Factors influencing subclinical atherosclerosis in patients with biopsy-proven nonalcoholic fatty liver disease

Taeang Arai, Masanori Atsukawa, Akihito Tsubota, Tadamichi Kawano, Mai Koeda, Yuji Yoshida, Tomohide Tanabe, Tomomi Okubo, Korenobufu Hayama, Ai Iwashita, Norio Itokawa, Chisa Kondo, Keiko Kaneko, Chiaki Kawamoto, Tsutomu Hatori, Naoya Emoto, Etsuko Iio, Yasuhiro Tanaka, Katsuhiko Iwakiri

1 Division of Gastroenterology and Hepatology, Nippon Medical School, Tokyo, Japan, 2 Core Research Facilities for Basic Science, Research Center for Medical Sciences, The Jikei University School of Medicine, Tokyo, Japan, 3 Division of Gastroenterology, Nippon Medical School Chiba Hokusho Hospital, Inzai, Japan, 4 Division of Pathology, Nippon Medical School Chiba Hokusho Hospital, Inzai, Japan, 5 Division of Endocrinology, Nippon Medical School Chiba Hokusho Hospital, Inzai, Japan, 6 Department of Virology and Liver Unit, Nagoya City University Graduate School of Medicinal Sciences, Nagoya, Japan

These authors contributed equally to this work.
* momogachi@yahoo.co.jp

Abstract

Although the presence of nonalcoholic fatty liver disease (NAFLD) is known to be related to subclinical atherosclerosis, the relationship between the severity of NAFLD and subclinical atherosclerosis is not clear. This study aimed to clarify the factors related to subclinical atherosclerosis, including the histopathological severity of the disease and PNPLA3 gene polymorphisms, in NAFLD patients. We measured brachial-ankle pulse wave velocity (baPWV) as an index of arterial stiffness in 153 biopsy-proven NAFLD patients. The baPWV values were significantly higher in the advanced fibrosis group than in the less advanced group (median, 1679 cm/s vs 1489 cm/s; \( p = 5.49 \times 10^{-4} \)). Multiple logistic regression analysis revealed that older age (\( \geq 55 \) years) (\( p = 8.57 \times 10^{-3}; \text{OR} = 3.03 \)), hypertension (\( p = 1.05 \times 10^{-3}; \text{OR} = 3.46 \)), and advanced fibrosis (\( p = 9.22 \times 10^{-3}; \text{OR} = 2.94 \)) were independently linked to baPWV \( \geq 1600 \) cm/s. NAFLD patients were categorized into low-risk group (number of risk factors = 0), intermediate-risk group (\( \geq 1 \)), and high-risk group (\( \geq 2 \)) based on their risk factors, including older age, hypertension, and biopsy-confirmed advanced fibrosis. The prevalence of baPWV \( \geq 1600 \) cm/s was 7.1% (3/42) in the low-risk group, 30.8% (12/39) in the intermediate-risk group, and 63.9% (46/72) in the high-risk group. Non-invasive liver fibrosis markers and scores, including the FIB-4 index, NAFLD fibrosis score, hyaluronic acid, Wisteria floribunda agglutinin positive Mac-2-binding protein, and type IV collagen 7s, were feasible substitutes for invasive liver biopsy. Older age, hypertension, and advanced fibrosis are independently related to arterial stiffness, and a combination of these three factors may predict risk of arteriosclerosis in NAFLD patients.
Introduction

Nonalcoholic fatty liver disease (NAFLD) is a major chronic liver disease, with a worldwide prevalence of approximately 25% [1, 2]. The disease is associated with the risk of progression to liver cirrhosis and hepatocellular carcinoma in some patients [3, 4]. The prognosis of NAFLD patients depends on the advancement of liver fibrosis [5–7] and is less favorable than that of healthy individuals. Furthermore, numerous patients die of cardiovascular disease (CVD) rather than liver-related events [6–8]. NAFLD is a multifactorial disease mutually associated with metabolic syndrome [9]. Reportedly, the presence of NAFLD is associated with subclinical atherosclerosis, independent from conventional metabolic risk factors [10–12]. However, the association between the advancement liver fibrosis and subclinical atherosclerosis remains controversial. In most of the previous studies, NAFLD was diagnosed based on abdominal ultrasonography and serum alanine aminotransferase (ALT) level, but not liver biopsy. Only a few studies have investigated the association between histological severity and subclinical atherosclerosis in biopsy-diagnosed NAFLD patients [13–15].

Recently, a genome-wide association study (GWAS) and subsequent related studies have demonstrated that single nucleotide polymorphisms (SNPs) in the patatin-like phospholipase domain containing 3 gene (PNPLA3) are associated with the development and severity of NAFLD [16–18]. The association between the PNPLA3 SNP genotype and atherosclerosis in Italian NAFLD patients was previously reported [19]. Similarly, it has been confirmed in Japanese patients that the PNPLA3 is a susceptibility gene involved in the development and advancement of NAFLD [20, 21], though its association with subclinical atherosclerosis has not been investigated.

