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# Progression of non-alcoholic fatty liver disease and long-term outcomes: A nationwide paired liver biopsy cohort study

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Authors' contributions

Guarantor: The corresponding author (TGS) had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: All co-authors. Acquisition of data: JFL. Analysis: BR. Interpretation of data: All co-authors. Writing first draft of the manuscript: TGS and JFL. Critical revision of the manuscript for important intellectual content and approval of final version: All co-authors.

Disclaimer

No funding organization had any role in the design and conduct of the study; in the collection, management, and analysis of the data; or in the preparation, review, and approval of the manuscript.

Details of ethics approval

This study was approved by the Regional Ethics Committee, Stockholm, Sweden (Protocol number: 2014/1287-31/4 and 2018/972-32).

Transparency statement

Dr. Simon affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2023.08.008.

Conflict of interest

TGS reports research grants to the institution from Amgen, for work unrelated to this manuscript. HH reports research grants to his institution from Astra Zeneca, EchoSens, Intercept, Gilead, MSD and Pfizer, and board advisory for Bristol-Myers Squibb and Gilead. JFL has coordinated a study on behalf of the Swedish IBD quality register (SWIBREG), that has received funding from Janssen corporation. JFL has also received financial support from MSD developing a paper reviewing national healthcare registers in China. Finally JFL is currently discussing potential research collaboration with Takeda. RL serves as a consultant to Aardvark Therapeutics, Altimmune, Anylam/Regeneron, Amgen, Arrowhead Pharmaceuticals, AstraZeneca, Bristol-Myer Squibb, CohBar, Eli Lilly, Galmed, Gilead, Glympse bio, Hightide, Inipharma, Intercept, Inventiva, Ionis, Janssen Inc., Madrigal, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Merck, Pfizer, Sagimet, Theratechnologies, 89 bio, Terns Pharmaceuticals and Viking Therapeutics. In addition his institutions received research grants from Arrowhead Pharmaceuticals, Astrazeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Galectin Therapeutics, Galmed Pharmaceuticals, Gilead, Intercept, Hanmi, Intercept, Inventiva, Ionis, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Novo Nordisk, Merck, Pfizer, Sonic Incytes and Terns Pharmaceuticals. Co-founder of LipoNexus Inc. The remaining authors have no disclosures and no conflicts of interest to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

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## Abstract

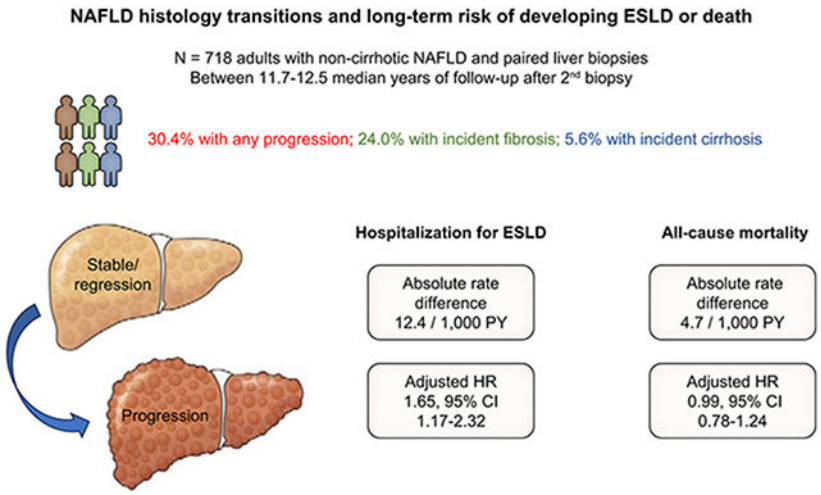
**Background & Aims:** More data are needed regarding the long-term impact of the histological progression of non-alcoholic fatty liver disease (NAFLD) on long-term outcomes, including end-stage liver disease (ESLD) and mortality.

**Methods:** We included Swedish adults with biopsy-confirmed non-cirrhotic NAFLD and ≥2 liver biopsies >6 months apart (1969-2017; n = 718). NAFLD was categorized at initial biopsy as simple steatosis, non-fibrotic steatohepatitis (NASH), or non-cirrhotic fibrosis. NAFLD progression was defined by histological changes between biopsies (*i.e.* incident NASH, incident fibrosis, fibrosis progression, cirrhosis). Using Cox regression, we estimated multivariable adjusted hazard ratios (aHRs) and 95% CIs for incident ESLD (*i.e.* hospitalization for decompensated cirrhosis, hepatocellular carcinoma or liver transplantation) and mortality, according to NAFLD progression *vs.* stable/regressed disease.

**Results:** At initial biopsy, 497 patients (69.2%) had simple steatosis, 90 (12.5%) had non-fibrotic NASH, and 131 (18.2%) had non-cirrhotic fibrosis. Over a median of 3.4 years between biopsies, 30.4% (218/718) experienced NAFLD progression, including 12.5% (62/497) with incident non-fibrotic NASH, 24.0% (141/587) with incident fibrosis, and 5.6% (40/718) with cirrhosis. Compared to stable/regressed disease, NAFLD progression was associated with significantly higher rates of developing incident ESLD (23.8 *vs.* 11.4/1,000 person-years [PY]; difference = 12.4/1,000 PY; aHR 1.65, 95% CI 1.17-2.32). While the highest ESLD incidence occurred with progression to cirrhosis (difference *vs.* stable/regressed disease = 56.3/1,000 PY), significant excess risk was also found with earlier transitions, including from simple steatosis to incident fibrosis (difference *vs.* stable/regressed disease = 18.9/1,000 PY). In contrast, all-cause mortality rates did not appear to differ when NAFLD progression was compared to stable/regressed disease (difference = 4.7/1,000 PY; aHR 0.99, 95% CI 0.78-1.24).

