Nonalcoholic fatty liver disease and risk of intracerebral hemorrhage

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Abstract  Background and aims: This study aimed to investigate the association between the steatosis severity of nonalcoholic fatty liver disease (NAFLD) and future intracerebral hemorrhage (ICH) risk.

Methods and results: We used data from the Kailuan study. Participants without a history of stroke, myocardial infarction, cancer, other liver diseases or alcohol abuse were enrolled. NAFLD and the severity of liver steatosis were assessed by abdominal ultrasonography. We stratified the participants into different groups according to the severity changes in liver steatosis status across the first 4-year follow-up period. The outcome was the first occurrence of ICH during the next 6-year follow-up period. Hazard ratios (HRs) and 95% CI of ICH were estimated using Cox models adjusted for potential risk factors. A total of 49,906 participants were enrolled in this study. During a median of 6.79 years of follow-up, 193 incident ICH cases were identified. Compared with persistent nonfatty liver participants, the hazard ratios (HRs) for participants with persistent mild steatosis, persistent moderate steatosis, persistent severe steatosis, alleviating steatosis, and aggravating steatosis were 1.28 (95% CI, 0.75–2.18), 2.33 (95% CI, 1.24–4.38), 1.63 (95% CI, 0.22–12.11), 1.41 (95% CI, 0.91–2.18), and 1.37 (95% CI, 0.94–2.00), respectively, in the fully adjusted model.

Conclusions: NAFLD with persistent moderate steatosis was significantly related to an increased risk of future ICH, independent of other conventional risk factors.

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1. Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing in parallel with obesity and is becoming the leading chronic liver disease worldwide [1]. Evidence suggests that patients with NAFLD are frequently accompanied by features of metabolic syndrome [2] and have higher overall mortality from cardiovascular disease [3]. In recent years, some studies have also suggested that NAFLD is associated with the development of ischemic stroke [4,5]. It is known that ischemic stroke and hemorrhagic stroke have several common underlying pathologies. In addition, previous studies found that other kinds of liver diseases were associated with an increased risk of intracerebral hemorrhage (ICH) [6,7]. Therefore, it is plausible to assume NAFLD might also be associated with the development of ICH. However, few studies have focused on the association between NAFLD and ICH to date.

Of note, NAFLD encompasses a wide spectrum range from steatosis to nonalcoholic steatohepatitis with varying amounts of steatosis and fibrosis [2]. It is important to stratify patients according to different severities of NAFLD in studies, as different severity stages might lead to different clinical outcomes. A previous study demonstrated that patients with a worse intensity of NAFLD have an increasingly high risk of incident cardiovascular events, but ICH itself was not regarded as an independent outcome [4]. However, none of the published studies have focused on the association between the severity of NAFLD steatosis and ICH to date. Furthermore, it is noteworthy that the severity of NAFLD steatosis could be aggravated or alleviated through weight loss, dietary changes and management of dyslipidemia during a period. The influence of NAFLD on patients is continuous, indicating that long-term NAFLD status might have a stronger impact on the development of ICH than contemporary NAFLD status. Therefore, we conducted a community-based follow-up analysis to estimate the association between changes in NAFLD steatosis severity across a period of follow-up and future ICH risk.

2. Methods

2.1. Study population

The Kailuan study is a community-based, prospective, observational study aimed at investigating the epidemiology of asymptomatic polyvascular abnormalities in Chinese adults. The details of the Kailuan study have been reported previously [8]. Briefly, 101,506 participants aged older than 18 years agreed to participate and underwent questionnaire assessments and laboratory and imaging examinations at baseline in 2006. The same assessments were repeatedly performed every two years from 2006 to 2016. There were 1204 participants with missing data on ultrasonography data of steatosis and 24,225 participants in 2010. In addition, we excluded 4876 participants with a history of stroke, myocardial infarction, cancer, or other known liver diseases, including chronic liver parenchymal disorders, acute fatty liver of pregnancy, hepatitis B, and hepatitis C in 2010; 2485 participants with missing data of alcohol intake; and 18,810 participants with excessive alcohol abuse until 2010 (excessive alcohol abuse was defined as men who drank 20 g per day and women with a drinking history for at least a year). Finally, a total of 49,906 participants were enrolled in this study. Baseline characteristics between participants included and excluded are shown in Supplementary Table 1. Our study was approved by the Ethics Committee of the Kailuan General Hospital. The Kailuan study was registered at the International Clinical Trials Registry Platform (Unique identifier: ChiCTR-TNRC-11001489). Informed consent was signed by all participants.

