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# Liver biopsy in the real world – Reporting, expert concordance, and correlation with a pragmatic clinical diagnosis

**Running Title: Liver biopsy in the real world**

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HPK, MOI, ARM, AGA, MWF, AJS, and ASB contributed to the design of the study and original draft; HPK, AGA and ASB contributed to data curation. All authors (HPK, MOI, ARM, AGA, MR, PN, ASL, PT, JT, MWF, AJS, ASB) contributed to the interpretation of the data, critically reviewed and edited the manuscript, and approved the final draft for submission. AGA was responsible for the analysis, in addition to methodology, validation, and visualization of the data. ASB is the guarantor of the article.

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**List of Abbreviations:** nonalcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); American Association for the Study of the Liver Diseases (AASLD); non-invasive test (NIT); nonalcoholic fatty liver (NAFL); NAFLD activity score (NAS)
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**Abstract**

**Objectives:** Patients with nonalcoholic steatohepatitis (NASH) and fibrosis stage ≥2 comprise a target population for pharmacotherapy. Liver biopsy, the reference standard for identifying this population, requires complete and accurate assessment of steatohepatitis and fibrosis. This study aimed to (1)investigate the completeness of real-world NASH-related pathology reports, (2)assess concordance between site pathologists and central expert interpretation of the histologic elements of NASH, (3)determine concordance between biopsy-diagnosed NASH and a pragmatic clinical definition of NASH.

**Methods:** Liver pathology reports from 222 patients across 38 TARGET-NASH sites were analyzed for documentation of the histologic features of NASH. Biopsy slides were overread by a blinded central expert pathologist. Concordance of histologic scores and interpretation was assessed. Histologic concordance with a clinical definition of NASH was determined. TARGET-NASH clinically defined NASH: elevated ALT, hepatic steatosis on biopsy or imaging, and ≥1 of the following: BMI ≥30kg/$m^2$, type 2 diabetes mellitus, dyslipidemia.

**Results:** Documentation of steatosis, lobular inflammation, portal inflammation, ballooning were missing from 21%, 35%, 46%, 40% of reports, respectively. There was slight-fair concordance (weighted kappa 0.01-0.35) between site and central pathologists for inflammatory features and moderate concordance (weighted kappa 0.56-0.57) for fibrosis staging. Clinical definition of NASH: 75-91% concordant (94-95% sensitive) with biopsy-diagnosed NASH.

**Conclusions:** There is substantial variability in reporting and grading NASH and fibrosis staging in clinical practice. This heterogeneity may adversely impact patient assessment and translation of practice guidelines into reality. The TARGET-NASH pragmatic clinical definition may serve as a valuable tool to accurately identify NASH patients in clinical practice.

**Keywords:** nonalcoholic fatty liver disease, steatosis, grading, staging, biopsy

## Introduction

The growing prevalence of nonalcoholic fatty liver disease (NAFLD) contributes to the increasing burden of cirrhosis and need for liver transplantation.(1,2) Those with nonalcoholic steatohepatitis (NASH) comprise a subset of individuals with NAFLD who have a more aggressive disease phenotype marked by inflammation and fibrosis that is associated with increased morbidity and mortality.(3–5) The American Association for the Study of the Liver Diseases (AASLD) practice guidelines recommend a biopsy to identify patients with NASH and advanced fibrosis.(6) Liver biopsy interpretation requires accurate, reliable, and consistent reporting of the key components that define NASH and fibrosis. As novel therapeutics continue to be developed, the ability to accurately diagnose individuals is fundamental in identifying patients who may benefit from interventions that may lead to a reduction or reversal in disease progression.