In this study, arterial stiffness was evaluated using brachial-ankle pulse wave velocity (baPWV), and factors influencing arterial stiffness, including histological findings and the PNPLA3 SNP, were investigated in Japanese biopsy-confirmed NAFLD patients. In addition, we performed a risk assessment for arteriosclerosis using clinical parameters.

Materials and methods

Patients

Among patients who visited Nippon Medical School Chiba Hokusoh Hospital and Nippon Medical School Hospital between August 2013 and July 2018, 153 patients aged 18 years or older underwent histological evaluation and were diagnosed with NAFLD, according to the European Association for the Study of the Liver guidelines as follows [22–24]: NAFLD was defined as the presence of steatosis in ≥5% of hepatocytes according to histological analysis. Exclusion criteria included 1) daily alcohol consumption ≥30 g for males and ≥20 g for females; 2) other chronic liver diseases, such as viral hepatitis B or C, autoimmune hepatitis, Wilson disease, and hemochromatosis; 3) secondary causes of steatosis, such as drug-induced fatty liver disease, total parenteral nutrition, and inborn errors of metabolism. A careful interview, clinical and laboratory evaluations, and image inspection were performed at the time of the liver biopsy in all patients.

The study protocol complied with the ethical guidelines established in accordance with the 2013 Declaration of Helsinki and was approved by the Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital (approval number: 603). All patients provided written informed consent prior to entry into this study.

Clinical and laboratory evaluation

Clinical and laboratory data were collected concurrently with liver biopsy. The body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Blood pressure
was measured in a seated position at least twice at an interval of several minutes, and the mean was calculated. Hypertension was diagnosed when systolic blood pressure was 135 mmHg or higher or diastolic blood pressure was 85 mmHg or higher and when patients were being treated with an antihypertensive drug [25]. Dyslipidemia was diagnosed when total cholesterol was 220 mg/dL or higher, high-density lipoprotein cholesterol (HDL cholesterol) was below 40 mg/dL, and/or triglycerides were 150 mg/dL or higher, as well as when patients were being treated with an antihyperlipidemic drug [26]. Type 2 diabetes was diagnosed according to the 2006 World Health Organization (WHO) criteria in addition to the presence of treatment with an oral hypoglycemic agent and insulin.

Laboratory evaluation included complete blood count, routine liver biochemistry (aspartate aminotransferase, ALT, total bilirubin, albumin, alkaline phosphatase, and gamma glutamyl transpeptidase), fasting lipids (total cholesterol, triglycerides, HDL cholesterol, and low-density lipoprotein cholesterol), fasting plasma glucose, hemoglobin A1c, and immunoreactive insulin. As an index of insulin resistance, the homeostasis model assessment-insulin resistance (HOMA-IR) was calculated using the following equation: HOMA-IR = fasting insulin (μU/mL) × plasma glucose (mg/dL)/405 [27]. Hyaluronic acid [28], type IV collagen 7s domain [29, 30], and Wisteria floribunda agglutinin positive Mac-2-binding protein (WFA+M2B) [30–32], all of which have been reported as useful liver fibrosis markers in NAFLD, were measured. In addition, the fibrosis scores such as the FIB-4 index [33] and NAFLD fibrosis scorer (NFS) [34] were calculated, as reported previously. DNA was extracted from each patient, and the PNPLA3 rs738409 was genotyped by using a PCR protocol based on TaqMan assays.

**Pulse wave velocity**

A noninvasive index of arterial stiffness, brachial-ankle PWV (baPWV), was measured using a volume-plethysmographic apparatus (form PWV/ABI; Colin, Co., Ltd., Komaki, Japan), under previously reported measurement conditions [35]: 1) the patients were examined after resting in the supine position for several minutes; 2) they refrained from ingesting caffeine and cigarette smoking starting 3 hours before measurement, as a rule; and 3) baPWV was measured in a quiet examination room controlled at a constant temperature (22˚C–26˚C). The mean of the bilateral baPWV values was used for analysis. baPWV was measured by skilled laboratory technicians who were blinded to patient information. Referring to previous reports, patients with baPWV ≥1,600 cm/s were defined as a risk group for cardiovascular events [36, 37].