**Conclusions:** In a nationwide, real-world cohort of patients with paired NAFLD biopsies, histological disease progression contributed to significantly higher rates of developing incident ESLD, but did not appear to impact all-cause mortality.

## Graphical Abstract




**Keywords**

NAFLD; liver histology; progression; survival; fibrosis; steatohepatitis

## Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the most common cause of chronic liver disease in Western countries, affecting over 30% of US and European adults.[1] Up to one-third of patients with NAFLD develop inflammatory steatohepatitis (NASH) and fibrosis, which can lead to cirrhosis, decompensated liver disease, and death.[2-4] It is now well-established that in NAFLD, mortality is highest with advanced fibrosis,[3-8] and previous studies using paired liver biopsies have helped begin to characterize rates of and risk factors for histological progression of NAFLD.[9-12] However, to date, no prior large study has examined dynamic changes in NAFLD histology in relation to major, long-term clinical outcomes.[13] Thus, on a nationwide level, it is unknown whether persons with stable or regressed NAFLD have improved survival and lower rates of developing end-stage liver disease (ESLD) compared to patients who experience NASH or fibrosis progression. This is particularly relevant for randomized-controlled trials of NAFLD therapeutics, which currently focus on short-term histological endpoints – including NASH resolution and/or improvement of ≥1 stage of fibrosis without worsening of NASH – as presumed surrogates for major clinical outcomes.[13,14] Such long-term outcomes data can inform the optimal design of NAFLD therapeutic trials, while also providing the necessary evidence base for public health strategies focused on preventing the development and progression of NAFLD.

Using a nationwide, real-world cohort of adults in Sweden with confirmed NAFLD and paired clinical liver biopsies, we examined dynamic changes in NAFLD histology in relation to incident ESLD and overall mortality. With detailed histopathology data and long-term follow-up for the entire country of Sweden, this unique cohort permits a more comprehensive assessment of the natural history and outcomes of NAFLD.

## Patients and methods

### Study population & cohort construction

This nationwide cohort study used the ESPRESSO (Epidemiology Strengthened by Histopathology Reports in Sweden) cohort, which prospectively recorded liver histopathology data from all 28 pathology departments in Sweden (1969-2017).[15] Each report includes a unique personal identity number, biopsy date, topography within the liver and morphology. We linked ESPRESSO to validated, nationwide registers containing prospectively recorded data regarding demographics, comorbidities, prescribed medications and death. ESPRESSO was approved by the Stockholm Ethics Board; informed consent was waived as the study was register-based.[16]

We included all adults aged ≥18 years between 1969-2017 with NAFLD confirmed by an initial liver biopsy, using a validated algorithm of liver topography codes, and Systematized Nomenclature of Medicine (SNOMED) codes corresponding to steatosis (supplementary methods), and who also had ≥1 subsequent liver biopsy submitted >6 months later.[17] As NAFLD represents a clinicopathologic diagnosis, we applied a validated algorithm to exclude any person with another etiology of liver disease or alcohol abuse/misuse, as well as any person with liver transplantation, emigration from Sweden or an ESLD outcome, recorded on or prior to the second biopsy (Tables S1 and S2).[17] As this study focused on histological progression of NAFLD, we further excluded anyone with cirrhosis on the initial biopsy.

At the initial biopsy, eligible patients were categorized into three groups (simple steatosis, non-fibrotic NASH, non-cirrhotic fibrosis), and further into five groups at the second biopsy (normal; simple steatosis; non-fibrotic NASH; non-cirrhotic fibrosis; cirrhosis), using established algorithms that employ SNOMED topography and morphology codes as previously described and validated in this cohort (with positive predictive values [PPVs] all ≥87% for each histology group;[17] see supplementary methods for details). Briefly, consistent with nationwide liver histopathology reporting recommendations for pathologists in Sweden,[18] simple steatosis was defined by ≥1 code for steatosis and no codes for inflammation or ballooning (*i.e.* M5400x or M4-) or fibrosis (*i.e.* M4900x) or cirrhosis (*i.e.* M4950x). NASH without fibrosis was defined by the presence of ≥1 code for steatosis plus ≥1 code for inflammation (*i.e.* M5400x or M4-), without any codes for fibrosis or cirrhosis. Non-cirrhotic fibrosis (*i.e.* F1-F3 fibrosis, with or without NASH) was defined by the presence of ≥1 code for steatosis plus ≥1 code for fibrosis (*i.e.* M4900x), but no codes for cirrhosis.

