PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Clinical characteristics in patients with nonalcoholic steatohepatitis in Japan: a case-control study using a 5-year large-scale claims database.
AUTHORS	Tokutsu, Kei; Ito, Kaoru; Kawazoe, Shigeki; Minami, Sota; Fujimoto, Kenji; Muramatsu, Keiji; Matsuda, Shinya

VERSION 1 – REVIEW

REVIEWER	Clayton-Chubb, Daniel Alfred Hospital, Gastroenterology
REVIEW RETURNED	17-May-2023

GENERAL COMMENTS	Thank you for the opportunity to review this paper. General comments: - The English could be tightened up/improved to improve readability; - How the NASH cohort was defined is slightly difficult to follow. It may be worth having a figure to show (for example) ICD code(s) for NASH --> ICD code(s) for a liver biopsy which dates before the NASH ICD code --> included as a NASH patient/participant? - When talking about obesity for a general audience it may be worth reminding non-Asian readers that the BMI cut-offs used as different in Asia to non-Asian countries Specific comments: - The paper says the NASH patients were 'matched' with controls; I don't think 'matched' is being used in the usual way here? - Ensure the decimal places/significant digits are uniform through the paper (e.g., 2 vs 1 decimal point for BMI) - 'Cancer' as an outcome might be more interesting if it was 'extra-hepatic cancer' - I'm not sure what: "In addition, patients who could be visually confirmed to have "hepatitis," "non-alcoholic," and "NASH" were also included in the analysis" means - The 'random selection of controls' - why was this done / how was the number of controls selected? - When excluding patients, why was autoimmune hepatitis not excluded? - In the 'Comorbidities as risk factors for NASH' section, the causality appears to be reversed (e.g., you say hepatic cirrhosis was associated with the greatest risk of NASH when in reality I presume you're saying that the most significantly increased odds of disease in NASH vs no-NASH is hepatic cirrhosis?) - In the discussion, the paragraph on diabetes treatments could be shortened - You say 'GSLT2' rather than SGLT2 at one point - Check the in-text referencing for 'Terai'

	- You give two different ORs for osteoporosis (page 9 line 26 and line 33), which should be reconciled - Are the ICD codes correct? E.g., for Cirrhosis, K743 appears to be for PBC? And in Japan, would non-biopsy proven Cirrhosis usually be recorded? - Figure 1(B) dyslipidemia Forest plot appears to have 3 dots? Comments about Statistics: - "We conducted descriptive statistics using multiple logistic regression models to analyze the relationship between NASH and sex, age, lifestyle-related diseases, death, and comorbidities for continuous data (Odd Ratio [OR], 95% confidence interval [95% CI]). Differences between the NASH and non-NASH groups were evaluated using paired-sample t-tests for continuous variables. Statistical significance was set at $p < 0.05$." This paragraph needs to be re-worded I think - multiple logistic regression isn't usually referred to as 'descriptive statistics', comorbidities weren't necessarily recorded as continuous data, and I don't think paired T-tests were used to compare NASH vs no-NASH groups? Comments about referencing: - Some of the references need some formatting amendments, e.g., 11 & 12 where the journal title isn't in usual short-form

REVIEWER	Adela, Ramu National Institute of Pharmaceutical Education and Research Guwahati, Department of Pharmacy Practice
REVIEW RETURNED	24-May-2023

GENERAL COMMENTS	The title is not interesting may be need to change clinical characteristics in NASH patients it is well known subject. However authors presented the data from the updated information. It is only focusing on clinical characteristics not define the NASH classifiers. Strengths were not mentioned in the strengths and limitations section Authors not identified proper key words Risk adjusted confounders was not mentioned the Table 4

VERSION 1 – AUTHOR RESPONSE

Reviewer1
Thank you for very much for your feedback and helpful suggestion. Below in the manuscript we address all the queries and problems you raised.

Reviewer's comment#1
The English could be tightened up/improved to improve readability.

Response#1
Thank you for your suggestion. We did a native check before submission, but we apologize for the insufficient final check. The English of the manuscript has been revised to improve readability. At the same time, we requested a native check of the revised manuscript again.

Reviewer’s comment#2
How the NASH cohort was defined is slightly difficult to follow. It may be worth having a figure to show (for example) ICD code(s) for NASH --> ICD code(s) for a liver biopsy which dates before the NASH ICD code --> included as a NASH patient/participant?

Response#2
Thank you for your helpful suggestion. Based on it, we have created a flow chart for a case-control study using the national health insurance (NHI) claims database and the long-term elderly health insurance (LEHI) claims database. The following figure was added to the methods section as Supplemental Fugure1.
This figure was added as Supplemental Figure 1 because the number of tables and figures in this journal is limited to 5.