**Histopathological evaluation**

Histopathological evaluation was performed by experienced pathologists blinded to the clinical and laboratory data of the patients. NAFLD was diagnosed when lipid droplet deposition was noted in 5% or more hepatocytes. Then, steatosis, lobular inflammation, ballooning, and liver fibrosis were semi-quantitatively evaluated according to the NASH CRN scoring system [38]: steatosis was graded 0–3 based on the percent of hepatocytes on biopsy specimens (0: <5%, 1: 5–33%, 2: 33–66%, 3: >66%). Lobular inflammation was graded 0–3 based on inflammatory foci per 200× field (0: no foci, 1: <2 foci, 2: 2–4 foci, 3: >4 foci). Ballooning was graded 0–2 based on the number of hepatocytes with this change (0: none, 1: few cells, 2: many cells/prominent ballooning). Fibrosis stage was evaluated as follows: F0 = no fibrosis, F1 = perisinusoidal or periportal fibrosis, F2 = perisinusoidal and portal/periporal fibrosis, F3 = bridging fibrosis, and F4 = cirrhosis. F3–4 was provisionally designated as advanced fibrosis.
Statistical analyses
Continuous variables were presented as medians and ranges, and categorical variables were presented as numbers and percentages. Continuous variables with skewed distribution were compared among or between groups using the Kruskal–Wallis test or the Mann–Whitney test, respectively. The Steel–Dwass test was applied when the Kruskal-Wallis test indicated a significant difference among groups. Multiple logistic regression analysis was used to identify the independent factors that were significantly associated with baPWV ≥1600 cm/s. The Cochran–Armitage test was used to investigate the changes in the prevalence of baPWV ≥1600 cm/s according to risk groups based on the number of risk factors including older age, hypertension, and advanced fibrosis. A receiver-operating characteristic (ROC) curve was generated in order to analyze the values of noninvasive markers and scores of fibrosis that most rationally predicted advanced fibrosis. All statistical analyses were performed using IBM SPSS version 17.0 (IBM Japan, Tokyo, Japan). The level of statistical significance was set at \( p < 0.05 \).

Results
Patients
Baseline characteristics of the 153 patients are shown in Table 1. There were 74 males and 79 females, and the median age was 57 years (range, 18–84 years). Regarding metabolic components, the median BMI was 28.8 kg/m\(^2\) (range, 18.1–44.9 kg/m\(^2\)). There were 58 patients with type 2 diabetes (37.9%), 70 with hypertension (45.8%), and 117 with dyslipidemia (76.5%). On pathological examination of the 153 liver biopsy specimens, the fibrosis stage was determined to be F0 for 24 (15.7%) patients, F1 for 49 (32.0%), F2 for 36 (23.5%), F3 for 30 (19.6%), and F4 for 14 (9.2%) patients, with advanced fibrosis (F3–4) in 44 (28.8%) patients. The median baPWV value was 1557 cm/s (range, 1018–2776 cm/s). Among the 153 patients, 61 (39.9%) had a baPWV ≥1600 cm/s. The \( PNPLA3 \) rs738409 was genotyped in 142 patients, and the distribution of the \( PNPLA3 \) polymorphisms was as follows: CC, GC, and GG genotypes were found in 40.1% (57/142), 40.1% (57/142), and 19.7% (28/142) of patients, respectively.

Relationship between baPWV and pathological severity of the disease
As shown in Fig 1, no correlation with baPWV was noted for inflammation and ballooning. On the other hand, baPWV significantly decreased with progression of liver steatosis \( (p = 4.16 \times 10^{-2}) \) and increased with progression of liver fibrosis \( (p = 7.52 \times 10^{-3}) \). When fibrosis stages were categorized into less advanced (F0-2) and advanced (F3-4) fibrosis groups, baPWV was significantly higher in the advanced fibrosis group \( (p = 5.49 \times 10^{-4}) \) (Fig 2).

Factors associated with baPWV ≥1600 cm/s
Multiple logistic regression analysis showed that the following three variables were independently linked to baPWV ≥1600 cm/s (Table 2): older age (≥55 years) \( (p = 8.57 \times 10^{-3}; \text{OR} = 3.03; 95\% \text{ CI} = 1.33–6.91) \), hypertension \( (p = 1.05 \times 10^{-3}; \text{OR} = 3.46; 95\% \text{ CI} = 1.65–7.28) \), and advanced fibrosis \( (p = 9.22 \times 10^{-3}; \text{OR} = 2.94; 95\% \text{ CI} = 1.31–6.63) \).

Prevalence of baPWV ≥1600 cm/s according to the clinical risk scores based on older age, hypertension, and advanced fibrosis diagnosed by liver biopsy
We classified NAFLD patients into three groups: low-risk group (number of risk factors = 0), intermediate-risk group (= 1), and high-risk group (≥2) based on the number of risk factors
linked independently to baPWV ≥1600 cm/s, including older age, hypertension, and advanced fibrosis, as described above. The prevalence of baPWV ≥1600 cm/s was 7.1% (3/42) in the low-risk group, 30.8% (12/39) in the intermediate-risk group, and 63.9% (46/72) in the high-risk group, respectively (p = 9.44 × 10⁻¹⁰) (Fig 3).