With these groupings, we defined the primary exposure: any NAFLD progression (*i.e.*, [a] incident NASH, [b] incident fibrosis, or [c] fibrosis progression, including progression to cirrhosis) *vs.* stable/regressed disease (with regression defined as, [a] fibrosis regression: *i.e.* from non-cirrhotic fibrosis to non-fibrotic NASH or simple steatosis, or [b] NASH regression: *i.e.* loss of inflammation/ballooning on the second biopsy). In separate analyses, we also evaluated individual categories of NASH and fibrosis progression or regression.

## Outcomes & covariates

Incident ESLD was defined as the first primary hospitalization discharge diagnosis for decompensated cirrhosis (*i.e.* ascites, spontaneous bacterial peritonitis, bleeding esophageal varices, hepatic encephalopathy or hepatorenal syndrome), incident hepatocellular carcinoma (HCC) or liver transplantation (see supplementary methods; Table S3). Overall mortality was ascertained from the Total Population Register, which prospectively records 93% of all deaths within 10 days, and the remaining 7% within 30 days.[19]

We collected detailed data regarding demographic, clinical and medication covariates (supplementary methods; Table S3). Age, sex, date of birth and emigration were ascertained from the Total Population Register.[20] Education was obtained from the LISA database.[21] Comorbidities were collected from the Patient Register, which prospectively records all data from hospitalizations (including surgeries), discharge diagnoses (1964-) and specialty outpatient care (2001-), with PPVs for diagnoses between 85-95%.[22] All multivariable models accounted for elapsed time between paired biopsies (months) and the following a *priori* covariates: age, sex, county of residence, calendar year, education level, cardiovascular disease and components of the metabolic syndrome (*i.e.* a 5-level variable: 1-point for diabetes, obesity, hypertension and/or dyslipidemia) (Table S3). For sensitivity analyses including medication covariates, we used the Prescribed Drug Register, which prospectively records all prescriptions dispensed from Swedish pharmacies (July 2005-), and is well-validated and virtually complete,[23] permitting comprehensive ascertainment of relevant medications including statins, low-dose aspirin (<163 mg), and antidiabetic medications.[23]

## Statistical analysis

We estimated rates of study outcomes according to NAFLD progression *vs.* stable/regressed disease. Follow-up began the day after the second liver biopsy, and continued to the first study outcome, emigration, or end of follow-up (December 31, 2019). Kaplan-Meier curves were constructed to calculate incidence rates and absolute rate differences with corresponding 95% CIs.

Using Cox proportional hazard models, we estimated multivariable adjusted hazard ratios (aHRs), accounting for time between biopsies and the aforementioned *a priori*-selected covariates, defined at initial biopsy date. Attributable risk percentages were estimated as (1–1/aHR). The proportional hazards assumption was assessed by examining the association between Schoenfeld residuals and time. In stratified models, we examined the associations between NAFLD progression and study outcomes according to sex, index biopsy date (1969-1989 *vs.* 1990-2005 *vs.* 2006-2017), and interval between biopsies (<5 years *vs.* ≥5 years) and we tested the significance of effect modification using the log likelihood ratio test.

We conducted several sensitivity analyses to test the robustness of our results. First, to ensure consistency with prior, smaller paired NAFLD biopsy cohorts,[10,11] we required >1 year between paired biopsies. Second, we constructed separate multivariable models further accounting for the potential confounding influence of aspirin, statins and antidiabetic medications, among the subset of patients with comprehensive medication use data available

on or before the start of follow-up (January 1, 2006). Third, we repeated our primary analysis of ESLD while accounting for all-cause mortality as a potential competing event. We also conducted separate analyses directly comparing NAFLD regression *vs.* progression, and comparing rates of study outcomes in patients with non-cirrhotic fibrosis with *vs.* without concurrent NASH on the second liver biopsy. Finally, to address potential selection bias – as patients undergoing repeat biopsy might be sicker and more likely to have poor outcomes, compared to those with one biopsy – we compared clinical characteristics and the proportions that developed ESLD in our paired biopsy cohort, *vs.* all Swedish patients with non-cirrhotic NAFLD and one clinical biopsy.

Statistical analyses were conducted using R software (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria) and survival package version 2.44 (Therneau, 2015, https://CRAN.R-project.org/package=survival). A two-sided $p < 0.05$ was considered statistically significant.

## Results

We included 718 adults with histologically confirmed NAFLD and ≥2 liver biopsies >6 months apart. Table 1 summarizes characteristics at the initial biopsy. Overall, 42.1% were female, with a mean age of 49.3 years, and 497 (69.2%) had simple steatosis, while 90 (12.5%) had non-fibrotic NASH, and 131 (18.2%) had non-cirrhotic fibrosis. Compared to patients with simple steatosis or non-fibrotic NASH on the initial biopsy, those with non-cirrhotic fibrosis were more likely to have features of the metabolic syndrome (Table 1).

### Histological changes between biopsies

Median time between biopsies was 3.4 years (range: 0.5–33.2 years). Table 2 outlines the histological distribution at each biopsy; at the second liver biopsy, 218 patients (30.4%) had disease progression, including 12.5% (62/497) with incident non-fibrotic NASH, 24.0% (141/587) with incident fibrosis, and 5.6% (40/718) with incident cirrhosis (Table 2). Among those without any fibrosis on the first biopsy (n = 587), 25 (4.3%) developed cirrhosis at the second biopsy. Patients with interval progression to cirrhosis were more likely to be older, and to have features of the metabolic syndrome (Table 3).