2.2. NAFLD and potential covariates assessment

The diagnosis of NAFLD and the assessment of liver steatosis severity were based on the results of abdominal ultrasonography using a high-resolution B-mode topographical ultrasound system with a 3.5 MHz probe (ACUSON X300, Siemens, Germany). Abdominal ultrasonography was performed for each participant at baseline in 2006 and after 4 years in 2010. Experienced radiologists were blinded to the baseline information and laboratory results of the participants. After excluding other causes of liver diseases and excessive alcohol intake, NAFLD was diagnosed by the presence of at least two of three abnormal findings: (1) diffusely increased echogenicity in the liver with echogenicity greater than that in the spleen or kidney; (2) deep attenuation of the ultrasound signal; and (3) poor visualization of intrahepatic structures. The severity of liver steatosis was differentiated by abdominal ultrasonography: nonfatty liver; mild steatosis when there was a diffuse increase in fine echoes in the liver parenchyma; moderate steatosis when there was slightly impaired visualization of the intrahepatic vessels and diaphragm; and severe steatosis when there was a diffuse increase in fine echoes with no visualization of the intrahepatic vessels and diaphragm [4]. We stratified the participants into six groups according to the changes in liver steatosis severity across the 4-year follow-up period. Including persistent nonfatty liver; persistent mild steatosis, persistent moderate and persistent severe steatosis when participants were diagnosed to have mild, moderate, and severe steatosis in both 2006 and 2010; alleviating steatosis when the degree of steatosis was alleviated in 2010 compared with the result in 2006; aggravating steatosis when the degree of steatosis became more severe in 2010 compared with the result in 2006.

Demographic variables, medical history, drinking condition, smoking status, education level, income level and physical activity were collected by trained investigators with questionnaires. A standardized questionnaire was used to assess the information about alcohol intake by trained investigators. Body mass index (BMI) was calculated as follows: BMI was calculated as body weight (kg) divided by the square of height (m²). Blood pressure was...
measured on the left arm in a seated position using a mercury sphygmomanometer following the standard recommended procedures. Two measures were taken after participants had rested for at least 5 min. The average of two readings was used. Hypertension was defined as a systolic blood pressure $\geq$ 140 mmHg or a diastolic blood pressure $\geq$ 90 mmHg, a previous history of hypertension, or current use of antihypertensive agents. An autoanalyzer (Hitachi 747; Hitachi, Tokyo, Japan) at the central laboratory of Kailuan Hospital was used to analyse the levels of fasting blood glucose (Fbg), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), high-sensitivity C-reactive protein (hsCRP) and liver function indices. Blood samples were collected under fasting conditions and within 4 h of preparation.

### 2.3. ICH identification and follow-up assessment

Participants were followed up face-to-face by trained investigators every 2 years until the occurrence of the first ICH or December 31, 2016, which came first. The first occurrence of ICH from 2010 to 2016 was included as the outcome. The identification of ICH has been described in detail in a previous study [9]. In brief, ICH was defined according to the World Health Organization criteria [10]. The outcome information was confirmed by checking medical records from medical insurance and discharge summaries from the 11 hospitals. For potential ICH patients, trained abstractors recorded their symptoms, signs and neuroimages, including computed tomography and magnetic resonance imaging. Trained physicians reviewed all neuroimages, and disagreements were resolved by another physician.

