact of NAFLD surrogate markers in a large cohort of patients that had been referred to coronary angiography. In contrast to a repeatedly demonstrated increased link of cardiovascular events in NALFD patients, we demonstrated that NAFLD surrogate markers were not independent risk factors for overall or cardiovascular mortality in patients with CAD. Thus, these markers may not be useful for primary prevention of cardiovascular events in patients with CAD.

Key words: NAFLD, NASH, CAD, cardiovascular, heart disease, fatty liver, risk score

Introduction

Liver diseases are a growing public health problem in many countries (34). Fatty liver disease alone is estimated to be prevalent in approximately 30% of the adult population across Europe but also in many other regions worldwide, e.g. the USA, Middle East, or South East Asia (35). In many cases, elevated liver enzymes, often detected coincidentally, may be first signs of a developing fatty liver disease. However, they are often overlooked as structured surveillance programs are not yet established in any country and awareness of the risk of chronic liver disease is mostly unsatisfactory anywhere in the world (8, 13, 14).

Untreated NAFLD may have significant consequences as developing liver fibrosis is the main determinant for a gradual increase in mortality (11) (25). However, fatty liver disease by itself also marks a significantly increased risk for cardiovascular injury. The mortality rate of NAFLD patients from myocardial infarction but also risk from non-fatal cardiovascular events is higher compared to the general population (4). Particularly women were demonstrated to suffer at an average ten years earlier from cardiovascular events (2). In addition, previous studies also showed a consistent association of fatty liver disease with an increased risk of subclinical atherosclerosis and subsequent functional restrictions (1, 15, 18). Thus, early detection of NAFLD may be essential not only to prevent liver related but also cardiovascular mortality.

Given the high prevalence of the disease, screening and surveillance for NAFLD must focus on high risk populations. CAD, as one of the major complications of the metabolic syndrome, may obviously identify a potential risk population. However, the relevance of co-existing NAFLD in patients with proven CAD remains widely unknown. We therefore aimed to investigate the role of NAFLD in CAD patients, especially in CV high-risk patients proven by coronary angiography using data from the large prospective LURIC (Ludwigshafen Risk and Cardiovascular Health) study.

Since selection of patients potentially at risk for NAFLD must in clinical routine be performed by primary care physicians, we therefore investigated the most commonly used scores i.e. fatty liver index (FLI, (6)), fibrosis-4 index (FIB-4, (26)), AST to platelet ratio index (APRI, (32)), and NAFLD fibrosis score (NFS, (3)) for their predictive value.

Methods

Subjects

The LURIC study recruited 3316 Caucasians referred for coronary angiography between 1997 and 2000 at the Ludwigshafen Heart Center in South-West Germany (33). Clinical indications for angiography were chest pain or a positive non-invasive stress test suggestive of myocardial ischemia. Individuals suffering from acute illnesses other than acute coronary syndrome, chronic non-cardiac diseases and a history of malignancy within the past five years were excluded. The ethics committee of the "Landesärztekammer Rheinland-Pfalz" approved the study (837.255.97 (1394)) that was conducted in accordance with the "Declaration of Helsinki". Informed written consent was obtained from all participants.

Definition of scores of liver function

We calculated four different scores to estimate liver function. The "fatty liver index" (FLI) was calculated according to Bedogni et al. (6), the FIB-4 score was calculated according to Sterling et al. (26), the "AST to platelet ratio index" (APRI) was calculated according to Wai et al. (32), and the "NAFLD fibrosis score" (NFS) was calculated according to Angulo et al. (3).

In accordance with recent expert reviews recommending FIB-4 score ≥1.3 to be used for further assessment and linkage to specialty care where additional technology to assess liver stiffness or serum fibrosis test will be available (22, 24, 36), we chose cut-off values of 1.3 and 2.67 for severity classification of liver fibrosis (none, mild, severe). These cutoffs also allowed data comparison to Chen et al. (10).

For the NFS a cut off of -1.455 was chosen to distinguish between low NFS (<= -1.455 and high (>= -1.455) probability of NAFLD-related advanced fibrosis as

158 described and validated previously (27).

159 Complete data for all scores was available for 2882 study participants. We further
160 excluded 14 study participants due to cirrhosis and/or dialysis and 12 participants
161 with implausibly low platelet values leaving a final sample size of 2856 participants.
162 The distribution of the non-invasive surrogate scores in LURIC is shown in
163 **Supplemental Figure 1**. The correlation between the different scores is shown in
164 **Supplemental Figure 2.**

165

166 *Assessment of alcohol consumption*

167 Data on alcohol consumption was obtained from the baseline questionnaire on
168 drinking habits of the LURIC study. Stated alcohol consumption was converted into g
169 of ethanol / day.

170 Potential liver damage due to alcohol was estimated using the alcoholic liver disease
171 to NAFLD index (ANI), a model specifically developed to predict the probability that
172 steatohepatitis is due to alcoholic liver disease. The model is based upon
173 aminotransferase levels, mean corpuscular volume (MCV), body mass index (BMI),
174 and sex: ANI = -58.5 + 0.637 (MCV) + 3.91 (AST/ALT) – 0.406 (BMI) + 6.35 for men
175 (12).

176

177 *Definition of clinical variables and endpoints*

178 The presence of a visible luminal narrowing (>20% stenosis) in at least one of 15
179 coronary segments was used to define coronary artery disease (CAD) according to
180 the classification of the American Heart Association. Diabetes mellitus was defined
181 according to guidelines of the American Diabetes Association as increased fasting
182 (≥126 mg/dl) and/or post-challenge (2 h after the 75 g glucose load >200 mg/dl)
183 glucose and/or elevated glycated haemoglobin (>6.5%) and/or history of diabetes.

Hypertension was defined as a systolic and/or diastolic blood pressure ≥140 and/or ≥90 mm Hg or a history of hypertension. The glomerular filtration rate was estimated by using the 2012 CKD-EPI eGFRcreat-cys equation (16).

Information on vital status was obtained from local registries. Death certificates, medical records of local hospitals, and autopsy data were reviewed independently by two experienced clinicians who were blinded to patient characteristics and who classified the causes of death. In cases of disagreement or uncertainty concerning the coding of a specific cause of death the decision was made by a principal investigator (W.M.).

849 (29.7%) participants died during a median follow-up of 9.8 years (range 0.1-11.3 years). Cardiovascular mortality (n=477) included the following categories: sudden cardiac death (n=229, 9.2%), fatal myocardial infarction (n=88, 3.5%), death due to congestive heart failure (n=127, 5.1%), death after intervention to treat coronary artery disease (n=20, 0.8%), fatal stroke (n=56, 2.0%), and other causes of death due to CAD (n=13, 0.5%). Non-cardiovascular death included fatal infection (n=61), fatal cancer (n=122) and other causes (n=117).

Information for vital status is complete for all participants but the cause of death of 16 deceased was unknown and these patients were included in calculations of all-cause mortality but not in calculations considering different causes of death.