### Incident ESLD

We recorded a total of 131 incident ESLD events over a median follow-up of 11.7 years after the second biopsy (IQR 5.2–17.9 years; range 0–38.9 years). Patients with any NAFLD progression had significantly higher rates of developing incident ESLD (23.8/1,000 person-years [PY]), compared to those with stable/regressed disease (11.4/1,000 PY), corresponding to an absolute rate difference of 12.4/1,000 PY and an aHR of 1.65 (95% CI 1.17-2.32) (Table 4). Overall, the highest absolute rates of ESLD occurred with progression from non-cirrhotic fibrosis to cirrhosis (80.1/1,000 PY; aHR 3.25, 95% CI 1.60-6.57). However, rates of incident ESLD were also significantly elevated in patients with more mild histological transitions, including those who progressed from simple steatosis to incident fibrosis (18.9/1,000 PY; aHR 2.88, 95% CI 1.70-4.87), and from non-fibrotic NASH to incident fibrosis (8.5/1,000 PY; aHR 2.44, 95% CI 1.33-6.41). In contrast, among patients initially

with simple steatosis, those who progressed to non-fibrotic NASH had similar rates of developing incident ESLD as those with stable/regressed disease.

For patients with any NAFLD progression, the attributable risk (AR) percentage for developing ESLD was 39.3%, suggesting that in this cohort, 39.3% of ESLD events could have been prevented, by preventing NAFLD progression. Among the subset of patients who progressed to cirrhosis from non-cirrhotic fibrosis, the corresponding AR percentage was further enhanced to 69.2%.

## Mortality

Over 12.5 median years of follow-up after the second biopsy (IQR 5.9-18.5 years; range 0.5-38.9 years), we recorded 107 deaths in patients with NAFLD progression (40.4/1,000 PY), and 234 deaths in patients with stable/regressed disease (35.7/ 1,000 PY; absolute difference 4.7/1,000 PY; aHR 0.99, 95% CI 0.78-1.24) (Table 3). Although mortality rates appeared slightly higher in patients who progressed to cirrhosis (43.7/1,000 PY), those differences were not statistically significant, nor did mortality rates differ substantially between groups of patients with more mild histological transitions (Table 3). Among patients with NAFLD progression, the AR percentage was <1% for overall mortality, suggesting that in this group, a negligible proportion of all deaths could have been prevented by preventing NAFLD progression (Table 3).

In stratified analyses, the associations between NAFLD progression and study outcomes did not differ significantly between men and women, or by calendar year of initial biopsy (*i.e.* 1969-1989, 1990-2005, and 2006-2017), or by interval between biopsies (<5 years *vs.* ≥5 years) (all $p$ interactions >0.05) (Table S4).

## Sensitivity and exploratory analyses

Our findings were consistent across numerous sensitivity analyses, including after requiring >1 year between paired biopsies (Table S5), and after restricting the cohort to patients with comprehensive medication use data available (*i.e.*, 2006-2017), and further adjusting our multivariable models for aspirin, statin and/or antidiabetic medication use (Table S6). Our findings also persisted for incident ESLD after applying competing risk regression, with all-cause mortality considered a competing event (Table S7), and further after directly comparing NAFLD progression *vs.* regression (difference for incident ESLD, 11.9/1,000 PY; not shown). Additionally, in exploratory analyses, we restricted the cohort to patients with non-cirrhotic fibrosis on the second biopsy, and those with concurrent NASH appeared to have higher rates of incident ESLD, compared to those without (difference, 22.2/1,000 PY) (Table S8); however, with relatively few events, these findings must be interpreted with caution, for they also might be explained by the presence of higher fibrosis stages in patients with NASH, because NASH is highly colinear with progressive fibrosis (Table S8). Finally, we compared clinical characteristics and progression to incident ESLD in our paired biopsy cohort *vs.* all Swedish adults with just one non-cirrhotic NAFLD biopsy (Table S9). Patients with paired biopsies had a slightly higher prevalence of cardiovascular disease and hypertension, but other clinical features and the case distribution were otherwise broadly similar between cohorts. Moreover, the proportions of patients who ultimately developed

ESLD in follow-up were similar (18.2% [paired biopsy cohort] *vs.* 20.9% [single biopsy cohort]) as were the overall incidence rates of ESLD (14.9/1,000 PY and 14.5/1,000 PY, respectively; not shown).

## Discussion

In this nationwide cohort of adults with histologically confirmed NAFLD and paired liver biopsies, we observed dynamic changes in the presence and severity of NASH and fibrosis, which in turn had a meaningful impact on the long-term incidence of ESLD. Specifically, NAFLD progression conferred a 65% higher relative risk of developing ESLD, and an absolute excess rate of 12.4/1,000 PY. As expected, the highest absolute ESLD incidence rates were found with progression to cirrhosis (56.3/1,000 PY); however, markedly elevated rates were also evident with earlier histological transitions between paired biopsies. By highlighting the marked excess ESLD risk that accompanies NAFLD progression, our findings underscore the enormous potential benefit of public heath initiatives designed to reverse NASH and fibrosis. They also provide strong evidence that preventing NAFLD progression could dramatically reduce the growing burden of ESLD. Indeed, extrapolating our data to recent population-based estimates[17,24,25] (see Supplementary information) suggests that effectively preventing NAFLD progression could help prevent over 16,500 cases of incident ESLD in the US, and more than 10,200 cases in Western Europe, each year.