Reviewer’s comment#3
When talking about obesity for a general audience it may be worth reminding non-Asian readers that the BMI cut-offs used as different in Asia to non-Asian countries

Response#3
Thank you for your suggestion. In response to the suggestion, we added the following sentence to the discussion section regarding BMI standards in Japan and WHO.

World Health Organization (WHO) diagnostic criteria define BMI 25 kg/m2 or more as overweight and BMI 30 kg/m2 or more as obese. In Japan, the definition of obesity as judged by the Japan Society for Study of Obesity is set as a BMI of 25 kg/m2 or more, which is lower than the WHO criteria. One of the reasons for this is that it is known that Japanese people are more likely to develop fatty liver if their BMI is less than 25 kg/m2 but are not obese, and they develop fatty liver at a high rate after their BMI exceeds 25 kg/m2.

Specific comments:
Reviewer’s comment#4
The paper says the NASH patients were 'matched' with controls; I don't think 'matched' is being used in the usual way here?

Response#4
Considering your suggestion, we changed the word “matched” to “compared.” The correction is as follows.

Methods
Study population and eligibility criteria
According to our definition, each patient classified as having NASH was compared with non-NASH comparators randomly selected from the original database by sex, birth year, and residential area.

Reviewer’s comment#5
Ensure the decimal places/significant digits are uniform through the paper (e.g., 2 vs 1 decimal point for BMI)

Response#5

We appreciate your suggestion and have implemented changes to ensure all values are consistently displayed to one decimal place. However, we would be grateful if you could accept the OR notation with two decimal places.

Reviewer's comment #6
'Cancer' as an outcome might be more interesting if it was 'extra-hepatic cancer.'

Response#6
Thank you for your very interesting comment on the outcome of the comorbidities. Your suggestion is very meaningful and may increase the value of this study. However, considering the deadline for resubmitting (June 30th), there is not enough time to reanalyze extra-hepatic cancer. Therefore, the relationship between NASH and extra-hepatic cancer was described in the limitation section as a future research topic.

Limitations

This study showed that NASH is significantly involved in the development of intrahepatic lesions such as cirrhosis and liver cancer. However, to better understand the complex etiology of NASH, it may be necessary to investigate its relationship with extrahepatic primary cancers, such as extra-hepatic cancer.

Reviewer's comment#7
I'm not sure what: "In addition, patients who could be visually confirmed to have "hepatitis," "non-alcoholic," and "NASH" were also included in the analysis" means.

Response#7
We apologize for confusing you with inappropriate descriptions. To extract accurate information, we not only extracted data using ICD-10, but we also verified visually that the recorded disease name character string in the claim data was "hepatitis," "non-alcoholic," and "NASH."
We have rewritten the relevant part as follows.

Study population and eligibility criteria

The inclusion criteria were patients of any age with a record of at least one episode of NASH during the study period from April 2015 to March 2020. An episode of NASH was defined as NASH diagnosis (ICD-10 K-75.8; other inflammatory liver diseases or K-76.0; other fatty liver). Furthermore, patients whose disease name string could be confirmed as "hepatitis," "non-alcoholic," and "NASH" in the claims data were also included in the analysis.

Reviewer's comment #8
The 'random selection of controls' - why was this done / how was the number of controls selected?

Response#8
Thank you for your remarks on this section. Considering the limitations of the computational power required for statistical analysis, we randomly assigned as many patients without NASH as possible to the non-NASH group rather than all patients without NASH in the available data set.

Reviewer's comment #9
When excluding patients, why was autoimmune hepatitis not excluded?

Response #9
The reason for excluding autoimmune hepatitis was that we received advice from a hepatic clinician during the protocol development of this study.

Currently, in Japan, the only way to confirm a diagnosis of NASH is through a liver biopsy. Therefore, we included patients diagnosed through both ICD-10 code and liver biopsy in our study. Additionally, we received clinical advice that a liver biopsy may also be used to confirm a diagnosis of autoimmune hepatitis. Considering that advice, we excluded patients with autoimmune hepatitis to obtain a purer sample of patients with NASH. We added the reason in the method section that patients with autoimmune hepatitis were excluded.