The general indications for performing liver biopsy in NAFLD/NASH patients are to confirm or exclude the diagnosis of NASH and to assess disease severity.(7, 8) Liver biopsy remains the reference standard for diagnosing and assessing the histologic features of NASH, particularly the reversible necro-inflammatory components, and has been used in drug development and clinical trials to enroll patients and assess disease response. These *clinical trial* biopsies are interpreted by central expert pathologists.(9–11) However, the utility of a liver biopsy in *routine clinical practice* is dependent on the accuracy and reliability of its interpretation and whether or not all essential features are reported. Indeed, in real-world clinical practice, there is a paucity of data regarding the completeness and quality of liver pathology reports.

Since liver biopsies are invasive procedures that require post-procedural monitoring, increase healthcare utilization, and may be associated with serious complications, they are not scalable to the population as a whole.(12,13) The desire to avoid or limit the use of invasive testing, as well as the need to be able to apply diagnostics to a large population of patients, has led to research into non-invasive tests (NIT) to diagnose and stage NASH.(14,15) Such NITs have not yet met regulatory approval. Thus, there is a

critical unmet need for an inexpensive, practical, non-invasive method for diagnosing and clinically staging NASH.

Therefore, the aims of this study were to (1) describe the completeness of real-world histologic interpretation and reporting in patients who had liver biopsies to evaluate for NASH, (2) evaluate the concordance of histologic interpretation of NASH-related pathology slides between local pathologists and a central expert pathologist, and (3) assess the concordance between a pragmatic TARGET-NASH clinical definition of NASH and a histologic determination of NASH.

## Methods

### TARGET-NASH Cohort

TARGET-NASH is an ongoing longitudinal, observational real-world cohort that includes patients with NAFLD managed at academic and community hepatology, gastroenterology, and endocrinology practices in the United States (NCT02815891).(16,17) The study is sponsored by Target Real World Evidence (Target RWE), a company designed to collect pragmatic clinical information from routine clinical practice and provide phase 4 post marketing surveillance of new medications where applicable. Clinical information, including demographics, comorbidities, concomitant medications, laboratory results, pathology reports, and imaging data, is abstracted from the electronic medical records for a period of up to three years prior to enrollment and for up to five years prospectively, as previously described.(16–20) Institutional review board approval was obtained for each study site prior to enrollment and all enrolled participants provided written informed consent.

### Study Population

The current analysis includes adult patients enrolled in TARGET-NASH between 08/11/2016 and 10/12/2020 with the following characteristics: (1) age 18 years or older; (2) from United States sites; (3) with a needle liver biopsy date on or after 01/01/2014; and (4) a site pathologist overall interpretation of NAFLD, NASH, or steatohepatitis. Patients with an inconclusive biopsy according to interpretation by the central

pathologist were excluded. Additionally, patients were excluded if they had wedge biopsies, biopsies of a liver explant, or for whom a formal pathology report was unavailable for comparison. Patients with comorbid non-NAFLD liver diseases (e.g., autoimmune hepatitis, chronic hepatitis C) were also excluded.

**Nonalcoholic Fatty Liver Disease Definitions and Severity**

Patients enrolled in TARGET-NASH have been diagnosed with NAFLD based on usual clinical practice by their treating physician. Once enrolled into TARGET-NASH, patients are classified via biopsy and/or clinical criteria as having nonalcoholic fatty liver (NAFL), NASH, or cirrhosis due to NAFLD as previously described.(16–20) NASH is defined by the presence of steatosis, ballooning, and inflammation, with or without fibrosis, based on histologic interpretation.(6) A pragmatic clinical diagnosis of NASH was created by a panel of NASH experts and is based on an elevated ALT (>19 IU/L for women, >30 IU/L for men), hepatic steatosis on biopsy or imaging, and at least one of the following: BMI ≥30 kg/$m^2$, type 2 diabetes mellitus (HbA1c >6.5% or identified by review of the patient's medical history), or dyslipidemia (identified by review of the patient's medical history).(16) Patients' clinical information from +/- 2 years of their liver biopsy date was reviewed to determine whether or not they met criteria for a clinical diagnosis of NASH.