Currently, randomized-controlled trials of NAFLD therapeutics focus on short-term endpoints – *i.e.* NASH resolution and/or the improvement of ≥1 fibrosis stage without worsening of NASH – because these are assumed to be proxies for major clinical outcomes, including ESLD.[13] However, robust data supporting those assumptions are scarce, and no prior large study with serial biopsies and long-term follow-up has quantified the impact of NAFLD progression or regression on major clinical outcomes. We found that any NAFLD progression contributed to markedly higher rates of experiencing major ESLD events, and this was particularly true for incident or progressive fibrosis. Given the rapidly growing burden of NAFLD-related ESLD, and its substantial associated morbidity, our data provide epidemiological support for the current design of NAFLD therapeutic trials.

Prior studies have demonstrated that at the time of index biopsy, NAFLD histological severity independently predicts all-cause mortality.[17,24,25] However, it is not clear whether subsequent dynamic changes in NAFLD histology – including disease regression – might impact that mortality risk. We did not observe differences in mortality between patients with NAFLD progression and those with stable/regressed disease. On the one hand, this could be due to the selected nature of our paired biopsy cohort, as patients with clear evidence of NAFLD resolution might be less likely to undergo repeat biopsy. On the other hand, even among the subgroup of patients with non-cirrhotic fibrosis on initial biopsy (and who would thus be less likely to have complete NAFLD resolution), mortality rates were similar regardless of subsequent regression, disease stability, or progression. This finding also raises the possibility that patients with NAFLD might be more likely to develop other major risk factors that in turn can impact mortality – and those risk factors may not regress in parallel with NAFLD regression. Given this uncertainty, future studies are needed to fully

characterize the impact of NAFLD progression and regression profiles on major risk factors for mortality, and on both all-cause and cause-specific survival.

Our findings confirm and extend prior research demonstrating the dynamic natural history of NAFLD, which includes both progression and regression.[9-11,26] However, to date, paired biopsy studies[9-11,27-29] have all been limited by small sample sizes (largest N = 446),[11] and lack of long-term follow-up for major clinical outcomes. By including a large, nationwide population with prolonged follow-up time, the current study permitted detection of important differences in rates of major hepatic events across a range of histological transitions. For example, compared to patients with stable simple steatosis, patients who developed incident fibrosis had an absolute excess ESLD incidence of 18.9/1,000 PY over 10 years; this translates to one additional excess ESLD event for every five patients that progress to fibrosis. For patients with existing fibrosis, the risks of further progression were even more stark: compared to those with stable/regressed fibrosis, those who developed cirrhosis had an absolute excess ESLD incidence of 56.3/1,000 PY, translating over 10 years to one additional ESLD event for every two patients with interval progression to cirrhosis. As the worldwide burden of NAFLD continues to increase, these findings highlight the need for better tools to predict which patients with NAFLD are at highest risk of experiencing accelerated fibrosis progression and adverse events, and who thus may benefit from more aggressive, early interventions.[6]

This study benefits from a large, nationwide population with comprehensive and prospectively recorded histopathology reflecting real-world clinical care. We used strict, well-validated definitions of histological categories (all PPVs ≥87%),[17] in registers with near-complete follow-up for the entire Swedish population.[20] Moreover, our large sample size and long follow-up time permitted calculation of more precise risk estimates according to trajectories of NAFLD histological transitions, while minimizing the inherent limitations of previous, smaller studies.

We acknowledge several limitations. First, this was a retrospective study, and the indication for repeat biopsy was not standardized, which could introduce selection bias. However, our case distribution and risk estimates are broadly consistent with prior cohorts with single biopsies;[7,17,30-33] moreover, when we analyzed Swedish patients with just one clinical biopsy, we observed similar patterns of comorbidities and similar proportions ultimately developed incident ESLD. Second, it is possible that our findings may not be generalizable to the broader population with NAFLD who do not undergo liver biopsy. However, analyses specifically focused on NASH are not possible using existing non-invasive tools, and we would highlight that even in the presence of this potential selection bias, our findings of disease progression and stability/regression and their respective relationships to major outcomes are novel and clinically important. Third, despite our well-validated histology definitions,[17] histopathology is subject to sampling error and inter-observer variability, we lacked data regarding pathologists' access to prior histology slides, and hepatocyte ballooning may have been underreported in older liver biopsies; yet, our prior validation study demonstrated the accuracy of our exposure definitions, and with our large sample size, we expect that the proportions with over- or under-staged NAFLD were relatively balanced. Fourth, despite careful adjustment for clinical, demographic and medication confounders,

residual confounding is possible, and we lacked detailed data regarding indication for liver biopsy, individual fibrosis stages, alcohol use, or changes in body weight or laboratory values, and it is possible that the development of metabolic risk factors may not have been recorded in the included registers. Moreover, consistent with other administrative datasets, the recorded prevalence of diabetes was low, which could lead to unmeasured confounding. Accordingly, future large-scale studies are needed to assess the influence of changes in NAFLD activity score categories or individual fibrosis stages on rates of major outcomes, as well as studies testing whether changes in metabolic factors, body weight or alcohol use that influence NASH or fibrosis can subsequently impact long-term outcomes. Finally, further research incorporating biomarkers and/or serial non-invasive assessments is needed to help better identify patients most at-risk for accelerated disease progression and adverse events.