Study population and eligibility criteria

Exclusion criteria were claims for any of the following conditions at any time: hepatitis B virus, hepatitis C virus, human immunodeficiency virus, alcoholic liver disease, toxic liver injury, copper metabolism disorder, autoimmune hepatitis, Gaucher's disease, lysosomal acid lipase deficiency, biliary cirrhosis, cholangitis, or iron metabolism disorder. ICD-10 codes were used to identify the patients with these diseases. It should be noted that given the expert opinion that a liver biopsy may be performed for a definitive diagnosis of autoimmune hepatitis to extract a purer sample in patients with NASH, patients with autoimmune hepatitis were excluded.

Reviwer's comment #10

In the 'Comorbidities as risk factors for NASH' section, the causality appears to be reversed (e.g., you say hepatic cirrhosis was associated with the greatest risk of NASH when in reality I presume you're saying that the most significantly increased odds of disease in NASH vs no-NASH is hepatic cirrhosis?)

Response #10

Thank you for your comment. Per your comment, we have revised the sentence as follows:

Comorbidities as risk factors for NASH

The OR with NASH and NASH-related comorbidities is shown in Table 4. In a multiple logistic regression model examining the association between NASH and developing comorbidities, compared to non-NASH, NASH was associated with the greatest risk of hepatic cirrhosis (OR 28.81, 95% CI, 21.79–38.08), followed by liver cancer (OR 18.38, 95% CI, 12.56–26.89), GERD (OR 3.08, 95% CI 2.53–3.73), colorectal adenomas (OR 2.54, 95% CI 1.25-5.16), colon cancer (OR 2.36, 95% CI 1.70–3.28), cancer (OR 2.16, 95% CI 1.79–2.62), SAS (OR: 1.82, 95% CI 1.20–2.76), CVD (OR 1.40, 95% CI 1.16–1.69), and osteoporosis (OR 1.25, 95% CI 1.02–1.53). No significant difference in comorbidities was observed for depression (OR 1.11, 95% CI 0.87–1.41), insomnia (OR 1.12, 95% CI 0.94–1.34), and CKD (OR 0.81, 95% CI 0.58–1.12).

Reviwer's comment #11

In the discussion, the paragraph on diabetes treatments could be shortened.

Response #11

Thank you for your suggestion. Per your comments, the section on diabetes treatment has been rewritten as follows.

Discussion

According to the NASH/NAFLD guidelines10, some therapeutic drugs for dyslipidemia, hypertension, and DM have been suggested to be effective for NASH, and aggressive treatment of patients with complications of these lifestyle-related diseases is recommended. Therefore, this survey investigated the proportion of prescriptions for antihyperlipidemic, hypertensive, and antidiabetic drugs. As a result, the proportion of prescriptions was 53.2% for statins and 45.9% for angiotensin receptor blockers (ARBs) in the NASH group, which was significantly higher than 21.4% for statins and 22.2% for ARBs in the non-NASH group, but the proportion of prescriptions was less than 50%. Premature mortality in NASH is related to both hepatic (cirrhosis and hepatocellular carcinoma) and extra-hepatic

complications, largely CVD. Many therapeutic agents have been tested but are still nonapproved, specifically for NASH. Also, presently, there is no drug with sufficient evidence of improving fibrosis in patients with NASH. It is anticipated that many clinical studies on drug therapy and development for NASH will be conducted in the future.

Reviwer's comment #12
You say 'GSLT2' rather than SGLT2 at one point.

Response #12
We deeply regret that our manuscript contained some typos. We have corrected the relevant parts. Nevertheless, please note that we have deleted the relevant part (GSLT2) in response to the comment on the Reviwer's commnet #11.

Reviwer's comment #13
Check the in-text referencing for 'Terai'

Response #13
Thank you for providing a thorough review. We apologize for the mistake in our final manuscript check and have since made the necessary corrections.

Reviwer's comment #14
You give two different ORs for osteoporosis (page 9 line 26 and line 33), which should be reconciled.

Response #14
We regret that there was an error in the description. Regarding your point, the description of the OR value on line 33 was wrong. The value has been revised.

Reviwer's comment #15
Are the ICD codes correct? E.g., for Cirrhosis, K743 appears to be for PBC? And in Japan, would non-biopsy proven Cirrhosis usually be recorded?

Response #15
The ICD-10 codes for the definition of comorbidities were determined based on expert opinion and others, so we believe the ICD-10 codes adopted in this study are appropriate.
Regarding Primary biliary cirrhosis (PBC), which you pointed out, liver biopsy is not mandatory for insurance claims (definitive diagnosis) of PBC in Japan. Therefore, at the doctor's discretion, PBC may be diagnosed based on test results other than a liver biopsy. Because we did not consider whether a liver biopsy was performed for data extraction of comorbidities, we determined that K743 was included in hepatic cirrhosis.
We hope that this answers your question.