**Pathology Reports**

Liver pathology reports from academic and community practices were analyzed by two readers (HPK, ASB) for documentation of steatosis, lobular inflammation, portal inflammation, hepatocyte ballooning, and fibrosis stage. Disagreements were adjudicated. Specific quantification of each histologic element was recorded using the NAFLD activity score criteria.(21) Additionally, an overall NASH activity score according to a standardized scoring system (NAS or Brunt grade) was recorded.(21,22)

**Central Read of Digitized Biopsy Slides**

Biopsy slides from 150 participants were scanned, digitized, and over-read by a blinded central expert pathologist. An overall interpretation was made, including categories of definite NASH, possible NASH, and not NASH. Definite NASH was defined as the

presence of steatosis, lobular inflammation, and unequivocal hepatocyte ballooning. Possible NASH was included as a category for sensitivity analysis given the heterogeneous distribution of NASH-related lesions and the potential for sampling error that might lead to inaccurate diagnosis and staging.(15,23) Possible NASH required the presence of steatosis, as well as lobular inflammation but no unequivocal ballooning. In the presence of cirrhosis and absent inflammation and ballooning, NASH was still considered possible if there was any amount of steatosis. Not NASH was defined as the absence of steatosis and hepatocyte ballooning. These definitions are summarized in Table 1. For biopsy slides with a central interpretation of definite or possible NASH, hepatic steatosis, lobular inflammation, portal inflammation, hepatocyte ballooning, and fibrosis stage were assessed. Each slide was also assigned scores using the NAS and Brunt grade.(21,22)

**Statistical Analysis**

Descriptive statistics were reported for baseline characteristics for all patients and by pathologist/biopsy read type, central vs. the local site pathologist. Continuous variables were summarized using the frequency, median, minimum, and maximum values. Categorical variables were summarized using the frequency and the percentage relative to those with non-missing values. Demographic information summarized included age at enrollment, gender, race, ethnicity, pathologist type (i.e., central vs. site), and site type. Pathologist documentation of hepatic steatosis, lobular inflammation, portal inflammation, hepatocyte ballooning, fibrosis, and standardized NAFLD scoring systems, NAS and Brunt grades, were reported as percentages of the total number of pathology reports. Pathologists' concordance for quantifying steatosis, lobular inflammation, portal inflammation, hepatocyte ballooning, and fibrosis, assessed using weighted kappa statistics, were reported.(24) The concordance between the central pathologist biopsy-defined NASH and the TARGET-NASH clinical definition were summarized using frequency counts and percentages. The sensitivity and specificity of the TARGET-NASH clinical definition were calculated in reference to the histologic definition as the reference standard. All analyses were performed in SAS, version 9.4, and JMP Pro, Cary, NC.

## Results

### Baseline Characteristics

A total of 222 pathology reports representing unique individuals from 38 TARGET-NASH sites were included and reviewed to assess pathology report completeness when evaluating NASH (Figure 1, Table 2). Of these, scanned pathology slides from 150 participants enrolled across 13 TARGET-NASH sites were scanned and digitized for interpretation by the central expert pathologist to evaluate concordances of histologic interpretation between local and central pathologists, and between a pragmatic TARGET-NASH clinical definition of NASH and a histologic determination of NASH. These pathology slides represented 150 unique patients with a median age of 55 years (range 21-75), 63% female, and 86% white; 75% were interpreted at academic (i.e., academic teaching or university affiliated hospital) sites and 25% at community (i.e., private practice) sites. Available scanned liver biopsy slides that were centrally read came from patients who were slightly younger (median age 55 vs. 58 years), more frequently Hispanic (19% vs. 10%), and were more commonly interpreted at academic sites (75% vs. 63%) as compared to patients whose biopsies were not available for central read.

### Histologic Reporting by Local Pathologists

Of the 222 pathology reports that were reviewed, documentation of steatosis, lobular inflammation, portal inflammation, and ballooning were missing from 21%, 35%, 46%, and 40% of reports, respectively. Grading of NASH was more commonly performed using the NAS compared to the Brunt criteria; however, 55% of reports did not include either of these validated grading scores. Fibrosis was frequently reported with only 5% of reports missing a fibrosis score and only 3% of reports missing any description of fibrosis (data not shown).