Prevalence of baPWV ≥1600 cm/s according to the clinical risk scores based on older age, hypertension, and advanced fibrosis as diagnosed by fibrosis markers and scores

Using ROC analyses for the diagnosis of advanced fibrosis, the area under the curve (AUC) of the fibrosis markers and scores were as follows: FIB-4 index (cut-off value = 2.49,
AUC = 0.836), NFS (9.47 × 10^{-2}, 0.874), hyaluronic acid (62.3 ng/mL, 0.871), WFA^+\text{-}M2BP (0.95 C.O.I, 0.816), and type IV collagen 7s (5.2 ng/mL, 0.842) (Fig 4). Next, we reclassified NAFLD patients into three risk groups based on the number of risk factors, including older age, hypertension, and advanced fibrosis, as diagnosed by each noninvasive fibrosis marker and score instead of by invasive liver biopsy. The prevalence of NAFLD patients with baPWV ≥1600 cm/s increased with a greater number of risk factors, even when advanced fibrosis was diagnosed by FIB-4 index (p = 9.25×10^{-6}), NFS (p = 7.01×10^{-8}), hyaluronic acid (p = 3.31×10^{-7}), WFA^+\text{-}M2BP (p = 2.49×10^{-7}), and type IV collagen 7s (p = 7.30×10^{-7}).

Fig 1. Box and whisker plots of baPWV values according to the severity of each histological component in NAFLD patients. baPWV, brachial-ankle pulse wave velocity; NAFLD, nonalcoholic fatty liver disease. * p < 0.05.

https://doi.org/10.1371/journal.pone.0224184.g001
In this study, we clarified that older age, hypertension, and advanced liver fibrosis were independently associated with arterial stiffness in Japanese biopsy-proven NAFLD patients. Arterial stiffness is evaluated by measuring PWV, which is widely used as a preclinical cardiovascular risk marker [39]. Several studies reported that the PWV in NAFLD patients is higher than that in healthy individuals and that the presence of NAFLD is associated with arterial stiffness independent from conventional metabolic risk factors [40–44]. On the other hand, only a few studies have investigated the association of the severity of NAFLD with arterial stiffness. Advanced fibrosis estimated based on NFS [45] and transient elastography [46] were reported to be associated with a high PWV value independent from conventional metabolic risk factors in patients diagnosed with NAFLD by ultrasonography. However, to our knowledge, the association between the severity of liver disease and PWV was histologically

![Fig 2. baPWV values in NAFLD patients according to fibrosis stage. baPWV values (median, 1489 cm/s) in the advanced fibrosis group (fibrosis stage = F3-4) were significantly higher than those (median, 1679 cm/s) in the less advanced fibrosis group (fibrosis stage = F0-2) ($p = 5.49 \times 10^{-4}$). baPWV, brachial-ankle pulse wave velocity; NAFLD, nonalcoholic fatty liver disease.](https://doi.org/10.1371/journal.pone.0224184.g002)

**Discussion**

In this study, we clarified that older age, hypertension, and advanced liver fibrosis were independently associated with arterial stiffness in Japanese biopsy-proven NAFLD patients. Arterial stiffness is evaluated by measuring PWV, which is widely used as a preclinical cardiovascular risk marker [39]. Several studies reported that the PWV in NAFLD patients is higher than that in healthy individuals and that the presence of NAFLD is associated with arterial stiffness independent from conventional metabolic risk factors [40–44]. On the other hand, only a few studies have investigated the association of the severity of NAFLD with arterial stiffness. Advanced fibrosis estimated based on NFS [45] and transient elastography [46] were reported to be associated with a high PWV value independent from conventional metabolic risk factors in patients diagnosed with NAFLD by ultrasonography. However, to our knowledge, the association between the severity of liver disease and PWV was histologically
investigated by liver biopsy in only 2 reports of a small number of patients from Turkey, with contradictory results; while the progression of histological liver fibrosis was an independent factor for a high PWV value in a study involving 100 biopsy-confirmed NAFLD patients [13], no difference was noted in PWV between patients with simple steatosis and patients with steatohepatitis in the other study involving 61 NAFLD patients [14]. Our analysis of a relatively large Japanese cohort was comparable to and supported the findings of the former study. In this study, baPWV was elevated with the progress of liver fibrosis, while it was decreased with the progress of hepatic steatosis. This paradox can be explained by the loss of hepatic fat in NAFLD patients with advanced fibrosis. This result may suggest that liver fibrosis affects the arterial stiffness more than the hepatic fat.

The arterial stiffness-promoting mechanism of the presence and severity of NAFLD independent of conventional metabolic risk factors remains unclear, though there is experimental evidence supporting that NAFLD and arterial stiffness develop and progress due to a common etiology [47–49]. First, chronic inflammation and oxidative stress, considered important factors for the development and progression of NAFLD, induce cardiovascular disorder. Second, it has been reported that the blood level of adiponectin, which has anti-inflammatory and anti-fibrosis activity, decreases due to an increase in adipose tissue and chronic inflammation, and this decrease then promotes NAFLD and arterial stiffness. Third, an influence of TGF-β, which plays an important role in the progression of liver fibrosis, on arterial stiffness has been suggested. To clarify the association between NAFLD and arterial stiffness, further studies are necessary.

