**Supplementary**

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# Supplementary Figure 1



# Supplementary Figure 2



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# Supplementary Table 1.

The open online reporting form is available at: <https://zh.surveymonkey.com/r/SN5BCVY>

**Supplementary Table 1: Invitation for project to evaluation CK-18 in NAFLD, a global individual patient data registry study.**

|  |  |
| --- | --- |
| 1. **Basic information of partners** | |
| Name |  |
| Institution |  |
| Email |  |
| Country |  |
| Data collection time |  |
| Would you like to be listed as an author? |  |
| 1. **Basic information of patients** | |
| Country |  |
| Number of patient |  |
| Age |  |
| Gender |  |
| Race |  |
| 1. **Physical and laboratory indicators of patients** | |
| BMI (kg/m2) |  |
| CK-18 M30 levels (U⁄L) |  |
| ALT (U⁄L) |  |
| 1. **Coexisting diseases of patients** | Hypertension  Diabetes mellitus |
| 1. **Patient's liver biopsy data (NAS)** | |
| Steatosis |  |
| Lobular inflammation |  |
| Ballooning |  |
| 1. **Fibrosis stage** |  |

ALT: Alanine aminotransferase; BMI: Body mass index; CK-18: Cytokeratin-18; NAFLD: Non-alcoholic fatty liver disease; NAS: NAFLD activity score.