In conclusion, this nationwide, real-world cohort of adults with paired NAFLD liver biopsies demonstrates that a substantial proportion of patients experience disease progression, which in turn contributes to a marked excess risk of developing ESLD. In contrast, we did not observe significant mortality benefit with NAFLD regression; if that is confirmed in future studies, then additional research will be needed to carefully define the optimal risk-benefit balance of emerging NAFLD therapeutics, as they become available. Nevertheless, given the growing burden of NAFLD-related ESLD, our data support public health initiatives designed specifically to prevent NAFLD progression.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data availability statement

No additional data are available due to Swedish regulations.

## Abbreviations

| | |
|---|---|
| **aHR** | adjusted hazard ratio |
| **ESLD** | end-stage liver disease |
| **NAFLD** | non-alcoholic fatty liver disease |
| **NASH** | non-alcoholic steatohepatitis |
| **PY** | person-years |
| **PPV** | positive predictive value |

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## Highlights

- In adults with NAFLD and paired clinical liver biopsies, a substantial proportion experienced progression between biopsies.
- NAFLD progression was associated with a markedly higher incidence of end-stage liver disease.
- In contrast, NAFLD progression was not associated with significantly worse overall mortality.

### Impact and implications

Currently, data are scarce regarding the long-term impact of histological progression or regression of non-alcoholic fatty liver disease (NAFLD) on subsequent risk of adverse clinical outcomes, including the development of end-stage liver disease and mortality. This is particularly important because randomized-controlled trials of NAFLD therapeutics currently focus on short-term histological endpoints as presumed surrogates for those major clinical outcomes. Thus, the results from this study can help inform the optimal design of future NAFLD therapeutic trials, while also providing the necessary evidence base for public health policies focused on preventing the development and progression of NAFLD.

**Table 1.**

Characteristics of patients with NAFLD at the initial biopsy date.

| Characteristic | All NAFLD N = 718 | Simple steatosis n = 497 | NASH without fibrosis n = 90 | Non-cirrhotic fibrosis n = 131 |
|---|---|---|---|---|
| Female | 302 [42.1] | 204 [41.1] | 43 [47.8] | 55 [42.0] |
| Age at the index date, years, mean [SD] | 49.3 [13.9] | 49.1 [14.1] | 48.2 [14.0] | 51.1 [13.1] |
| Year of index biopsy | — | — | — | — |
| 1969–1989 | 193 [26.9] | 152 [30.6] | 21 [23.3] | 20 15.3] |
| 1990–2000 | 354 [49.3] | 252 [50.7] | 43 [47.8] | 59 45.0] |
| 2001–2010 | 140 [19.5] | 78 [15.7] | 22 [24.4] | 40 30.5] |
| 2011–2017 | 31 [4.3] | 15 [3.0] | 4 [4.4] | 12 [9.2] |
| Nordic country of birth | 666 [92.8] | 459 [92.4] | 83 [92.2] | 124 [94.7] |
| Highest education level[1] | (Among 525) | (Among n = 345) | (Among n = 69) | (Among n = 111) |
| ≤9 years | 160 [30.5] | 105 [30.4] | 24 [34.8] | 31 27.9] |
| 10–12 years | 227 [43.2] | 145 [42.0] | 33 [47.8] | 49 [44.1] |
| ≥13 years | 130 [24.8] | 90 [26.1] | 11 [15.9] | 29 [26.1] |
| Unknown | 8 [1.5] | 5 [1.5] | 1 [1.5] | 2 [1.8] |
| Cardiovascular disease | 108 [15.0] | 75 [15.1] | 13 [14.4] | 20 [15.3] |
| Metabolic syndrome features[2] | 181 [25.2] | 118 [23.7] | 21 [23.3] | 42 [32.1] |
| Dyslipidemia | 23 [3.2] | 12 [2.4] | 1 [1.11] | 10 [7.6] |
| Diabetes | 45 [6.3] | 25 [5.0] | 6 [6.7] | 14 [10.7] |
| Hypertension | 77 [10.7] | 48 [9.7] | 10 [11.1] | 19 [14.5] |
| Obesity | 16 [2.2] | 9 [1.8] | 2 [2.2] | 5 [3.8] |

All variables reported as n [%] unless otherwise stated. For definitions of the NAFLD histological groups and all demographic and clinical covariates, see the Supplementary methods and Table S3.

HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

[1]Education level categories were based on compulsory school, high school, and college (see Supplementary methods for details). Education level was recorded in Sweden beginning in 1990, thus data presented are for persons with index dates on or after January 1, 1990. For all analyses, persons with index dates prior to 1990 had education level recorded as missing.

[2]Metabolic syndrome features was defined as any one of the following components of the metabolic syndrome (*i.e.* dyslipidemia, diabetes, hypertension and/or obesity), as outlined in the Methods and defined in Table S3.