### Concordance of Histologic Interpretation of NASH Features

Of the 150 biopsy slides that were centrally interpreted, there was substantial discordance in the quantification of NASH components and fibrosis staging by local

versus central expert pathologists (Table 3). Based on kappa scores, there was slight to fair concordance (weighted kappa range 0.01-0.31) for steatosis, lobular inflammation, portal inflammation, and hepatocyte ballooning. Fair concordance was observed for overall assessment of disease using the NAS (weighted kappa – 0.35; 95% CI 0.23, 0.46) and Brunt grading criteria (weighted kappa – 0.34; 95% CI 0.11, 0.58), and concordance was moderate for fibrosis staging with a weighted kappa statistic of 0.57 (95% CI 0.48, 0.66). When limited to concordance among biopsies with advanced fibrosis or cirrhosis (F 3-4), concordance was substantial (weighted kappa - 0.61 95% CI 0.52, 0.71).

**Concordance of TARGET-NASH Clinically-Defined NASH vs Biopsy-Defined NASH**

Of the 150 centrally-interpreted biopsy slides, 103 (69%) were interpreted as definite NASH, 23 (15%) possible NASH, and 24 (16%) not NASH according to histologic interpretation. The 23 biopsy specimens that were judged to be only possible NASH based upon central reading of the liver biopsy were all from patients who met pre-established clinical criteria for NASH. Despite the presence of several histological features of NASH including evidence of steatosis (23/23), portal and/or lobular inflammation (22/23), and fibrosis (16/23), the absence of definitive ballooning in all of these specimens precluded the histological definition of NASH (Table 4). Across the study population 31 patients had significant fibrosis (fibrosis stage ≥ 2) but no ballooning.

In 113 (75%) cases, there was concordance between the TARGET-NASH clinical and histologic diagnoses, where 96 cases were determined to represent NASH and 16 cases were determined as not meeting criteria for NASH (Figure 1). The remaining 37 (25%) patients included 31 patients with a clinical diagnosis of NASH but without definite histologic NASH. However, 23 of these 31 cases were classified as representing possible NASH by the central pathologist. When categorizing these cases as histologic NASH, the concordance between clinical and biopsy-driven NASH diagnosis increased to 91%. Only 6 of the 150 patients (4%) with biopsy-diagnosed

NASH did not meet all the criteria for clinically-defined NASH, due to the absence of obesity (BMI ≥30 mg/kg$^2$), although all were overweight (BMI ≥25 mg/kg$^2$). Using the biopsy-defined definite NASH diagnosis as the reference standard, the clinical NASH definition was 94% sensitive (95% CI 87.8, 97.8) and 34% specific (95% CI 20.9, 49.3). When defining biopsy-defined NASH as those with definite or possible NASH, the sensitivity and specificity of the clinical definition increased to 95% (95% CI 89.9, 98.2) and 67% (95% CI 44.7, 84.4), respectively, with a positive predictive value of 94% and a negative predictive value of 73% (Table 5).

**Discussion**

In this real-world observational cohort of patients with NAFLD, there is substantial heterogeneity in the local histological reporting of NASH with a large proportion of reports missing important descriptors of NASH disease activity, as well as only slight to moderate concordance between local and central pathologists when interpreting specific features of NASH and stages of fibrosis. Of note, we also found that the TARGET-NASH clinical definition of NASH was accurate in identifying biopsy-defined NASH, as confirmed by a central expert pathologist, in the vast majority of cases.