Statistical analyses

The distribution of continuous variables was examined by visual inspection of histograms and density plots as well as by comparing mean and median values. When normally distributed, continuous data are presented as the mean and standard deviation (SD). Non-normally distributed variables are presented as the median and 25th and 75th percentile. Categorical data are presented as percentages. Statistical

differences between groups and continuous variables were determined using ANOVA. Non-normally distributed variables were log-transformed before entering analysis. The chi-square test was used for categorical variables. Correlations between the non-invasive surrogate scores were assessed by Spearman's rho. Cox proportional hazard models were built to assess the effect of the surrogate scores on all-cause mortality and cardiovascular mortality. The proportional hazard assumption was checked by examination of scaled Schoenfeld residuals. For the generation of hazard ratio plots the non-invasive surrogate scores were modelled as restricted cubic splines 0,1.

All tests were two-sided and a p value < 0.05 was considered statistically significant. All analyses were carried out using R v4.0.2 (http://www.r-project.org) and IBM SPSS v25. Hazard ratio plots were drawn using the R-package 'rms' (v6.0-0). Harrell's C was calculated using the R-package 'hmisc' (v4.4-0), ROC curves were calculated and compared using the method of Delong as implemented in the R package 'pROC' (v1.16-2) and the NRI for censored survival data was calculated using the R-package 'nricens' (v1.6). The correlation plot was drawn using the R package 'correlation' (v0.2-1). The optimal cut-off for prediction models was calculated using the R package 'OptimalCutpoints' (v.1.1-4).

Results

Study demographics

After a follow-up time of 9.8 years (median) 2007 study participants were still alive while 849 had died.

Deceased study participants were older ($p<0.001$), more often male ($p=0.011$) and suffered more often from coronary artery disease (CAD, $p<0.001$), diabetes mellitus ($p<0.001$) and hypertension ($p<0.001$) (**Table 1**). In contrast, main determinants of the metabolic syndrome such as BMI ($p<0.001$), LDL cholesterol ($p=0.001$), HDL cholesterol ($p<0.001$), and blood pressure ($p<0.001$) showed only slight differences, which however must be considered statistically significant except for triglycerides (TG, $p=0.493$). Regarding the algorithms developed to estimate liver function, highest significance was obtained for FIB-4 and NFS scores that were higher in the group of deceased study participants.

Association of NAFLD and liver fibrosis scores with all-cause mortality

We stratified our cohort according to quartiles of the different scores and examined their association with all-cause and cardiovascular mortality (**Figure 1, Supplemental Figure 3**). There was a highly significant direct association of higher quartiles of FIB-4 and NFS with higher cardiovascular and all-cause mortality.

We also calculated hazard ratio plots modelling the scores as restricted cubic splines (**Supplemental Figures 3+4**). For all-cause mortality, we observed significant associations for all scores. However, for cardiovascular mortality only the FIB-4 and the NFS showed a steadily increasing association with higher risk.

To better define the causes of the higher mortality we next examined a number of clinical and biochemical markers according to the quartiles of our non-invasive surrogate scores. For FIB-4 we noticed a highly significant direct association with

self-reported alcohol intake and the alcoholic liver disease to NAFLD index (ANI, p<0.001) (**Table 2a**). This was not the case for the NFS in which there was no association at all with alcohol intake and the ANI (**Table 2b**, p=0.617). High-sensitive CRP, a marker of inflammation, increased with increasing NFS (p<0.001) but not with increasing FIB-4 (p=0.212). Both markers have in common increasing levels of NT-proBNP (p<0.001) and higher percentages of participants suffering from chronic disease (CAD, T2DM, hypertension, all p<0.001). Results for the other liver damage scores are presented in **Supplemental tables 1-2**.

Performance of non-invasive surrogate scores in risk prediction models

We next sought to examine whether the surrogate scores could serve as independent predictors of mortality by adjusting the analyses for conventional cardiovascular risk factors (age, sex, BMI, hypertension, diabetes mellitus, smoking, LDL-C, HDL-C) as well as alcohol intake and medication (aspirin, coumarins, diuretics, beta blocker, ACE inhibitors, AT2 receptor blockers, oral antidiabetic drugs, cortisol, anti-gout medication, statins). **Table 4** shows the results of the adjusted analyses. Adjusting the non-invasive surrogate scores for all confounders neither FIB-4 nor NFS remained significantly associated with all-cause or cardiovascular mortality. However, the highest quartile of the FLI (p=0.015) did show significant associations with mortality, at least all-cause mortality. Hazard ratio plots for all scores with full adjustment for cardiovascular confounders are shown in the Supplement (**Supplemental Figures 6+7**). In contrast, when adjusting the Cox regression models only for variables that had not already been used for the calculation of the scores, higher quartiles of FIB-4 (p<0.001) and NFS (p<0.001) were significantly associated with both all-cause and cardiovascular mortality (**Table 3**).

We also analysed the subgroup of LURIC participants with angiographically proven

CAD at baseline. In multivariate adjusted analysis no significant association with mortality was observed (**Supplemental Table 3**).

Combination of FIB-4 and NFS with cardiovascular risk scores

We investigated whether the addition of the surrogate scores to established risk prediction algorithms for persons with or without a clinical history of cardiovascular disease would improve risk prediction. Therefore, we selected a subgroup of patients that had not experienced myocardial infarction or stroke at study baseline (n = 1231) and tested both the ESC heart score and the Pooled Cohort Equation (PCE) with and without non-invasive surrogate scores **(suppl. table 4)**. The inclusion of FIB-4 only marginally increased the conventional AUC and Harrell's C for the endpoint all-cause mortality (PCE+FIB-4: p=0.468, ESC+FIB-4: 0.128). The inclusion of NFS did not lead to any improvement in risk prediction (PCE+NFS: p=0.995, ESC+NFS: 0.688). We additionally calculated the net-reclassification-index (NRI) and found no significant improvement, neither for FIB-4 nor for NFS.

In a subgroup of patients with stable coronary artery disease (n = 1232) we tested the incremental predictive value of the surrogate scores in comparison with the Marshner score and the VILCAD score. For the Marshner Score we observed only slight improvements in the AUC that were not statistically significant (Marshner+FIB-4: p=0.051, Marshner+NFS: 0.516). However, we observed larger improvements when adding FIB-4 or NFS to the VILCAD score (VILCAD+FIB-4: p<0.001, VILCAD+NFS: 0.007) and both the categorical and the continuous NRI increased.