To the best of our knowledge, this is the first report to analyze factors associated with arterial stiffness, including the PNPLA3 SNP genotype. Two studies on the association between the carotid intima-media thickness, a surrogate marker of subclinical atherosclerosis as is PWV, and the PNPLA3 SNP genotype in Italian NAFLD patients have been reported, but the

### Table 2. Univariate and multivariate logistic regression analysis of factors associated with baPWV ≥1600 cm/s.

| Factors                          | Category          | Univariate | Multivariate |
|---------------------------------|-------------------|------------|--------------|
|                                 |                   | OR 95% CI  | p value      | OR 95% CI   | p value      |
| Age (years)                     | Older age (≥55)   | 4.86 2.29–10.32 | 3.84 × 10⁻³ | 3.03 1.33–6.91 | 8.57 × 10⁻³ |
| Gender                          | Female            | 1.32 0.69–2.52 | 0.683        |              |              |
| BMI (kg/m²)                     | By 1 kg/m² down   | 1.04 0.97–1.12 | 0.274        |              |              |
| Total-cholesterol (mg/dL)       | By 1 mg/dL down   | 1.01 1.00–1.02 | 0.194        |              |              |
| HDL-cholesterol (mg/dL)         | By 1 mg/dL up     | 1.01 0.98–1.04 | 0.621        |              |              |
| Triglyceride (mg/dL)            | By 1 mg/dL down   | 1.00 1.00–1.01 | 0.129        |              |              |
| Plasma glucose (mg/dL)          | By 1 mg/dL up     | 1.00 0.99–1.01 | 0.716        |              |              |
| Insulin (µU/mL)                 | By 1 µU/mL up     | 1.02 0.98–1.05 | 0.345        |              |              |
| HOMA-IR                         | By 1 up           | 1.04 0.95–1.13 | 0.402        |              |              |
| Diabetes                        | Presence          | 1.56 0.80–3.04 | 0.188        |              |              |
| Hypertension                    | Presence          | 4.45 2.23–8.90 | 2.35 × 10⁻³ | 3.46 1.65–7.28 | 1.05 × 10⁻³ |
| Smoking                         | no                | 1.13 0.59–2.19 | 0.708        |              |              |
| PNPLA3 genotype                 | GG                | 1.53 0.67–3.52 | 0.313        |              |              |
| Liver steatosis                 | 1 grade down      | 1.53 0.92–2.56 | 9.92 × 10⁻² |              |              |
| Liver inflammation              | 1 grade down      | 1.23 0.73–2.08 | 0.438        |              |              |
| Liver ballooning                | 1 grade down      | 1.18 0.67–2.06 | 0.567        |              |              |
| Liver fibrosis stage            | Advanced fibrosis | 4.65 2.20–9.82 | 5.53 × 10⁻³ | 2.94 1.31–6.63 | 9.22 × 10⁻³ |

baPWV, brachial-ankle pulse wave velocity; OR, odds ratio; CI, confidence interval; BMI, body mass index, HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; PNPLA3, patatin-like phospholipase domain containing 3.

https://doi.org/10.1371/journal.pone.0224184.t002
findings were contradictory. Petta et al. reported that the \textit{PNPLA3} GG genotype was significantly associated with the severity of carotid atherosclerosis in young NAFLD patients [19], but Di Costanzo et al. found no association between the carotid intima-media thickness and the \textit{PNPLA3} SNP genotype and indicated that complications due to metabolic abnormalities influenced the carotid intima-media thickness, which is well known [50]. In our study, no association was noted between baPWV and the \textit{PNPLA3} SNP genotype, but further investigation is necessary regarding the influence of the \textit{PNPLA3} SNP genotype on atherosclerosis in light of racial differences in the morbidity of atherosclerosis and the \textit{PNPLA3} SNP genotype distribution.

In this study, older age, hypertension, and advanced fibrosis were additively related to arterial stiffness in NAFLD patients, and it is possible to speculate the risk of arteriosclerosis progression by combining these three factors. Furthermore, we showed that the risk assessment of atherosclerosis progression in clinical practice is possible by substituting noninvasive liver fibrosis markers and scores for histological diagnosis by invasive liver biopsy. Clinicians
should pay attention to cardiovascular events in NAFLD patients with high fibrosis marker levels and scores as well as older age and hypertension, and further care should be taken when these factors overlap.

There were some limitations in this study. First, the number of patients, especially those with advanced fibrosis, was relatively small. Second, as described above, there are racial differences in atherosclerosis and the PNPLA3 genotype. To make a definitive conclusion, it may be necessary to re-confirm the results of this study in independent validation cohorts with different characteristics, races, and/or ethnicities.

In conclusion, older age, hypertension, and advanced liver fibrosis were found to be independent factors associated with arterial stiffness in Japanese biopsy-proven NAFLD patients. Furthermore, the combination of older age, hypertension, and advanced fibrosis based on noninvasive fibrosis markers and scores may predict the risk of arteriosclerosis progression in NAFLD patients in clinical practice.
Supporting information

S1 Fig. Box and whisker plots of baPWV values according to each fibrosis marker and score such as FIB-4 index (A), NFS (B), hyaluronic acid (C), WFA+-M2BP (D), and type IV collagen 7s (E). baPWV, brachial-ankle pulse wave velocity; FIB-4, fibrosis-4; NFS, NAFLD (nonalcoholic fatty liver disease) fibrosis score; WFA+-M2BP, Wisteria floribunda agglutinin positive Mac-2-binding protein.