**Table 2.**

Distribution of NAFLD histology[1] at the first and second liver biopsies.

| Empty Cell | Second biopsy, n (%)[1] | | | | |
|---|---|---|---|---|---|
| | **Normal** | **Simple steatosis** | **NASH without fibrosis** | **Non-cirrhotic fibrosis** | **Cirrhosis** |
| **First biopsy, n** | — | — | — | — | — |
| **Simple steatosis, n = 497** | 103 (20.7) | 223 (44.9) | 62 (12.5) | 91 (18.3) | 18 (3.6) |
| **NASH without fibrosis, n = 90** | 4 (4.4) | 17 (18.9) | 37 (41.1) | 25 (27.8) | 7 (7.8) |
| **Non-cirrhotic fibrosis, n = 131** | 11 (8.4) | 29 (22.1) | 18 (13.7) | 58 (44.3) | 15 (11.5) |

NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

[1]NAFLD was defined from liver histology using a validated algorithm, as outlined in the Methods and Supplementary methods. Proportions shown represent the % of individuals in each NAFLD histological category at the second biopsy, divided by the total number of individuals in the original histological category at the time of the first liver biopsy.

**Table 3.**

Characteristics of patients with NAFLD at the second biopsy date.

| Characteristic | All NAFLD N = 718 | Normal n = 118 | Simple steatosis n = 269 | NASH without fibrosis n = 117 | Non-cirrhotic fibrosis n = 174 | Cirrhosis n = 40 |
|---|---|---|---|---|---|---|
| Female | 302 [42.1] | 59 [50.0] | 100 [37.2] | 53 [45.3] | 71 [40.8] | 19 [47.5] |
| Age, years, mean [SD] | 55.0 [13.9] | 57.2 [14.4] | 53.6 [13.8] | 55.5 [13.8] | 53.7 [14.2] | 61.3 [9.6] |
| Time between biopsies, years, median, [min, max] | 3.4 [0.5, 34.7] | 3.4 [0.5, 23.9] | 3.4 [0.5, 33.2] | 2.4 [0.5, 21.1] | 4.3 [0.5, 25.8] | 3.9 [0.5, 34.7] |
| Year of follow-up biopsy | — | | — | — | — | — |
| 1969–1989 | 102 [14.2] | 21 [17.8] | 41 [15.2] | 26 [22.2] | 9 [5.2] | 5 [12.5] |
| 1990–2000 | 243 [33.8] | 41 [34.8] | 86 [32.0] | 44 [37.6] | 57 [32.8] | 15 37.5] |
| 2001–2010 | 266 [37.1] | 35 [29.7] | 114 [42.4] | 36 [30.8] | 70 [40.2] | 11 27.5] |
| 2011–2017 | 107 [14.9] | 21 [17.8] | 28 [10.4] | 11 [9.4] | 38 [21.8] | 9 [22.5] |
| Nordic country of birth | 666 [92.8] | 109 [92.4] | 251 [93.3] | 105 [89.7] | 162 [93.1] | 39 [97.5] |
| Highest education level[1] | (Among n = 616) | (Among n = 97) | (Among n = 228) | (Among n = 91) | (Among n = 165) | (Among n = 35) |
| ≤9 years | 196 [31.8] | 28 [28.9] | 78 [34.2] | 27 [29.7] | 45 [27.3] | 18 51.4] |
| 10–12 years | 249 [40.4] | 37 [38.1] | 94 [41.2] | 42 [46.2] | 65 [39.4] | 11 [31.4] |
| ≥13 years | 146 [23.7] | 20 [20.6] | 54 [23.7] | 19 [20.9] | 29.7] | 4 [11.4] |
| Unknown | 25 [4.1] | 12 [12.4] | 2 [0.9] | 3 [3.3] | 6 [3.6] | 2 [5.7] |
| Cardiovascular disease | 191 [26.6] | 39 [33.1] | 63 [23.4] | 29 [24.8] | 48 [27.6] | 12 [30.0] |
| Dyslipidemia | 87 [12.1] | 10 [8.5] | 26 [9.7] | 12 [10.3] | 32 [18.4] | 7 [17.5] |
| Diabetes | 136 [18.9] | 17 [14.4] | 44 [16.4] | 17 [14.5] | 46 [26.4] | 12 [30.0] |
| Hypertension | 171 [23.8] | 28 [23.7] | 49 [18.2] | 21 [18.0] | 59 [33.9] | 14 [35.0] |
| Obesity | 32 [4.5] | 9 [7.6] | 8 [3.0] | 4 [3.4] | 7 [4.0] | 4 [10.0] |
| Any metabolic syndrome feature[2] | 329 [45.8] | 59 [50.0] | 109 [40.5] | 47 [40.2] | 91 [52.3] | 23 [57.5] |
| Available medication use data[3] | (Among n = 205) | (Among n = 40) | (Among n = 53) | (Among n = 24) | (Among n = 74) | (Among n = 14) |
| Aspirin | 40 [19.5] | 10 [25.0] | 10 [18.9] | 4 [16.7] | 12 [16.2] | 4 [28.6] |
| Statin | 64 [31.2] | 10 [25.0] | 19 [35.9] | 7 [29.2] | 22 [29.7] | 6 [42.9] |
| Antidiabetic medication | 57 [27.8] | 6 [15.0] | 13 [24.5] | 7 [29.2] | 24 [32.4] | 7 [50.0] |

NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

All variables reported as n [%], unless otherwise stated. For definitions of the NAFLD histological groups and all demographic and clinical covariates, see Supplementary methods and Table S3.