In the appropriate context, liver biopsy remains a necessary step in staging the activity and severity of NASH and in excluding or identifying comorbid liver diseases. While liver biopsy remains the reference standard for assessing the histologic elements of NASH, the utility of a liver biopsy is limited by its interpretation, reporting, and selection of who receives a liver biopsy.(17) The degree of heterogeneity and missing components in pathologic reporting observed in this study limits the value of these biopsies in routine clinical care. Furthermore, the concordance for biopsy interpretation was moderate at best. Useful reporting should clearly document and quantify hepatic steatosis, lobular and portal inflammation, hepatocyte ballooning, and degree of fibrosis, with the goal of accurately staging disease severity and being able to differentiate between NAFL and NASH. When not all the diagnostic criteria for NASH are reached, it is useful to underline in the descriptive remarks of the histological report if there are elements of steatosis and associated with ballooning or lobular inflammation or, due to possible

sampling error, NASH cannot be entirely excluded. A descriptive conclusion should be provided in these cases. Recommendations for such reporting have recently been put forth by the AASLD NASH Task Force, which was composed of a multidisciplinary team of hepatologists and pathologists and underscores the importance of these constituencies working closely together.(25) Such heterogeneity and lack of reliability in reporting and interpretation may adversely impact patient assessment and application of new NASH therapies. Utilizing specific standardized scoring mechanisms may improve concordance between pathologists' interpretations and increase the utility of performing liver biopsies, which are not without complications.

Another important limitation of performing liver biopsies is related to the heterogeneous or uneven distribution of NASH-related lesions throughout the liver parenchyma.(15,23) Therefore, even in the presence of optimal histologic interpretation and reporting, liver biopsies are subject to sampling error that can lead to inaccurate diagnoses and disease staging. According to our histologic definitions of NASH, slides were determined to represent definite NASH, possible NASH, and not NASH. All cases of possible NASH were related to the absence of unequivocal hepatocyte ballooning. In the correct clinical context, therefore, it is reasonable to conclude that these cases represent true NASH. This additional limitation of liver biopsies brings into question the role of liver biopsy in NAFLD and contributes to our understanding that the utility of liver biopsies for clinical decision making in the real world may not be practical.

In light of the current limitations of liver biopsy and histologic interpretation, one important unmet need is a pragmatic and accurate clinical definition for the diagnosis of NASH. With the growing prevalence of NAFLD, it has become increasingly important to be able to screen for and diagnose NASH early, ideally using noninvasive methods with clinical information that is readily available. As an observational cohort, TARGET-NASH developed a clinical definition of NASH with such pragmatism in mind, only requiring information that is available as part of routine care. This is in contrast to studies that have evaluated, or are evaluating, specific biomarkers to identify that NASH that include variables that are not routinely measured. (14) Using a biopsy interpretation for definite

NASH, the TARGET-NASH clinical diagnosis was able to detect 94% of NASH cases as interpreted by a central expert pathologist. The performance of the TARGET-NASH clinical definition improved when including cases of biopsy-defined possible NASH in the reference standard, with favorable operating characteristics that would not only allow for the detection of cases but reasonably rule them out, as well. The high sensitivity we observed is essential when using a tool or test to screen for a disease.

There are some limitations of the current study that must be acknowledged. In evaluating the TARGET-NASH clinical definition of NASH, we were only able to assess for concordance between a clinical diagnosis and a biopsy-defined diagnosis rather than validating the clinical definition. A validation study would require large cohorts of individuals with and without clinical NASH to undergo liver biopsies, something that is beyond the scope of what is possible through the TARGET-NASH cohort. Another possible limitation is that the subset of slides available for central interpretation represented only 13 of the original 38 sites and more often came from academic sites. If there is a difference in the histologic reporting between academic vs. community sites, then the assessment of concordance of histologic interpretation may be subject to bias. Further limitations include missing data such pathology reports that were unavailable for comparison and race/ethnicity descriptors that were unavailable in two patients.

This study highlights important and relevant limitations of liver biopsy interpretation and reporting in the real-world clinical setting. As liver biopsy currently remains the reference standard for diagnosing and staging NASH, major efforts must be made to improve the comprehensiveness and consistent reporting of histologic findings to improve the utility of biopsies in patient assessment and management. Additionally, this study importantly demonstrates that a pragmatic clinical definition of NASH can be used to accurately identify individuals who may require more aggressive management and benefit from future therapeutics. This clinical NASH definition developed by TARGET-NASH warrants further investigation in future validation studies.