# Supplementary file 2. Detailed information of cohorts in this study.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Center** | **Country** | **Enrollment dates** | **Study design** | **PMID if data were used for publication** | **Eligibility criteria** | **Liver biopsy reading** | **Upper limit of ALT** | **Time interval between CK-18 M30 testing and liver biopsy (day/month)** |
| Angers cohort | France | From June 2013 to June 2018 | Prospective cross-sectional single center study | PMID: 29577364 | Inclusion: Liver biopsy scheduled of the evaluation of NAFLD | Central reading by a single expert pathologist | 49 IU/L | Within 24 h |
| Nice cohort | France | From January 2003 and April 2009 | Prospective, cross-sectional | PMID: 21050233 | All patients were negative for hepatitis B and C viral markers, autoantibodies indicative of autoimmune hepatitis, and had negligible alcohol consumption (< 20 g ⁄ day). Alcohol abuse was also excluded by interviewing the patients’ relatives. Patients with a history of inflammatory disease (including rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease), current infections, recent history of cancer (<5 years), and severe pulmonary or cardiac disease were not enrolled in the study. All patients underwent a Roux-en-Y divided gastric bypass, and a surgical liver biopsy was obtained during surgery. | Two expert pathologists blinded to clinical data | 31 IU/L for women and 41 IU/L for men | Within 24 h |
| Athens cohort | Greece | From January 2004 to December 2007 | Cross-sectional | PMID: 19881359 | Inclusion: NAFLD diagnosis based on  (a) increased ALT on ≥3 separate monthly determinations within the last 6 months, (b) absence of any other possible cause of liver injury, and (c) evidence of hepatic steatosis on liver biopsy  Exclusion: malignancy, decompensated liver disease, inadequate liver biopsy specimen (<1.5 cm), mean daily alcohol use >30/20 g for M/F, or positive HBsAg/anti-HCV/anti-HIV | Reading by a single expert pathologist | 40 U/L | Within 24 h |
| Kuala Lumpur cohort | Malaysia | From May 2012 to October 2015 | Prospective, cross-sectional | PMID: 30825254  PMID: 25184298 | Inclusion:  The subjects of this study were those screened and enrolled for a clinical trial at the Gastroenterology and Hepatology Clinic, University of Malaya Medical Centre. Consecutive adult NAFLD patients (>18 years old) were considered for inclusion into the clinical trial. The diagnosis of NAFLD was based on ultrasonography findings of fatty liver and exclusion of significant alcohol intake, use of medications that can cause hepatic steatosis, viral hepatitis B and C infection, and other causes of chronic liver disease where indicated. NAFLD patients with serum ALT or AST levels ≥40 IU/L were offered screening for the trial, which included a liver biopsy. Screening was also offered when there were other reasons for NASH to be suspected (e.g., significant liver fibrosis based on liver stiffness measurement, obese patients with metabolic syndrome).  Exclusion:  Patients in the clinical trial who were on insulin were excluded from this study to limit the influence of supra-physiological results of insulinemia induced by insulin therapy. | Single expert pathologist blinded to clinical data | 65 U/L | Within 24 h |
| Hangzhou cohort | China | From May 2015 to August 2016 | Cross-sectional | / | Inclusion:  (1) Age: 18–75, no limitation for ethnic and gender  (2) BMI <35 kg/m2  Patients with NASH based on liver biopsy obtained within 6 months before randomization. The histological evidence of NASH was defined as NAS) ≥4 (according to NASH CRN) with a minimum 1 score for steatosis, lobular inflammation, and hepatocyte ballooning, respectively.  (3) Without history of significant alcohol consumption for a period of >3 months within 5 years (<10 g/day for female and <20 g/day for male).  The lab test results should meet the requirements:  ① ALT < 5 times of normal upper limit  ② Creatinine (Cr) <normal upper limit  ③ ALB >3.5g/L  ④ NR = 0.8–1.3  ⑤ FPG <126mg/dL (7 mmol/L) and/or 2 h PPG <200 mg/dL (11.1 mmol/L) and/or HbA1C <6.5%  Exclusion criteria:  (1) Evidence of other form of acute or chronic liver disease. (virus hepatitis, hereditary hemochromatosis, hepatolenticular degeneration, alcoholic liver disease, drug-induced hepatopathy).  (2) Known heart failure of New York Heart Association class 2, 3, or 4.  (3) Wear of cardiac pacemaker.  Hypothyroidism (TSH >2 times of upper normal limit).  (4) History of disease affecting drug absorption, distribution, metabolism (inflammatory bowel disease, gastrointestinal surgery, chronic pancreatitis, gluten allergy, vagotomy).  (5) Positivity of antibody to human immunodeficiency virus.  Inability to safely obtain liver biopsy.  Known intolerance to vitamin E. | Expert pathologists | 52 U/L | Within 1 week |
| Sydney cohort | Australia | From 1999 to 2015 | Prospective cross-sectional single center study. | / | All patients were referred for the investigation of abnormal liver tests or steatosis detected by ultrasound. All patients had an alcohol intake of <20 g/day (males) or <10 g/day (females). The diagnosis of fatty liver disease was established by liver biopsy in all cases. We carefully excluded patients with liver disease of other etiology. | Routine reading by a single expert pathologist, not specifically blinded but unaware of CK-18 M30 measurement | / | Within 24 h |
| Bern cohort | Switzerland | From September 2017 to October 2019 | Single center, retrospective cohort study | / | Inclusion:  Patient >18 years; Patient undergoing liver biopsy for NAFLD between January 1, 2017 and November 1, 2019 at University Hospital Bern Bauchzentrum Hepatologie; Written general consent  Exclusion:  Concomitant steatosis-inducing drugs (methotrexate, amiodarone, tamoxifen, systemic corticotherapy); excessive alcohol consumption (>210 g/week in men or >140 g/week in women); other causes of chronic liver disease (e.g., chronic viral hepatitis B or C, hemochromatosis, autoimmune hepatitis, cholestatic liver diseases, alpha1 antitrypsin deficiency, Wilson disease …); decompensated cirrhosis (encephalopathy, variceal bleeding, liver failure, ascites); systemic infection; HCC; no measurement of CK-18 M30 available | Central reading by 1 expert pathologist, not specifically blinded but usually unaware of CK-18 M30 measurement | Male: >50 U/L; female:>35 U/L | Within 6 months |
| Wenzhou cohort | China | From 2017/01 to 2018/03 | Prospective cross-sectional single center study | PMID: 34152626  PMID: 31519069 | Inclusion: age 18–75 years; BMI <35 kg/m2; US, CT, or MRI imaging showing fatty liver disease; abnormal ALT but <5 ULN; no alcohol drinking history or daily alcohol intake <20 g for male and 10 g for female | Routine reading by a single expert pathologist | / | Within 2 days |
| Shanghai Ruijin cohort | China | From May 2019 to October 2020 | Cross-sectional | / | Inclusion:  NASH confirmed by liver biopsy  Exclusion:  Viral hepatitis, alcoholic liver disease, DILI, and WD | Reading by expert pathologists | 35 U/L | Within 24 h |
| Tianjin cohort | China | From August 2015 to November 2020 | Prospective, cross-sectional, single center, study | / | Inclusion:  1. NAFLD diagnosed by liver biopsy  2. agree to participate this project | Double blind and reading by pathologists | 40 U/L | Within 1 week |
| Mainz cohort | Germany | From August 2015 to October 2017 | Prospective study | PMID: 31342533 | Inclusion: biopsy‐confirmed NAFLD  Exclusion: Exclusion criteria were a history of bariatric surgery, BMI <18.5 or >45 kg/m2, instable cardiovascular or pulmonary diseases, immune‐mediated or musculoskeletal disease, liver cirrhosis, malignancy, and steatogenic or anticoagulation medication. Alcohol threshold was defined according to the EASL Guideline | Locally, blinded experts histopathologists | 35 U/L women  50 U/L men | Within 6 months |
| Hong Kong cohort | China | From 2004 to 2010 | Cross-sectional | PMID: 22314419  PMID: 23066946 | Inclusion:  - Age >18 years  - Biopsy-proven NAFLD  - Informed written consent  Exclusion:  - Excessive alcohol consumption (>30 g/day in men or >20 g/day in women)  - Secondary causes of hepatic steatosis  - Positive HBsAg or anti-HCV  - Histological evidence of other liver diseases | Single expert pathologist who was blinded to the clinical data | 53 U/L for men and 47 U/L for women | Within 1 week |
| Shanghai Xinhua cohort | China | From 2013 to 2014 | Prospective study | PMID: 27465946 | Inclusion:  - Age >18 years  - Biopsy-proven NAFLD  - Liver sample had to be ≧16 mm and at least 6 portal tracts  - Informed written consent  We excluded men who has a history of alcohol drinking habit or the ethanol intake per week >140 g in men (70 g in women) in the past 12 months, refusal to undergo liver biopsies, patients with secondary causes of hepatic steatosis (such as use of systemic corticosteroids), positive anti-hepatitis C virus antibody, or contraindications TE (e.g., pregnancy, ascites, with pacemaker implanted, and non-healing wounds in right upper quadrant abdomen). | The liver biopsies were interpreted by three experienced hepatopathologists, who were blinded to clinical data, and a consensus must be reached if disagreement | 40 U/L | Within 7 days |
| Istanbul cohort | Turkey | From 2009 to 2012 | Cross-sectional | / | Inclusion: Liver biopsy was performed in presence of the following indications: (1) evidence of hepatic steatosis on liver ultrasound; (2) abnormal liver enzymes or hepatomegaly or splenomegaly confirmed on imaging studies; and (3) exclusion of secondary causes of hepatic fat accumulation (e.g., significant alcohol consumption [>21 units of alcohol per week for men and >14 units of alcohol per week for women] and a previous history of steatogenic drugs use. Finally, all patients had biopsy-proven NAFLD diagnosis.  Exclusion: Being under the age of 18, presence of viral hepatitis, drug-induced liver disease, autoimmune hepatitis, genetic liver diseases, malignancy, low platelet count (<100,000/mL), and absence of steatosis >5% on liver biopsy. | Single blind reading by single expert hepatopathologist | 40 U/L | Within 6 months |

ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body Mass Index; CRN: Clinical Research Network; CK-18: Cytokeratin-18; FPG: Fasting plasma glucose; HCC: Hepatocellular carcinoma; INR: International normalized ratio; NAFLD: Non-alcoholic fatty liver disease; NAS: NAFLD activity score; NASH: Non-alcoholic steatohepatitis; PPG: Postprandial plasma glucose.

# Supplementary Table 3: Ln CK-18 M30 concentration in NAFL, borderline NASH, and NASH populations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Center** | **NASH (*n* = 855)** | **Borderline NASH (*n* = 649)** | **NAFL (*n* = 153)** | ***P*-value** |
| Angers (France) | 5.86 ± 0.710† | 5.39±0.650\* | 4.94±0.531 | <0.001 |
| Athens (Greece) | 5.96±0.674† | 5.69±0.404\* | 5.15±0.225 | <0.001 |
| Sydney (Australia) | 6.01±0.977† | 5.40±1.103\* | 4.47±0.672 | <0.001 |
| Hong Kong (China) | 6.34±0.880† | 6.03±0.756\* | 5.47±0.529 | <0.001 |
| Kuala Lumpur (Malaysia) | 6.33±0.695† | 5.81±0.579 | 5.57±1.107 | <0.001 |
| Nice (France) | 5.93 ± 0.587† | 5.30 ± 0.383 | 5.42 ± 0.397 | <0.001 |
| Shanghai Xinhua (China) | 6.10 ± 0.655† | 5.62 ± 0.549 | 5.21 ± 0.720 | 0.007 |
| Bern (Switzerland) | 5.67 ± 0.551 | 5.62 ± 0.670 | 5.31 ± 0.639 | 0.643 |
| Istanbul (Turkey) | 4.93 ± 0.934† | 4.30 ± 0.945\* | 3.38 ± 0.802 | <0.001 |
| Wenzhou (China) | 5.57 ± 1.105† | 4.91 ± 0.909 | 4.42 ± 0.800 | <0.001 |
| Mainz (Germany) | 6.46 ± 1.088 | 5.95 ± 0.595 | Insufficient NAFL data to calculate | 0.037 |
| Hangzhou (China) | 5.33 ± 0.761 | 5.31 ± 1.017 | Insufficient NAFL data to calculate | 0.960 |
| Shanghai Ruijin (China) | 5.35 ± 0.765 | 4.32 ± 0.704 | Insufficient NAFL data to calculate | <0.001 |
| Tianjin (China) | 5.25 ± 0.977 | 4.64 ± 0.748 | Insufficient NAFL data to calculate | 0.184 |
| Total | 5.70 ± 0.962† | 5.29 ± 0.880\* | 4.89 ± 0.821 | <0.001 |

CK-18: Cytokeratin-18; NAFL: Non-alcoholic fatty liver; NASH: Non-alcoholic steatohepatitis.

\*CK-18 M30 content is significantly different in NAFL and borderline NASH populations.

†CK-18 M30 content is significantly different in NAFL and NASH populations.