Reviwer's comment #16
Figure 1(B) dyslipidemia Forest plot appears to have 3 dots?

Response #16
Thank you very much for your detailed review. As demonstrated below, we have eliminated the unnecessary dots. We replaced Figure 1(B). Please note that the notation of Figure1(B) has been changed to Figure2 due to this revision of the manuscript.

Comments about Statistics:
Reviwer's comment #17

"We conducted descriptive statistics using multiple logistic regression models to analyze the relationship between NASH and sex, age, lifestyle-related diseases, death, and comorbidities for continuous data (Odd Ratio [OR], 95% confidence interval [95% CI]). Differences between the NASH and non-NASH groups were evaluated using paired-sample t-tests for continuous variables. Statistical significance was set at $p < 0.05$."
This paragraph needs to be re-worded I think - multiple logistic regression isn't usually referred to as 'descriptive statistics', comorbidities weren't necessarily recorded as continuous data, and I don't think paired T-tests were used to compare NASH vs no- NASH groups?

Response #17
Thank you very much for your valuable suggestions. As you pointed out, categorical variables in the difference between the NASH and the non-NASH groups were evaluated using Pearson's chi-squared test, and continuous variables were evaluated using independent t-test.
The relevant paragraph was rewritten as follows.

(Page 7, Line 33 – Page 8, Line 2)
Methods
Statistical Analyses
Descriptive statistics were conducted using logistic regression analysis to analyze the relationship between NASH and sex, age, lifestyle-related diseases, death, and comorbidities (Odd Ratio [OR], 95% confidence interval [95% CI]). Differences between the NASH and non-NASH groups were evaluated using Pearson's chi-squared test for categorical variables and independent t-tests for continuous variables. Statistical significance was set at $p < 0.05$.
Comments about referencing:
Reviwer's comment #18
Some of the references need some formatting amendments, e.g., 11 & 12 where the journal title isn't in usual short-form.

Response #18
Thank you very much for your thorough review. We have made the necessary corrections to the journal titles in the references mentioned.

Reviewer: 2
Thank you for very much for your feedback. We hope that we addressed the problems you raised.

Comments to the Author:
Reviwer's comment #1
The title is not interesting may be needed to change clinical characteristics in NASH patients it is well known subject. However, authors presented the data from the updated information.

Response #1
Thank you for your suggestion. Based on your comment, we have modified the study title. The new title is: " Clinical characteristics in patients with nonalcoholic steatohepatitis in Japan: a 5-year large-scale claim database analysis"

Reviwer's comment #2
It is only focusing on clinical characteristics not define the NASH classifiers.

Response #2
As you mentioned, this study aimed to identify the clinical characteristics of patients and did not examine the pathological classifiers of NASH. The inability to comprehend pathological classification

is due to the lack of recorded medical examination results in the Japanese medical insurance claim database.
We have noted the points you mentioned in the methods section. The contents are as follows.

(Page 7, Line 23-24)
Methods
Data Collection

Baseline data on all patient characteristics (age, sex), date of death (if data were recorded), prescribed drugs for treating NASH, and NASH-related comorbidities were collected. Age and sex were obtained as of April 2015. The dates of death and prescribed drugs for treating NASH-related comorbidities were obtained at any time during the study period. Height, weight, and laboratory test values were also obtained from patients' available data at any time during the study period. BMI was calculated from the data of height and weight recorded.

Information on the pathological classification of NASH could not be obtained due to the unavailability of medical examination test results in the NHI and LEHI claims databases.

Reviwer's comment #3
Strengths were not mentioned in the strengths and limitations section.

Response #3
Thank you for your suggestion. The strengths and limitations section has been modified as follows.

(Page 2, Line 29 – Page 3, Line 4)
Strengths and Limitations of this study:

- In this study, analysis was performed using claim data covering a wide range of age groups, including elderly patients.
- To extract patients with NASH with high purity, data extraction was limited to patients with a history of liver biopsies. A long-term observation period of 5 years was established.
- Japan has several public health insurance systems, but the data used in this study were collected from the NHI and therefore did not cover the whole of Japan.
- It should be recognized that the data are secondary use and that some information is missing.

Reviwer's comment #4
Authors not identified proper key words.

Response #4
Thank you very much for your thorough review. We reselected the keywords as follows.
Public Health, Hepatobiliary disease < GASTROENTEROLOGY, Case-Control studies

Reviwer's comment #5
Risk adjusted confounders was not mentioned the Table 4.