### 2.4. Statistical analysis

Continuous variables are described by medians and interquartile range (IQR) and compared using the Kruskal–Wallis test because of skewed distributions. Categorical variables are presented as frequencies and percentages and were compared using the chi-square test. The Kaplan–Meier curve and log-rank test were performed to present cumulative ICH risk for each steatosis severity group. The hazard ratios and 95% CI of the incidence of ICH were estimated from Cox proportional hazard models. In model 1, we adjusted for age, sex, BMI, smoking status, and physical activity in 2006. Model 2 was adjusted for model one plus education levels, history of diabetes, hypertension, dyslipidemia, antidiabetic agents, antihypertensive agents, lipid lowering agents, antiplatelet and anticoagulant agents, Fbg and hs-CRP in 2006. Model 3 was adjusted for all of the variables mentioned above in 2010. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC, USA). A two-sided $P < 0.05$ was considered statistically significant.

### 3. Results

#### 3.1. Baseline characteristics

A total of 49,906 participants were enrolled in our study, with a median age of 49.28 years. During 548,659 person-years of follow-up between 2010 and 2016, 193 incident ICH cases were identified. There were significant differences in age, sex, educational status, BMI, systolic blood pressure, Fbg, hsCRP, history of diabetes mellitus and hypertension, and antihypertensive, antidiabetic, and antiplatelet agent use between participants with and without ICH. Compared with participants who did not develop ICH, participants with ICH were more likely to be male, have an older age, have a lower level of education, have a higher BMI, have higher systolic blood pressure, have higher Fbg and hsCRP levels, be more likely to suffer from hypertension and diabetes, and have higher proportions of current antihypertensive, antidiabetic and antiplatelet agent use (Table 1).

Among 49,906 participants enrolled in this study, the numbers (%) of persistent nonfatty liver, persistent mild, persistent moderate, persistent severe, alleviating, and aggravating liver steatosis were 25,833 (51.76), 4031 (8.08), 1655 (3.32), 210 (0.42), 6276 (12.58) and 11,901 (23.85), respectively.

#### 3.2. Correlation between changes in liver steatosis severity and ICH risk

Over a median follow-up of 6.79 years, a total of 193 ICH events were identified. Including 0.29% (76 out of 25,833) of persistent nonfatty liver participants, 0.45% (18 out of 4031) of NAFLD participants with persistent mild steatosis, 0.79% (13 out of 1655) of NAFLD participants with persistent moderate participants, 0.48% (1 out of 210) of NAFLD participants with persistent severe participants, 0.51% (32 out of 6276) of NAFLD participants with aggravating steatosis participants and 0.45% (53 out of 11,901) of NAFLD participants with alleviating steatosis. Compared with the nonfatty liver group, participants with persistent mild, moderate, severe, alleviating, and aggravating steatosis showed a trend of increasing risk of ICH. After adjusting for potential confounding factors, a significant association remained between NAFLD participants with persistent moderate steatosis and ICH. The HR values for participants with persistent moderate steatosis in the three different adjusted models were 2.20 (95% CI, 1.17–4.14), 1.98 (95% CI, 1.05–3.73) and 2.33 (95% CI, 1.24–4.38), respectively. The association between other liver steatosis severity groups and ICH did not reach statistical significance in the fully adjusted model (Table 2). The results of the Kaplan–Meier curve and log-rank test also suggested a higher ICH risk in NAFLD participants with persistent moderate steatosis than in others, especially those with nonfatty liver disease ($P = 0.0061$; Figure 1).
4. Discussion

In this large, population-based long-term follow-up study of 49,906 adults, we found that persistent moderate liver steatosis in participants with NAFLD was independently associated with an increased risk of ICH after adjustment for demographic characteristics, potential ICH risk factors and relevant comorbidities.