For the calculation of optimal FIB-4 and NFS cutoffs for the prediction of all-cause and cardiovascular mortality in CAD patients we split our cohort into a training set (N=2285) and a test set (N=571). Values were calculated using the Youden Index (defined as sensitivity + specificity -1) with the scores entered as continuous. Cut-off

values for FIB-4 were 0.9106 and 0.8537 for all cause and cardiovascular mortality, respectively. For NFS optimal cut-off values were -0.8221 for both all-cause and cardiovascular mortality. Despite a significant HR, AUC remained low even with optimal cut-off values **(supplemental table 4).**

Discussion

A close correlation between NAFLD, coronary atherosclerosis, and cardiovascular mortality has been suggested in several retrospective studies. A large Korean, cross-sectional study observed a significant association of NAFLD with the presence of coronary artery calcification as detected by means of CT scan (9). These findings were further substantiated by a meta-analysis on 16 studies involving 16,433 NAFLD but also large prospective studies. In conclusion, NAFLD was demonstrated to be associated with increased coronary artery calcification independent of traditional risk factors (17) (31). A smaller study also from Korea including a total of 308 patients 118 of whom suffered from NAFLD demonstrated that hepatic steatosis and fibrosis are associated with subclinical myocardial dysfunction (18). Finally, a recent meta-analysis of observational studies indicated that NAFLD is significantly associated with an increased risk of fatal and non-fatal cardiovascular events. However, the observational design of the studies included did not allow to prove that NAFLD causes cardiovascular disease (28).

However, clinical relevance of these pathological finding and cardiac events remains controversial particularly with respect to a potentially increased mortality. In a recent retrospective evaluation of the third National Health and Nutrition Examination Survey higher FLI and NAFLD LFS were associated with increased liver disease mortality, but not with other mortality outcomes in the US population. Overall and

cardiovascular disease mortality was not associated with higher liver fat scores (30). Also, investigating data of 56,995 adult transplants within the United Network for Organ Sharing-Standard Transplant Analysis and Research (UNOS STAR) dataset revealed a lower hazard of all-cause and similar cardiovascular mortality in NASH patients compared to the non-NASH HCV-negative group. Similarly, the presence of NAFLD in patients admitted for acute ischemic stroke does not appear to be associated with more severe stroke or with worse in-hospital outcome (29).

However, if in turn the occurrence / presence of NALFD in patients with CAD is associated with prognosis remains widely unexplored. Since surveillance of patients for NAFLD will most likely have to rely on screening high risk populations, we aimed to investigate the association of NAFLD and liver fibrosis with all-cause and cardiovascular mortality in patients with confirmed cardiovascular disease (21). In contrast to other (smaller) studies that previously had investigated the impact of NAFLD and fibrosis scores on survival prediction, all study participants in LURIC had received a coronary angiography. The risk of cardiovascular disease in these patients was immanent as 63% of all deaths were due to cardiovascular events (cardiac death, stroke, **suppl. table 7**). Furthermore, all participants were treated for cardiovascular diseases at a single center, Ludwigshafen hospital, in southern Germany. Thus, any bias originating from treatment differences between different medical centers can be excluded.

So far, the only other study investigating cardiovascular mortality in CAD patients dependent on NAFLD and liver fibrosis scores had comparable numbers but up to 25% of patients (depending on the investigated subgroup) did not undergo coronary angiography. In addition, it should be noted that we adjusted our risk prediction models for all cardiovascular risk factors in contrast to Chen et al. who omitted in their models risk factors that were already used in the calculation of the scores, e.g.

360 age in case of FIB-4 or NFS (10). Using a similar adjustment strategy like Chen et al.
361 both FIB-4 and NFS remained significantly associated with both all-cause and
362 cardiovascular mortality (**Table 3, Suppl. Table 6).** However, since for example age
363 alone is a strong predictor of cardiovascular mortality, we believe that adjustment for
364 those factors is essential for specific evaluations of the impact of fatty liver disease
365 (7). Overall, these differences may account for the differences in the performance of
366 NAFLD and liver fibrosis scores between the Chinese and our study. Chen et al. had
367 reported higher fibrosis scores are associated with increased risks of all-cause and
368 cardiovascular mortality among CAD patients (10). Ethnicity differences between
369 China and Europe may further contribute to differences in observed results.
370 Of interest Baars et al. recently reported that liver enzymes correlated with stenose
371 Diameter (5). This comes in parallel with a few other recent publications investigating
372 fibrosis measured by means of MRT report a possible link to an increased
373 cardiovascular disease (CVD) risk (19, 20). However, these fibrosis measurements
374 were correlated to the Framingham risk score or the coronary artery calcium score
375 but not to angiographically proven CVD. Thus, a clear correlation between liver
376 fibrosis and CVD needs to be proven. We therefore applied the Friesinger Score and
377 the Gensini score that quantify the severity of CAD to the tables as well as the
378 number of study participants suffering from 1,2 or 3-vessel disease which were partly
379 linked to survival in our patients. Even in case this had an impact on fibrosis and
380 surrogate scores, patients with high scores must be assumed to have an even higher
381 risk for cardiovascular mortality. However, our work demonstrated that surrogate
382 markers for fibrosis based on those liver enzymes do not serve as a predictor of
383 higher cardiovascular mortality in patients with already diagnosed cardiovascular
384 disease.
385 As clearly demonstrated in our cohort multivariate adjustment for variables has a

significant impact on the assessment of mortality risk using NAFLD and liver fibrosis scores. A fact that certainly needs more attention in future studies.

Adjusting reference cut off values may be necessary for NAFLD and / or liver fibrosis tests in different settings. Data by Sesti et al. demonstrated that advanced fibrosis, is associated with cardiovascular organ damage independent of other known factors (23). However, optimal cut off values were not validated for CAD centred cohorts. In our hands, optimal cut-off (Youden index) for FIB-4 was 0.9106 and 0.8537 for all-cause mortality and cardiovascular mortality, respectively. Since this cut-off value is lower compared to NAFLD centred cohorts one may speculate that for all-cause mortality FIB-4 may well differentiate between high- and low-risk groups. Thus, given the need of identifying high-risk groups for surveillance as prevalence of NAFLD may not allow surveillance of all patients with elevated liver enzymes FIB-4 may well serve as a predictor of all-cause mortality in CAD patients.

Although non-invasive surrogate markers NFS or FIB-4 were not able to independently predict cardiovascular mortality they may be useful in combination with established risk scores for cardiovascular mortality. Our previously established Vienna and Ludwigshafen Coronary Artery Disease (VILCAD) risk score, at the time of publication significantly outperforming established risk score based on conventional cardiovascular risk factors was based on routinely available set of risk factors, measures of cardiac function, and comorbidities. Adding the aspect of fatty liver disease and fibrosis to this score improved the discriminatory power for a 10-year survival even more. However, other established cardiovascular risk scores (Marshner, ESC, PCE) did not benefit from such a combination.

Lastly, it currently remains unclear if our findings can be extrapolated to patients with CVD risk profiles that have not undergone angiography. It certainly is important to keep in mind, that the selection and referral of patients had an impact on this cohort.

Conclusion

Common NALFD and fibrosis scores were associated with increased overall and cardiovascular mortality in a cohort of high CV risk patients in unadjusted analyses or in analyses only adjusted for risk factors that were not included in the calculation of the NAFLD and fibrosis scores. In contrast, we did not observe an association with increased risk after multivariate adjustment when modelling the scores as quartiles and an association only with all-cause mortality when using spline models. Therefore, FIB-4 and NFS are no independent predictors of cardiovascular mortality in patients at medium-to-high cardiovascular risk.