[1]Education level categories were based on compulsory school, high school, and college (see supplementary methods for details). Education level was recorded beginning in 1990, thus data presented are for persons with the second liver biopsy on or after January 1, 1990.

[2]Metabolic syndrome features was defined as any one of the following components: dyslipidemia, diabetes, hypertension and/or obesity, as outlined in the Methods and defined in Table S3.

[3]Medication use data was ascertained from the Prescribed Drug Register, which began on July 1, 2005; thus, data presented are for persons with index dates on or after January 1, 2006, as outlined in the Methods.

**Table 4.**

Incident ESLD[1] and all-cause mortality with histological progression of NAFLD between paired biopsies[*].

| First biopsy[*] | Second biopsy[*] | n | Total events, n | Person-Years | Incidence per 1,000 person-years[2] (95% CI) | Absolute rate difference[2] (95% CI) | Adjusted HR[3] (95% CI) |
|---|---|---|---|---|---|---|---|
| **Incident ESLD** | | | | | | | |
| All non-cirrhotic NAFLD | Stable or regression | 500 | 72 | 6,313 | 11.4 [8.9-14.4] | 0 (Ref.) | 1 (Ref.) |
| | Progression (any) | 218 | 59 | 2,476 | 23.8 [18.1-30.7] | 12.4 [5.8-19.1] | 1.65 [1.17-2.32] |
| Simple steatosis | Stable or regression | 326 | 33 | 4,352 | 7.6 [5.2-10.7] | 0 (Ref.) | 1 (Ref.) |
| | Incident NASH | 62 | 10 | 832 | 12.0 [5.8-22.1] | 4.4 [−3.5-12.3] | 1.30 [0.60-2.82] |
| | Incident fibrosis | 109 | 31 | 1,173 | 26.4 [18.0-37.5] | 18.9 [9.2-28.5] | 2.88 [1.70-4.87] |
| NASH without fibrosis | Stable or regression | 58 | 12 | 820 | 14.6 [7.6-25.6] | 0 (Ref.) | 1 (Ref.) |
| | Incident fibrosis | 32 | 8 | 346 | 23.1 [10.0-45.6] | 8.5 [−9.6-26.5] | 2.44 [1.33-6.41] |
| Non-cirrhotic fibrosis | Stable or regression | 116 | 27 | 1,140 | 23.7 [15.6-34.5] | 0 (Ref.) | 1 (Ref.) |
| | Incident cirrhosis | 15 | 10 | 125 | 80.1 [38.4-147.3] | 56.3 [5.9-106.7] | 3.25 [1.60-6.57] |
| **All-cause mortality** | | | | | | | |
| All non-cirrhotic NAFLD | Stable or regression | 500 | 234 | 6,563 | 35.7 [31.2-40.5] | 0 (Ref.) | 1 (Ref.) |
| | Progression (any) | 218 | 107 | 2,650 | 40.4 [33.1-48.8] | 4.7 [−4.2-13.6] | 0.99 [0.78-1.24] |
| Simple steatosis | Stable or regression | 326 | 151 | 4,479 | 33.7 [28.6-39.5] | 0 (Ref.) | 1 (Ref.) |
| | Incident NASH | 62 | 36 | 850 | 42.4 [29.7-58.7] | 8.6 [−6.2-23.5] | 1.12 [0.75-1.67] |
| | Incident fibrosis | 109 | 51 | 1,263 | 40.4 [30.1-53.1] | 6.7 [−5.7-19.0] | 1.22 [0.86-1.73] |
| NASH without fibrosis | Stable or regression | 58 | 31 | 841 | 36.9 [25.1-52.4] | 0 (Ref.) | 1 (Ref.) |
| | Incident fibrosis | 32 | 13 | 376 | 34.5 [18.4-59.1] | −2.3 [−25.1-20.6] | 0.67 [0.31-1.46] |
| Non-cirrhotic fibrosis | Stable or regression | 116 | 52 | 1,243 | 41.8 [31.2-54.9] | 0 (Ref.) | 1 (Ref.) |
| | Incident cirrhosis | 15 | 7 | 160 | 43.7 [17.6-90.1] | 1.9 [−32.4-36.3] | 0.58 [0.23-1.47] |

ESLD, end-stage liver disease; HR, hazard ratio; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

[*] NAFLD was defined from liver histology, as outlined in the Methods and Supplementary methods. For definitions of NAFLD progression and stable/regressed disease, see Methods.

[1] Incident ESLD was a composite endpoint defined by ≥1 inpatient primary diagnosis for cirrhosis, liver decompensation event (ascites, spontaneous bacterial peritonitis, esophageal variceal hemorrhage, hepatorenal syndrome or hepatic encephalopathy), incident hepatocellular carcinoma or liver transplantation, using validated definitions as per the supplementary methods and Table S3.

[2] Confidence intervals for incidence rates and absolute rate differences were approximated by the Poisson distribution.

[3] The multivariable-adjusted model accounted for matching factors (age, sex, county of residence and calendar year) with further adjustment for time between biopsies, education level, cardiovascular disease, and components of the metabolic syndrome (*i.e.*, diabetes, obesity, hypertension and/or dyslipidemia). For details and definitions, see the Methods, Supplementary methods and Table S3.