To date, several studies have reported that NAFLD is related to an increased risk of cardiovascular disease. A large meta-analysis of participants from 34 studies showed that NAFLD and the severity of NAFLD were associated with increased incident coronary artery disease and other adverse cardiovascular risk factors, including hypertension and atherosclerosis [11]. Another meta-analysis of 34,043 individuals from 16 observational studies also found that NAFLD patients, especially those with more severe liver stenosis, had a higher risk of overall
cardiovascular events (including death, myocardial infarction, coronary revascularizations, and stroke) [5]. In recent years, studies have provided further evidence that NAFLD is an independent risk factor for ischemic stroke. A large community-based cohort study in China demonstrated that NAFLD and the severity of NAFLD were associated with a higher risk of ischemic stroke events [4]. A meta-analysis with 135,602 individuals showed that a stepwise increment of steatosis of NAFLD can significantly increase the risk of carotid atherosclerosis and future ischemic stroke risk [12]. Endothelial dysfunction, oxidative stress, inflammation, insulin resistance and altered lipid metabolism might be the underlying mechanisms [13]. However, unlike ischemic stroke, few studies have focused on the relationship between NAFLD and another subtype of stroke, ICH. The results have not been consistent. A previous study reported that compared with those without NAFLD, participants with NAFLD had a significantly higher frequency of ICH [14]. However, in this study, the authors only used univariate analysis to estimate the relationship between NAFLD and ICH, which limited the credibility of the results. Another population-based cohort study of 456,100 participants demonstrated that gamma-glutamyl transferase (GGT), a surrogate marker of NAFLD, was significantly associated with a higher risk of ICH, independent of alcohol consumption [15]. In contrast, another study with a small sample size of 128 participants demonstrated that NAFLD did not affect ICH development or severity [16]. Recently, a recent meta-analysis including 64 studies with 135,602 individuals showed that participants with NAFLD did not have significantly higher odds of developing hemorrhagic stroke than participants without NAFLD (OR: 1.85, 95% CI: 0.20–17.40) [12]. However, as NAFLD is a reversible disease, observational studies based on a single time-point measurement could not indicate dynamic changes. Our study results add new evidence for the relationship between the evolution of liver steatosis severity in patients with NAFLD across 4 years and the risk of future ICH. The results of the current study showed that compared with patients with persistent nonfatty liver, NAFLD participants with persistent moderate steatosis had a significantly higher risk of incident ICH even in the fully adjusted model. The contradictory results might be partially explained by ethnic differences and the relatively small sample size in the previous study. Some of the studies did not or only partially adjusted for potential confounders. In addition, our study was the first to take the changes in liver steatosis severity over a period into consideration. Some studies have demonstrated that only advanced-stage liver disease, but not mild liver disease, contributes to the occurrence of ICH. Two meta-analyses showed that patients with nonalcohol-related cirrhosis, a severe end stage of chronic liver disease that might develop from NAFLD [2], had an increased risk of ICH. Worse liver dysfunction, coagulopathy and imbalance of prohemorrhagic derangements might be the underlying mechanisms [17,18].

Unexpectedly, we did not find the same result in NAFLD participants with persistent severe liver steatosis. However, this result should be interpreted with caution, as the nonsignificant association between persistent severe steatosis and ICH might be due to insufficient sample size in this group. Another possible explanation is that a hypercoagulation state develops in severe NAFLD, which may attenuate the hemorrhagic tendency. Increasing evidence suggests that severe liver disease might be accompanied by mixed coagulopathy, which leads to thrombotic processes or hemorrhage. It is possible that prothrombotic and hypercoagulation due to a significant inflammatory state play a more important role in severe NAFLD than in mild and moderate NAFLD [19]. Thus, patients with severe NAFLD were more likely to develop ischemic cardiovascular and cerebrovascular diseases rather than ICH. However, we were unable to confirm this speculation in that we did not have information on the coagulation status of the participants. Further studies with a larger number of participants are needed to confirm the results.