Conflict of interest

W.M. reports grants from Siemens Healthineers, grants and personal fees from Aegerion Pharmaceuticals, grants and personal fees from AMGEN, grants from Astrazeneca, grants and personal fees from Sanofi, grants and personal fees from Alexion Pharmaceuticals, grants and personal fees from BASF, grants and personal fees from Abbott Diagnostics, grants and personal fees from Numares AG, grants and personal fees from Berlin-Chemie, grants and personal fees from Akzea Therapeutics, grants from Bayer Vital GmbH, grants from bestbion dx GmbH, grants from Boehringer Ingelheim Pharma GmbH Co KG, grants from Immundiagnostik GmbH, grants from Merck Chemicals GmbH, grants from MSD Sharp and Dohme GmbH, grants from Novartis Pharma GmbH, grants from Olink Proteomics, other from Synlab Holding Deutschland GmbH, all outside the submitted work. B.K.K. reports lecture fees and/or advisory board memberships and/or study participation from Astellas, Bayer, Boehringer Ingelheim, Chiesi, Riepharm, Pfizer, Servier, and Vifor Pharma. M.E.K. is employed with SYNLAB Holding Deutschland GmbH and reports lecture fees from Bayer outside the submitted work.

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Figures and tables

Figure 1: Kaplan-Meier curves for cardiovascular mortality for quartiles of FLI (A), APRI (B), FIB-4 (C), NFS (D).

Table 1: Study characteristics according to vital status (mean ± SD or median (25^{th} to 75^{th} percentile)

Table 2 (A): Study characteristics according to FIB-4 quartiles. **Table 2 (B)** Study characteristics according to NFS quartiles

Table 3: Cox regression models with multivariate adjustment for variables not included in score formulas (A) Analysis and comparison of quartiles (B) Analysis and comparison using established cut off values.

Table 4: Association of non-invasive surrogate score s with all-cause and cardiovascular mortality, adjusted for cardiovascular risk factors. (A) Analysis and comparison of quartiles (B) Analysis and comparison using established cut off values.

Supplemental material

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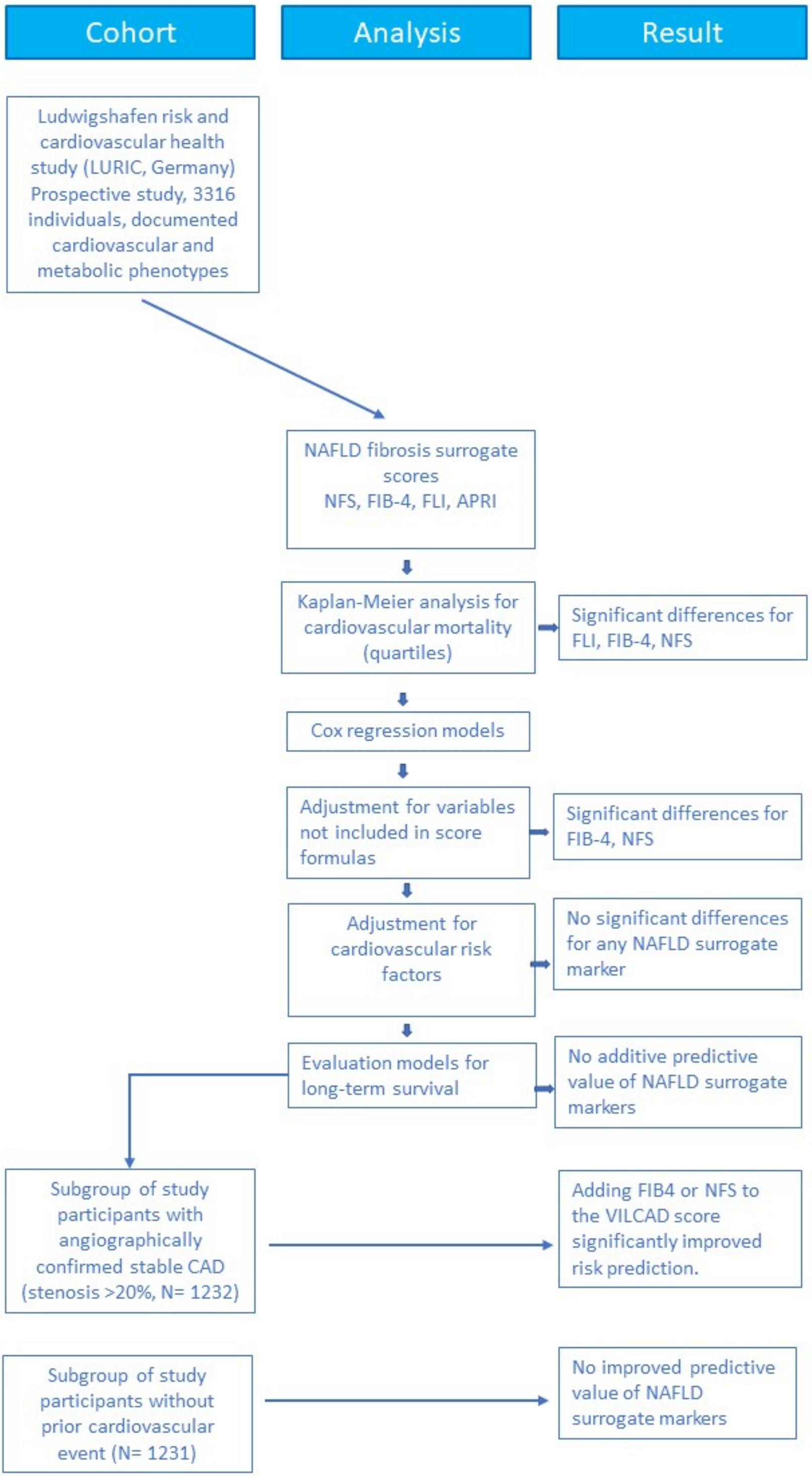