(TIF)

S1 File. Exel. Raw data including pathological findings and baPWV.
(XLSX)

Acknowledgments

The authors wish to thank all medical doctors from all institutions who were involved in this study.

Author Contributions

Data curation: Tadamichi Kawano, Mai Koeda, Tomohide Tanabe, Tomomi Okubo, Kore-nobu Hayama, Ai Iwashita, Norio Itokawa, Keiko Kaneko, Tsutomu Hatori, Naoya Emoto, Etsuko Iio, Yasuhiro Tanaka.

Formal analysis: Taeang Arai, Chisa Kondo.

Investigation: Taeang Arai, Tadamichi Kawano, Mai Koeda, Yuji Yoshida, Tomomi Okubo, Korenobu Hayama, Ai Iwashita, Norio Itokawa, Chisa Kondo, Keiko Kaneko, Tsutomu Hatori, Etsuko Iio.

Supervision: Akihito Tsubota, Chiaki Kawamoto, Yasuhiro Tanaka, Katsuhiko Iwakiri.

Writing – original draft: Taeang Arai, Masanori Atsukawa.

Writing – review & editing: Masanori Atsukawa, Akihito Tsubota.

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016; 64: 73–84. https://doi.org/10.1002/hep.28431 PMID: 26707369

2. Hashimoto E, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. J Gastroenterol. 2011; 46: 63–9. https://doi.org/10.1007/s00535-010-0311-8 PMID: 20844903

3. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 2006; 43: S99–S112. https://doi.org/10.1002/hep.20973 PMID: 16447287

4. Tanaii M, Hashimoto E, Tobari M, Kodama K, Tokushige K, Yamamoto M, et al. Clinicopathological investigation of steatohepatitic hepatocellular carcinoma: A multicenter study using immunohistochemical analysis of adenoma-related markers. Hepatol Res. 2018; 48: 947–955. https://doi.org/10.1111/hepr.13203 PMID: 30058778

5. Le MH, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, et al. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PLoS One. 2017; 27: e0173499. https://doi.org/10.1371/journal.pone.0173499 PMID: 28346543

6. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2015; 149: 389–397. https://doi.org/10.1053/j.gastro.2015.04.043 PMID: 25935633

7. Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017; 67: 1265–1273. https://doi.org/10.1016/j.jhep.2017.07.027 PMID: 28803953
8. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology. 2005; 129: 113–21. https://doi.org/10.1053/j.gastro.2005.04.014 PMID: 16012941

9. Lonardo A, Nascimbeni F, Taghert G, Bernardi M, Bonino F, Bugianesi E, et al. AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. Dig Liver Dis. 2017; 49: 471–483. https://doi.org/10.1016/j.dld.2017.01.147 PMID: 28215516

10. Oni ET, Agatston AS, Blaha MJ, Fialkow J, Cury R, Sposito A, et al. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? Atherosclerosis. 2013; 230: 258–67. https://doi.org/10.1016/j.atherosclerosis.2013.07.052 PMID: 24075754

11. Zhou YY, Zhou XD, Wu SJ, Fan DH, Van Poucke S, Chen YP, et al. Nonalcoholic fatty liver disease contributes to subclinical atherosclerosis: A systematic review and meta-analysis. Hepatol Commun. 2018; 2: 376–392. https://doi.org/10.1002/hepc.202666 PMID: 30133541

12. Gummesson A, Strömberg U, Schmidt C, Kullberg J, Angerström O, Lindgren S, et al. Non-alcoholic fatty liver disease is a strong predictor of coronary artery calcification in metabolically healthy subjects: A cross-sectional, population-based study in middle-aged subjects. PLoS One. 2013; 8: e202666. https://doi.org/10.1371/journal.pone.0202666

13. Sunbul M, Agirbasli M, Durmus E, Kivrak T, Akin H, Aydin Y, et al. Arterial stiffness in patients with non-alcoholic fatty liver disease is related to fibrosis stage and epicardial adipose tissue thickness. Atherosclerosis. 2014; 237: 490–3. https://doi.org/10.1016/j.atherosclerosis.2014.04.024 PMID: 25463079

14. Ozturk K, Uygun A, Guler AK, Demirci H, Ozdemir C, Cakir M, et al. Nonalcoholic fatty liver disease is an independent risk factor for atherosclerosis in young adult men. Atherosclerosis. 2015; 240: 380–6. https://doi.org/10.1016/j.atherosclerosis.2015.04.009 PMID: 25875390

15. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Zenari L, et al. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care. 2006; 29: 1325–30. https://doi.org/10.2337/dc06-0135 PMID: 16732016