Response #5
Thank you for your suggestion. Table 4 (shows that the ORs with NASH and NASH-related comorbidities adjusted for sex, age, and lifestyle-related diseases. This point is described in the main text.
Note that Table4 has become Table3 with this revision.

(Page 13, Line 14-15)
Comorbidities as risk factors for NASH

The OR with NASH and NASH-related comorbidities, adjusted for sex, age, and lifestyle-related diseases, is shown in Table 3.

VERSION 2 – REVIEW

REVIEWER	Clayton-Chubb, Daniel Alfred Hospital, Gastroenterology
REVIEW RETURNED	19-Jul-2023

GENERAL COMMENTS	Thank you for your comprehensive response to my original comments/suggestions, it's much appreciated. Some further minor comments/queries: 1. In the abstract you report: "Individuals with female hypertension..." -- do you mean hypertension was different only in the female subpopulation? Or that there were more females, and more hypertension, in the NASH group? 2. Considering the general medical nature of this journal, perhaps in the 'strengths and limitations' section where you note: "・ To extract patients with NASH with high purity, data extraction was limited to patients with a history of liver biopsies", it may be worth adding something like "a history of liver biopsies which may have selected for a group with more severe NASH", if you agree with that thought? 3. Page 4 Line 41 - the sentence ends prematurely - "blood tests in patients with obesity or ." 4. Page 4 Lines 47-48 - "As NASH is often asymptomatic and cirrhosis may already be diagnosed at the time of diagnosis" --> Cirrhosis may already be PRESENT at the time of diagnosis 5. There are a few statements like "This finding also suggests that NASH prevalence is higher in older females" -- it may be worth commenting on that potentially there is a selection bias in this sample (i.e., those who present to healthcare, those who get blood tests, those who agree to have a liver biopsy, etc.), and so given it's non-randomised and not necessarily representative of all patients drawing firm conclusions about exact age/gender rates is limited ? Thank you for the opportunity to review this revised manuscript.

VERSION 2 – AUTHOR RESPONSE

Reviewer’s comment #1

In the abstract you report: "Individuals with female hypertension..." -- do you mean hypertension was different only in the female subpopulation? Or that there were more females, and more hypertension, in the NASH group?

Response #1

Thank you for pointing out the unclear wording. Our analysis showed that there were more female and more patients with hypertension in the NASH group. In response to your suggestion, we have rewritten the relevant part as follows.

Results part of the abstract

The proportions of females, patients with hypertension, patients with dyslipidemia, and patients with type 2 diabetes were higher in the NASH group.

Reviewer’s comment #2

Considering the general medical nature of this journal, perhaps in the 'strengths and limitations' section where you note: ". To extract patients with NASH with high purity, data extraction was limited to patients with a history of liver biopsies", it may be worth adding something like "a history of liver biopsies which may have selected for a group with more severe NASH", if you agree with that thought?

Response #2

Thank you very much for your useful suggestion. We have rewritten the relevant part as follows.

Strengths and Limitations of This Study

- Data extraction was limited to patients with a history of liver biopsies, which may have been considered to include a group with more sever NASH.

Review’s comment #3

Page 4 Line 41 - the sentence ends prematurely - "blood tests in patients with obesity or ."

Response #3

We had checked everything carefully, but we overlooked “DM” which had been removed during some revising process. We have rewritten the relevant part as follows. We thank you for finding the omission of the checking.

blood tests in patients with obesity or DM [3]

Review’s comment #4

Page 4 Lines 47-48 - "As NASH is often asymptomatic and

cirrhosis may already be diagnosed at the time of diagnosis"

--> Cirrhosis may already be PRESENT at the time of diagnosis

Response #4

Thank you for pointing it out. Correct the corresponding part.

Reviewer’s comment #5

There are a few statements like "This finding also suggests that NASH prevalence is higher in older females" -- it may be worth commenting on that potentially there is a selection bias in this sample (i.e., those who present to healthcare, those who get blood tests, those who agree to have a liver biopsy, etc.), and so given it's non-randomised and not necessarily representative of all patients drawing firm conclusions about exact age/gender rates is limited ?

Response #5

Thank you for your very important feedback. Epidemiological studies in Japan have also shown that the prevalence of NASH is higher in older female. The present study supports the results of previous studies. On the other hand, as you pointed out, the sample size of our study may contain some selection biases. Based on your valuable suggestion, we added a description of selection bias to the limitations section.

Limitations

Furthermore, the study results suggest that the prevalence of NASH is higher in older female. However, there is a possibility of selection bias (e.g., those who visited a healthcare provider, had a blood test, or agreed to undergo a live biopsy).