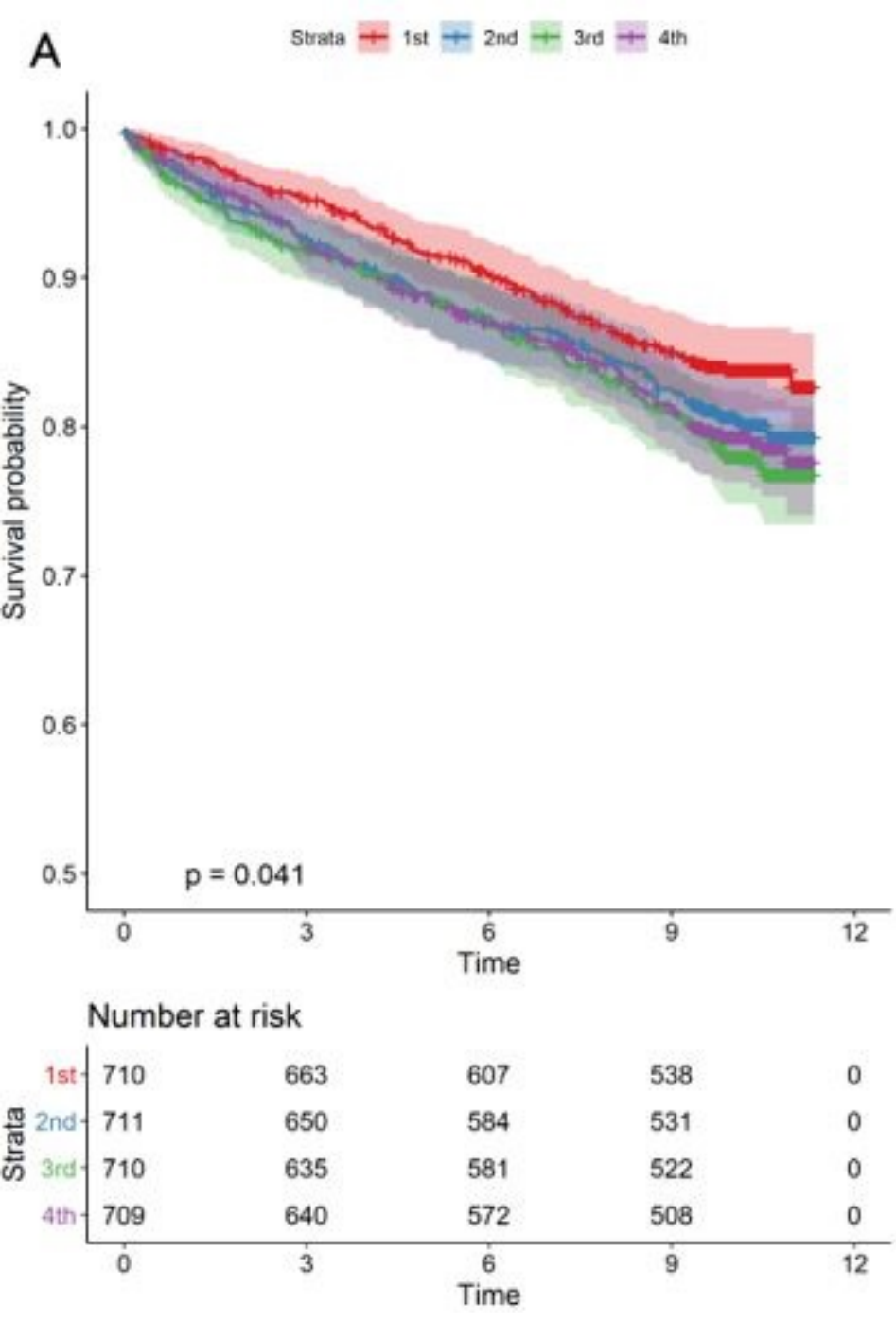

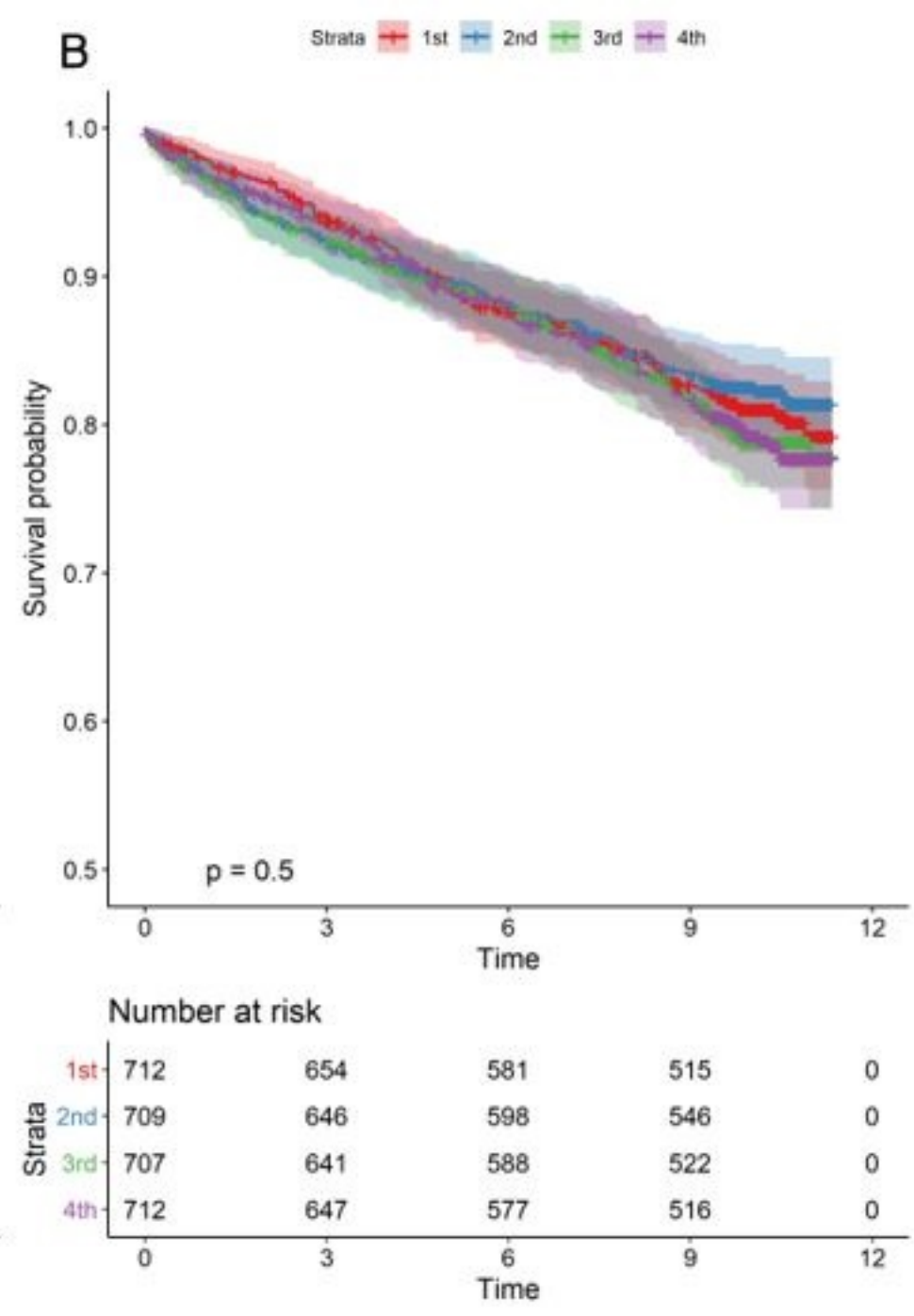

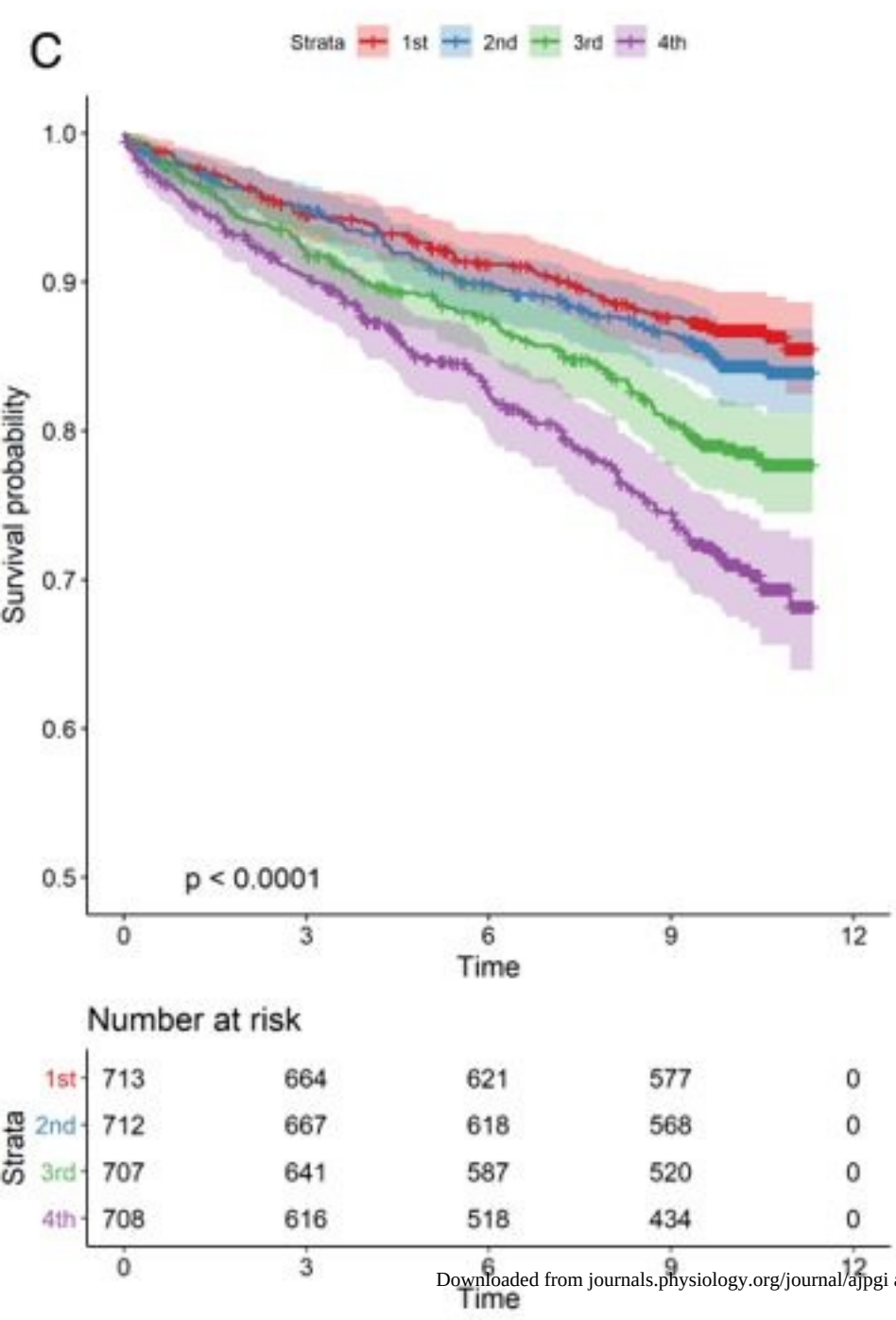