16. Romeo S, Kozlitina J, Xing C, Pertsemidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008; 40: 1461–5. https://doi.org/10.1038/ng.257 PMID: 18820647

17. Soookoian S, Pirolo CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of non-alcoholic fatty liver disease. Hepatology. 2011; 53: 1889–94. https://doi.org/10.1002/hep.24283 PMID: 2138108

18. Koo BK, Joo SK, Kim D, Bae JM, Park JH, Kim JH, et al. Additive effects of PNPLA3 and TM6SF2 on the histological severity of non-alcoholic fatty liver disease. J Gastroenterol. 2018; 53: 1277–1285. https://doi.org/10.1002/jgh.14056 PMID: 29193269

19. Petta S, Valenti L, Marchesini G, Di Marco V, Licata A, Cammà C, et al. PNPLA3 GG genotype and carotid atherosclerosis in patients with non-alcoholic fatty liver disease. PLoS One. 2013; 8: e74089. https://doi.org/10.1371/journal.pone.0074089 PMID: 24092707

20. Kawaguchi T, Sumida Y, Umemura A, Matsuou K, Takahashi M, Takamura T, et al. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. PLoS One. 2012; 7: e38322. https://doi.org/10.1371/journal.pone.0038322 PMID: 22719876

21. Seko Y, Sumida Y, Tanaka S, Mori K, Taketani H, Ishiba H, et al. Development of hepatocellular carcinoma in Japanese patients with biopsy-proven non-alcoholic fatty liver disease: Association between PNPLA3 genotype and hepatocarcinogenesis/fibrosis progression. Hepatol Res. 2017; 47: 1083–1092. https://doi.org/10.1111/hepr.12840 PMID: 27862719

22. Marchesini G, Day CP, Dufour JF, Canbay A, Nobili V, Ratzu V, et al. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016; 64: 1388–402. https://doi.org/10.1016/j.jhep.2015.11.004 PMID: 27062661

23. Bugianesi E, Rosso C, Cortez-Pinto H. How to diagnose NAFLD in 2016. J Hepatol. 2016; 65: 643–4. https://doi.org/10.1016/j.jhep.2016.05.038 PMID: 27401791

24. Nascimbeni F, Pais R, Bellentani S, Day CP, Ratzu V, Loria P, et al. From NAFLD in clinical practice to answers from guidelines. J Hepatol. 2013; 59: 859–71. https://doi.org/10.1016/j.jhep.2013.05.044 PMID: 23751574

25. Shimamoto K, Ando K, Fujita T, Hasebe N, Higaki J, Horuchi M, et al. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2014). Hypertens Res. 2014; 37: 253–390. https://doi.org/10.1038/hr.2014.20 PMID: 24705419

26. Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, et al. Executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases. J Atheroscler Thromb. 2019; 26: 774–797. https://doi.org/10.5558/jat.2019.26.8.774 PMID: 31786398
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28: 412–9. https://doi.org/10.1007/bf00280883 PMID: 3899825

28. Suzuki A, Angulo P, Lymp J, Li D, Satomura S, Lindor K. Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with non-alcoholic fatty liver disease. Liver Int. 2005; 25: 779–86. https://doi.org/10.1111/j.1478-3231.2005.01064.x PMID: 15998429

29. Yoneda M, Mawatari H, Fujita K, Yonemitsu K, Kato S, Takahashi H, et al. Type IV collagen 7S domain is an independent clinical marker of the severity of fibrosis in patients with nonalcoholic steatohepatitis before the cirrhotic stage. J Gastroenterol. 2007; 42: 375–81. https://doi.org/10.1007/s00535-007-2014-3 PMID: 17530362

30. Ogawa Y, Honda Y, Kessoku T, Tomeno W, Imajo K, Yoneda M, et al. Wisteria floribunda agglutinin-positive Mac-2-binding protein and type 4 collagen 7S: useful markers for the diagnosis of significant fibrosis in patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol. 2018; 33: 1795–1803. https://doi.org/10.1111/jgh.14156 PMID: 29633352

31. Abe M, Miyake T, Kuno A, Imai Y, Sawai Y, Hino K, et al. Association between Wisteria floribunda agglutinin-positive Mac-2 binding protein and the fibrosis stage of non-alcoholic fatty liver disease. J Gastroenterol. 2015; 50: 776–84. https://doi.org/10.1007/s00535-014-1007-2 PMID: 25326152

32. Atsukawa M, Tsubota A, Okubo T, Arai T, Nakagawa A, Itokawa N, et al. Serum Wisteria floribunda agglutinin-positive Mac-2 binding protein more reliably distinguishes liver fibrosis stages in non-alcoholic fatty liver disease than serum Mac-2 binding protein. Hepatol Res. 2018; 48: 424–432. https://doi.org/10.1002/hepr.13046 PMID: 29274190