The pathophysiological and causative mechanisms linking NAFLD and ICH are not yet definitive. The significant relationship between NAFLD and metabolic risk factors for ICH might be a possible explanation. NAFLD was once considered a hepatic manifestation of metabolic syndrome since they share common risk factors. Growing evidence further suggests that NAFLD may itself induce the development of metabolic syndrome [19]. Among the clinical characteristics related to metabolic syndrome, hypertension, diabetes, and obesity are all well-known causative risk factors for ICH [20,21]. Among them, hypertension is the most common cause of ICH. A recent meta-analysis indicated that NAFLD is associated with a 1.6-fold increased risk of developing hypertension [22]. Consistently, NAFLD patients were found to have a higher prevalence of hypertension and other metabolic risk factors mentioned above in both previous and the current study [23,24]. This suggests that hypertension, along with other metabolic factors, might be mediators of the association between NAFLD and ICH. However, it is noteworthy that in the current study, the association between NAFLD participants with persistent moderate steatosis and ICH remained significant even after we adjusted for hypertension and other potential covariates. This indicates that a higher risk of metabolic risk factors in NAFLD patients is at least not the only reason for the increase in ICH risk. Another underlying mechanism is that patients with NAFLD usually have a higher risk of liver dysfunction, which might result in hemostatic abnormalities, including impaired synthesis of clotting factors and heightened fibrinolysis, thrombocytopenia and platelet dysfunction, which can further induce hemorrhagic tendencies [25].

Previous studies reported that elevated aminotransferase and GGT levels, lower platelet counts and disrupted platelet function were associated with an increased risk of ICH [15,21,26]. In our study, participants with NAFLD had higher serum aminotransferase levels than those without. However, we did not observe lower platelet counts in patients with NAFLD. Hemostatic parameters and platelet function were not available in the current study. Further studies are needed to confirm the roles of these factors in...
the relationship between NAFLD and ICH. In addition, a heavier fibrosis burden in more severe NAFLD [27,28], endothelial dysfunction triggered by increased visceral fat accumulation [29], and vascular inflammation in patients with NAFLD might also contribute to the development of ICH through vascular wall damage and blood–brain barrier breakdown [15,30–32].

The strength of this study is the population-based database with a well-characterized individual cohort. The large sample size of our study population assured the statistical power for our results. However, a few limitations in our study deserve attention. First, we could not determine the causal relationship between NAFLD and ICH due to the observational study design. Second, we used the abdominal ultrasound method to diagnose and quantify the severity of liver steatosis, while liver biopsy is the standard criterion for NAFLD diagnosis. However, liver biopsy is an invasive method that is not applicable for screening healthy populations. Abdominal ultrasonography, on the other hand, is a noninvasive, low-cost, safe and accessible imaging technique that has been widely used for fatty liver diagnosis in clinical practice. The ultrasonography evaluation of liver steatosis is mainly qualitative, with a grading conveniently classified as mild, moderate or severe [33]. In fact, a meta-analysis has demonstrated that conventional ultrasonography allows for reliable and accurate detection of ≥5% histologically defined liver steatosis (82% sensitivity and 80% specificity), as well as moderate to severe liver steatosis (85% sensitivity and 85% specificity), compared to liver histology [34]. Nevertheless, further studies are needed to confirm our results using other methods to assess NAFLD and liver steatosis severity, such as ultrasonographic fatty liver indicator (US-FLI), controlled attenuation parameter (CAP) and magnetic resonance imaging. Third, we measured alcohol consumption with a questionnaire; although conducted by trained investigators, measurement error might still exist and be a cause of residual confounding. Fourth, information on coagulation indices, gamma-glutamyl transferase levels and platelet function was not available in our study, which made us unable to determine their role in the association between NAFLD and ICH. Finally, we were not able to conduct subgroup analyses because of the relatively small ICH sample sizes in some of the groups. Future studies with larger sample sizes and in other races are needed to confirm our results.

5. Conclusion

In conclusion, NAFLD participants with persistent moderate steatosis were independently associated with an increased risk of future ICH compared with those without fatty liver. This indicates that screening and treatment for patients at high risk of NAFLD might become a potential way to predict and reduce ICH risk. Future studies in other races with larger populations are still needed to confirm this result. Moreover, deeper insight into the potential mechanisms of this association is needed for prevention and therapeutic purposes and awaits further studies.

Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2022.08.010.

Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>NAFLD</td>
<td>nonalcoholic fatty liver disease</td>
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<td>ICH</td>
<td>intracerebral hemorrhage</td>
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<td>HR</td>
<td>hazard ratio</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>FBG</td>
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<td>LDL-C</td>
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<td>hsCRP</td>
<td>high-sensitivity C-reactive protein</td>
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<td>GGT</td>
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