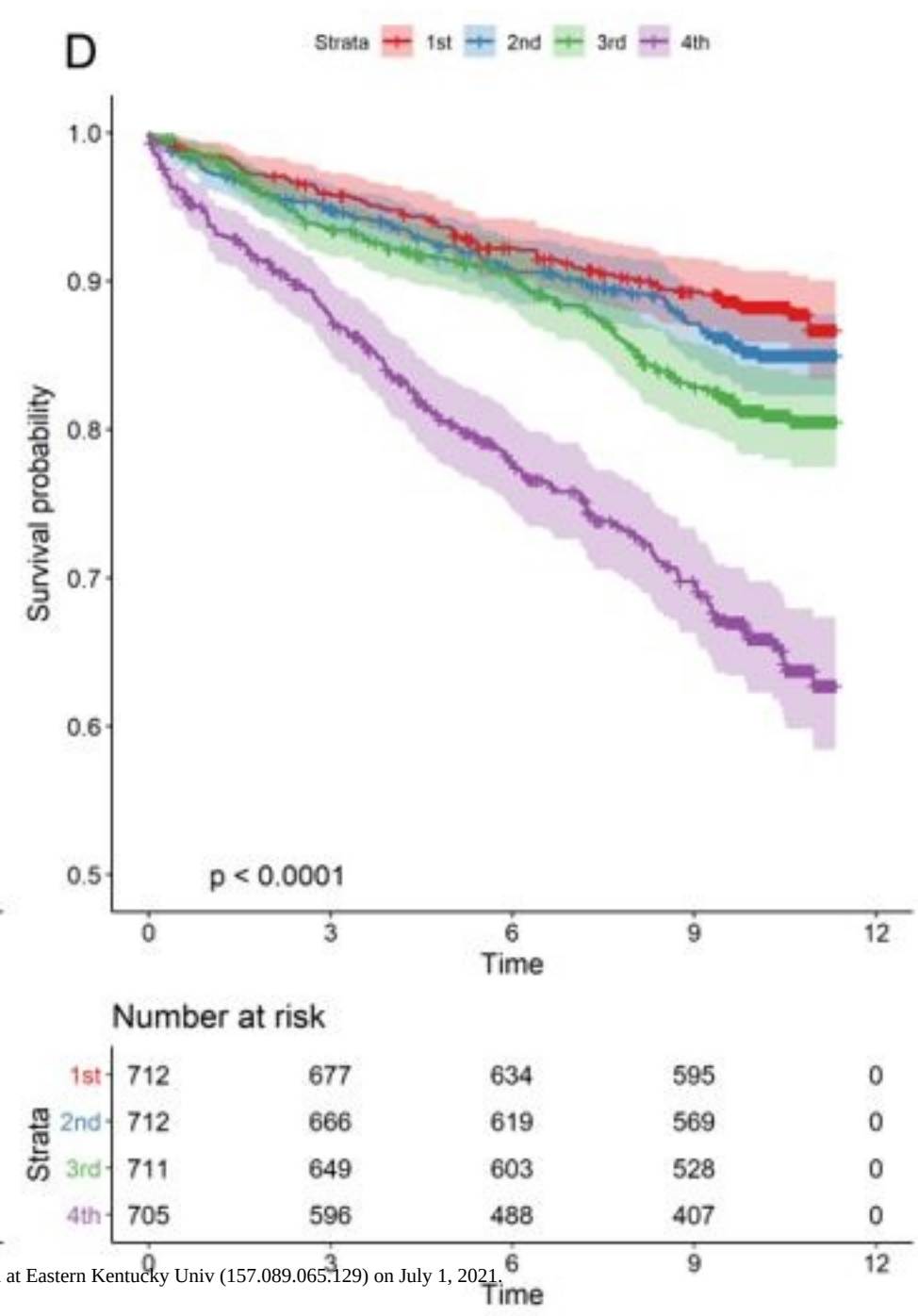

Table 1: Study characteristics according to vital status (mean ± SD or median (25^{th} to 75^{th} percentile)