33. Sumida Y, Yoneda M, Hyogo H, Itoh Y, Ono M, Fuji H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterol. 2012; 12: 2. https://doi.org/10.1186/1471-230X-12-2 PMID: 22221544

34. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007; 45: 846–54. https://doi.org/10.1002/hep.21496 PMID: 17939509

35. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012; 30: 445–8. https://doi.org/10.1097/jhj.0b013e32834f4a0b PMID: 22276144

36. Kim HJ, Nam JS, Park JS, Cho M, Kim CS, Ahn CW, et al. Usefulness of brachial-ankle pulse wave velocity as a predictive marker of multiple coronary artery occlusive disease in Korean type 2 diabetes patients. Diabetes Res Clin Pract. 2009; 85: 30–4. https://doi.org/10.1016/j.diabres.2009.03.013 PMID: 19398141

37. Yambe M, Tomiyama H, Hirayama Y, Guiniza Z, Takata Y, Koj Y, et al. Arterial stiffening as a possible risk factor for both atherosclerosis and diastolic heart failure. Hypertens Res. 2004; 27: 625–31. https://doi.org/10.1291/hyres.27.625 PMID: 15750255

38. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummins OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005; 41: 1313–21. https://doi.org/10.1002/hep.20701 PMID: 15915461

39. Tomiyama H, Matsumoto C, Shina K, Yamashina A. Brachial-A ngiography: Current Status and Future Directions as a Useful Marker in the Management of Cardiovascular Disease and/or Cardiovascular Risk Factors. J Atheroscler Thromb. 2016; 23: 128–46. https://doi.org/10.5551/jat.32979 PMID: 26558401

40. Salvi P, Ruffini R, Agnoletti D, Magnani E, Pagliarani G, Comandini G, et al. Increased arterial stiffness in nonalcoholic fatty liver disease: the Cardio-GOOSE study. J Hypertens. 2010; 28: 1699–707. https://doi.org/10.1097/HJH.0b013e32833a7de6 PMID: 20467324

41. Huang RC, Beilin LJ, Ayonrin de O, Mori TA, Olynyk JK, Burrows S, et al. Importance of cardiometabolic risk factors in the association between nonalcoholic fatty liver disease and arterial stiffness in adolescents. Hepatology. 2013; 58: 1306–14. https://doi.org/10.1002/hep.26495 PMID: 23703776

42. Huang Y, Bi Y, Xu M, Ma Z, Xu Y, Wang T, et al. Nonalcoholic fatty liver disease is associated with atherosclerosis in middle-aged and elderly Chinese. Arterioscler Thromb Vasc Biol. 2012; 32: 2321–6. https://doi.org/10.1161/ATVBAHA.112.252957 PMID: 22814750

43. Lee YJ, Shim JY, Moon BS, Shin YH, Jung DH, Lee JH, et al. The relationship between arterial stiffness and nonalcoholic fatty liver disease. Dig Dis Sci. 2012; 57: 196–203. https://doi.org/10.1007/s10620-011-1819-3 PMID: 21750929
44. Li N, Zhang GW, Zhang JR, Jin D, Li Y, Liu T, et al. Non-alcoholic fatty liver disease is associated with progression of arterial stiffness. Nutr Metab Cardiovasc Dis. 2015; 25: 218–23. https://doi.org/10.1016/j.numecd.2014.10.002 PMID: 25456154

45. Chen Y, Xu M, Wang T, Sun J, Sun W, Xu B, et al. Advanced fibrosis associates with atherosclerosis in subjects with nonalcoholic fatty liver disease. Atherosclerosis. 2015; 241: 145–50. https://doi.org/10.1016/j.atherosclerosis.2015.05.002 PMID: 25786358

46. Leite NC, Villela-Nogueira CA, Ferreira MT, Cardoso CR, Salles GF. Increasing aortic stiffness is predictive of advanced liver fibrosis in patients with type 2 diabetes: the Rio-T2DM cohort study. Liver Int. 2016; 36: 977–85. https://doi.org/10.1111/liv.12994 PMID: 26509555

47. Fargion S, Porzio M, Fracanzani AL. Nonalcoholic fatty liver disease and vascular disease: state-of-the-art. World J Gastroenterol. 2014; 20: 13306–24. https://doi.org/10.3748/wjg.v20.i37.13306 PMID: 25309067

48. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med. 2010; 363: 1341–50. https://doi.org/10.1056/NEJMra0912063 PMID: 20879883

49. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. Nat Rev Gastroenterol Hepatol. 2013; 10: 627–36. https://doi.org/10.1038/nrgastro.2013.149 PMID: 23958599

50. Di Costanzo A, D’Erasmo L, Polimeni L, Baratta F, Coletta P, Di Martino M, et al. Non-alcoholic fatty liver disease and subclinical atherosclerosis: A comparison of metabolically- versus genetically-driven excess fat hepatic storage. Atherosclerosis. 2017; 257: 232–239. https://doi.org/10.1016/j.atherosclerosis.2016.12.018 PMID: 28027788