Variable	Survivors	Deceased	P*
Age (years)	60.5(10.4)	68.3(9.14)	<0.001
Female sex (%)	32.2	27.3	0.011
BMI (kg/m^2)	27.6(3.98)	27.3(4.31)	0.033
LDL-C (mg/dl)	117(35)	113(33.2)	0.001
HDL-C (mg/dl)	39.4(11)	37.3(10.8)	<0.001
TG (mg/dl)	146(110-199)	147(108-199)	0.493
HbA1c (%)	6.2(1.08)	6.77(1.53)	<0.001
SysBP (mmHg)	139(23)	146(24.8)	<0.001
DiaBP (mmHg)	81.1(11.4)	80.6(11.7)	0.368
eGFR ($ml/min/1.73m^2$)	85.3(17.6)	71.3(21.8)	<0.001
hsCRP (mg/L)	2.92(1.2-7.41)	5.07(2.06-11.2)	<0.001
NT-proBNP (pg/mL)	213(86-531)	800(297-2160)	<0.001
Coronary artery disease (%)	73.2	87.9	<0.001
Hypertension (%)	70.2	80	<0.001
T2DM (%)	34.2	57.2	<0.001
Smokers (%)	20.2	17.6	0.116
Liver scores			
FLI	52.6(26.9)	55.5(26.4)	0.007
FIB-4	0.751(0.569-0.988)	0.927(0.684-1.25)	<0.001
APRI	4.43(3.38-6.25)	4.71(3.35-6.52)	0.068
NFS	-1.62(1.35)	-0.859(1.42)	<0.001

* t-test, non-normally distributed variables were logarithmically transformed before analysis

Table 2

A	FIB-4 quartiles				
Variable	First	Second	Third	Fourth	P*
Age (years)	54.8(10.9)	61.7(9.24)	65.2(8.34)	69(8.22)	<0.001
Female sex (%)	32.6	33.7	29.8	25.4	0.001
BMI (kg/m2)	27.4(4.33)	27.6(4.08)	27.6(3.89)	27.3(4)	0.444
LDL-C (mg/dl)	119(35.8)	119(32.9)	116(34.4)	112(33.6)	<0.001
HDL-C (mg/dl)	37.3(10.5)	38.7(10.7)	39(10.5)	39.9(11.5)	<0.001
TG (mg/dl)	155(112-210)	149(112-204)	148(112-201)	134(102-188)	<0.001
FGLUC (mg/dl)	102(93.6-117)	102(93.9-118)	102(93.2-118)	103(94.2-120)	0.442
HbA1c (%)	6.28(1.27)	6.33(1.29)	6.31(1.25)	6.32(1.18)	0.632
SysBP (mmHg)	135(23.5)	140(22.5)	144(23.3)	146(23.7)	<0.001
DiaBP (mmHg)	80(11.8)	80.7(11.5)	81.7(11.2)	81.5(11.2)	0.002
eGFR (ml/min/1.73m2)	89.9(20.3)	83.5(18.4)	79.7(19)	73.7(19.3)	<0.001
Cholinesterase (U/L)	5850(1380)	5860(1270)	5760(1220)	5340(1360)	<0.001
Alcohol intake (g eth/d)	2(0-21.7)	2.4(0-24)	3.72(0-24)	6(0-24)	<0.001
Alcoholic liver disease to NAFLD index (ANI)	-6.36(4.78)	-5.9(4.91)	-5.09(4.62)	-3.28(5.06)	<0.001
hsCRP (mg/L)	3.99(1.48-9.66)	3.08(1.3-8.36)	2.93(1.19-7.4)	3.55(1.35-9.05)	0.212
NT-proBNP (pg/mL)	195(70.5-636)	224(88.2-630)	292(122-876)	528(196-1340)	<0.001
Adiponectin (Âµg/L)	7.58(5.33-11)	8.5(5.65-12.5)	8.36(5.83-12.5)	9.74(6.44-15.1)	<0.001
Leptin (Âµg/L)	10.1(5.62-18.3)	10.6(6.6-18.4)	10.7(6.8-18.8)	9.8(6.1-16.7)	0.743
Friesinger Score	5(1-8)	5(2-8)	6(3-9)	6(3-9)	<0.001
Gensini Score	26(2-57)	28(4-57.5)	32(9-70)	37.2(12.8-68.2)	<0.001
Coronary artery disease (%)	72.7	75.4	80.3	83.1	<0.001
1-vessel disease (%)	31	28.1	27.4	26.8	<0.001
2-vessel disease (%)	17.6	18.5	20.2	20	<0.001
3-vessel disease (%)	24.1	28.8	32.7	36.3	<0.001
Heart failure (%)	26.7	28.7	32.8	42.8	<0.001
Hypertension (%)	62.6	72.4	77.8	78.3	<0.001
T2DM (%)	36.7	37.5	39.6	45.9	<0.001
Smokers (%)	32.6	21.4	12.2	12.8	<0.001
B	NFS Quartiles				
Variable	First	Second	Third	Fourth	P
Age (years)	55.1(11.4)	62.1(8.9)	64.5(8.83)	69.4(7.75)	<0.001
Female sex (%)	33.1	31.2	29.3	29.1	0.314
BMI (kg/m2)	25.9(3.52)	27.1(3.37)	28(4.04)	29.1(4.59)	<0.001
LDL-C (mg/dl)	121(35.1)	116(33.5)	116(34.7)	111(34.3)	<0.001
HDL-C (mg/dl)	39.4(11.5)	39.5(10.7)	38.5(10.9)	37.5(10.6)	<0.001
TG (mg/dl)	144(108-201)	145(109-198)	147(111-201)	146(110-198)	0.531
FGLUC (mg/dl)	98.4(92.3-107)	100(93.2-111)	104(95.2-122)	115(101-145)	<0.001
HbA1c (%)	5.94(0.781)	6.13(1.06)	6.44(1.37)	6.95(1.45)	<0.001
SysBP (mmHg)	133(22.6)	141(23.7)	143(23.6)	147(23)	<0.001
DiaBP (mmHg)	79.5(11.7)	81.4(11.3)	81.7(11.9)	81.3(11.1)	0.003
eGFR (ml/min/1.73m2)	89.8(19.3)	82.8(18.9)	79.5(18.2)	71.8(19.9)	<0.001
Cholinesterase (U/L)	5940(1370)	5830(1230)	5730(1300)	5390(1370)	<0.001
Alcohol intake (g eth/d)	2.8(0-24)	3.46(0-24.7)	3.28(0-24)	3.08(0-24)	0.869
Alcoholic liver disease to NAFLD index (ANI)	-5.08(4.66)	-5.26(4.64)	-5.36(5.21)	-5.18(5.51)	0.617
hsCRP (mg/L)	3.2(1.23-8.63)	3.02(1.25-7.64)	3.46(1.32-8.19)	4.28(1.74-10.4)	<0.001
NT-proBNP (pg/mL)	195(72-556)	239(99-684)	307(120-867)	561(203-1510)	<0.001
Adiponectin (Âµg/L)	8.1(5.68-12.1)	8.71(5.92-13.1)	7.94(5.59-12.6)	9.11(5.79-13.1)	<0.001
Leptin (Âµg/L)	8.6(5.32-15)	9.7(6.5-17.3)	11.2(6.8-18.6)	11.7(7.12-21.1)	<0.001
Friesinger Score	4(1-7)	5(2-8)	6(3-9)	6(3-9)	<0.001
Gensini Score	22(1-54.5)	29.2(3.5-61.6)	34.2(13.8-72.1)	36.2(13.5-68.6)	<0.001
Coronary artery disease (%)	69.1	76.8	81.2	83.4	<0.001
1-vessel disease (%)	30.6	30.6	28.2	26.4	0.055
2-vessel disease (%)	16	18	22	19.3	0.029
3-vessel disease (%)	22.5	28.2	31	37.7	<0.001
Heart failure (%)	24.3	24.6	36.4	47.9	<0.001
Hypertension (%)	58.4	73.1	77.9	83.4	<0.001
T2DM (%)	12.8	25	46.8	79.5	<0.001
Smokers (%)	31.1	19.6	15.4	12	<0.001

*ANOVA, non-normally distributed variables were logarithmically transformed before analysis

Table 3: Cox regression models with multivariate adjustment for variables not included in score formulas

A		All-cause mortality		Cardiovascular mortality	
Score	Quartile	HR (95%CI)	P*	HR (95%CI)	P*
FLI					
	1st	1	-		
	2nd	0.896(0.747-1.07)	0.236	1.03(0.817-1.31)	0.786
	3rd	0.913(0.758-1.1)	0.333	1.09(0.86-1.38)	0.477
	4th	1.14(0.949-1.38)	0.158	1.2(0.939-1.53)	0.145
FIB-4					
	1st	1	-	1	-
	2nd	1.15(0.934-1.41)	0.189	1.16(0.892-1.5)	0.273
	3rd	1.5(1.23-1.83)	<0.001	1.53(1.19-1.96)	0.001
	4th	2.28(1.9-2.75)	<0.001	2.11(1.67-2.67)	<0.001
APRI					
	1st	1	-	1	-
	2nd	0.821(0.684-0.986)	0.035	0.839(0.666-1.06)	0.136
	3rd	0.914(0.766-1.09)	0.321	0.942(0.754-1.18)	0.602
	4th	1.04(0.87-1.24)	0.680	1.01(0.81-1.27)	0.905
NFS					
	1st	1	-	1	-
	2nd	1.27(1.01-1.59)	0.044	1.22(0.916-1.64)	0.172
	3rd	1.63(1.31-2.04)	<0.001	1.54(1.17-2.04)	0.002
	4th	3.21(2.61-3.94)	<0.001	3.12(2.41-4.04)	<0.001

B	All-cause mortality		Cardiovascular mortality	
	HR (95%CI)	P	HR (95%CI)	P
FLI				
<30	1		1	
30-60	1.04(0.857-1.25)	0.717	1.17(0.916-1.49)	0.210
>60	1.11(0.923-1.34)	0.264	1.22(0.955-1.55)	0.113
FIB-4				
<1.3	1		1	
1.3-2.67	2.12(1.80-2.50)	<0.001	1.90(1.53-2.34)	<0.001
>2.67	1.94(1.07-3.53)	0.029	1.41(0.581-3.4)	0.450
APRI				
0.5-1.5	1		1	
>1.5	0.556(0.297-1.04)	0.067	0.49(0.231-1.04)	0.062
NFS				
<-1.455	1		1	
-1.455-0.676	1.84(1.58-2.14)	<0.001	1.94(1.6-2.36)	<0.001
>0.676	4.13(3.30-5.15)	<0.001	3.61(2.69-4.85)	<0.001

* FLI: adjusted for age, sex, diabetes mellitus, hypertension, smoking, LDL-C, HDL-C, medication and alcohol intake; FIB-4 adjusted for sex, BMI, diabetes mellitus, hypertension, smoking, LDL-C, HDL-C, medication and alcohol intake; APRI adjusted for age, sex, BMI, diabetes mellitus, hypertension, smoking, LDL-C, HDL-C, medication and alcohol intake; NFS adjusted for sex, hypertension, smoking, LDL-C, HDL-C, medication and alcohol intake;

Table 4: Association of liver scores with all-cause and cardiovascular mortality, adjusted for cardiovascular risk factors*

A	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	P	HR (95% CI)	P
FLI				
1st	1		1	
2nd	0.996(0.805-1.23)	0.968	1.08(0.819-1.41)	0.600
3rd	1.08(0.858-1.37)	0.499	1.2(0.889-1.61)	0.238
4th	1.43(1.07-1.92)	0.015	1.3(0.896-1.89)	0.167
FIB-4				
1st	1		1	
2nd	0.832(0.662-1.04)	0.113	0.859(0.645-1.14)	0.297
3rd	0.972(0.777-1.22)	0.803	1.04(0.787-1.38)	0.780
4th	1.13(0.904-1.41)	0.285	1.06(0.8-1.41)	0.680
APRI				
1st	1		1	
2nd	0.935(0.766-1.14)	0.512	1.06(0.827-1.37)	0.632
3rd	1.05(0.863-1.27)	0.649	1.13(0.885-1.44)	0.329
4th	1.05(0.86-1.27)	0.652	1.07(0.83-1.37)	0.614
NFS				
1st	1		1	
2nd	0.946(0.745-1.2)	0.645	0.899(0.667-1.21)	0.487
3rd	0.946(0.743-1.2)	0.651	0.854(0.632-1.16)	0.306
4th	1.17(0.903-1.52)	0.232	1.02(0.738-1.41)	0.905

B	All-cause mortality		Cardiovascular mortality	
	HR (95%CI)	P	HR (95%CI)	P
FLI				
<30	1		1	
30-60	1.05(0.854-1.29)	0.653	1.14(0.879-1.48)	0.321
>60	1.19(0.927-1.52)	0.173	1.20(0.877-1.65)	0.251
FIB-4				
<1.3	1		1	
1.3-2.67	1.30(1.09-1.55)	0.003	1.15(0.917-1.43)	0.230
>2.67	1.24(0.677-2.26)	0.490	0.90(0.37-2.19)	0.816
APRI				
0.5-1.5	1		1	
>1.5	0.642(0.341-1.21)	0.170	0.551(0.258-1.18)	0.124
NFS				
<-1.455	1		1	
-1.455-0.676	1.08(0.903-1.28)	0.415	1.03(0.829-1.29)	0.770
>0.676	1.58(1.21-2.08)	0.001	1.14(0.80-1.62)	0.472

*adjusted for age, sex, BMI, diabetes mellitus, hypertension, smoking, LDL-C, HDL-C, medication and alcohol intake