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**Statement of Interests**:

i. MOI is a consultant for Target RWE.
ii. ARM and AGA are employees of Target RWE.

iii. MR has served on scientific advisory boards or received speaker's honoraria for Allergan, Boehringer-Ingelheim Pharma, Bristol-Myers Squibb, Eli Lilly, Fishawack Group, Gilead Sciences, Novartis Pharma, Intercept Pharma, Inventiva, Novo Nordisk, Target NASH. He is also a consultant for Terra Firma and has been involved with clinical trial research for Boehringer Ingelheim, Danone Nutricia Research and Sanofi-Aventis.

iv. ASL has research grants from BMS, Gilead, and Target RWE. She is also an advisor/ and consultant for BMS and Target RWE, and serves on the DSMB of Novo Nordisk.

v. MWF is Chief Medical Officer for TARGET RWE and receives personal fees and is a stockholder in the company.

vi. AJS is President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect Inversago and Galmed. He has served as a consultant to Astra Zeneca, NGM Bio, Conatus, Salix, Tobira, Takeda, Jannsen, Gilead, Terns, Birdrock, 89 Bio, Siemens, Amgen, Regeneron, Alnylam, Genentech, Roche, Merck, Valeant, Boehringer-Ingelheim, Bristol Myers Squibb, Lilly, Hemoshear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exhalenz and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Zydus, Nordic Bioscience, Albireo, Prosciento, Surrozen. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, Astra Zeneca, Pfizer, Novo Nordisk, Boehringer Ingelhiem, Malinckrodt, Cumberland and Novartis. He receives royalties from Elsevier and UptoDate.

vii. ASB is a consultant for Target RWE, Pfizer Inc, and Novo Nordisk.

viii. All other authors declare that they have no conflicts of interest to disclose.

**Figure 1. Consort diagram for centrally interpreted liver biopsies and concordance between biopsy-defined NASH vs clinically-defined NASH**

Diagram reflects the selection of the population for this study based on data from the TARGET-NASH study. Liver biopsy reports were obtained for participants from participating study sites; scans of biopsy slides were available for a subset of these biopsies and were read by a central pathologist. Concordance between the central pathologist diagnosis and that based on the clinical definition was assessed.

**Table 1. Histologic definitions of definite NASH, possible NASH, and not NASH**

| | Steatosis | Ballooning | Inflammation |
|---|---|---|---|
| **Definite NASH** | Present, > 5% | Unequivocal hepatocellular ballooning | Present |
| **Possible NASH *without cirrhosis*** † | Present, >5% | Absent | Lobular inflammation present |
| **Possible NASH *with cirrhosis*** ‡ | Present, >5% | One may be present but not required | |
| **Not NASH** | Absent, <5% | Absent | Absent or present from another etiology |

†Possible NASH without cirrhosis was present with >5% steatosis and the presence of lobular inflammation but no unequivocal ballooning

‡Possible NASH as the etiology for cirrhosis was considered possible if only steatosis was present due to the possibility of “burnt out NASH” as ballooning and inflammation may disappear with advanced disease

**Table 2. Baseline characteristics for the study population and stratified by biopsy read type**

| Summary | Centrally Read Biopsy (N = 150) | Non-Centrally Read Biopsy (N = 72) |
|---|---|---|
| Age at Study Entry (years) | | |
| Median (n) | 55.0 (150) | 57.5 (72) |
| Min - Max | 21.0 - 75.0 | 30.0 - 74.0 |

| Summary | Centrally Read Biopsy (N = 150) | Non-Centrally Read Biopsy (N = 72) |
|---|---|---|
| Gender, n (%) | | |
| n | 150 | 72 |
| Female | 95 (63.3%) | 44 (61.1%) |
| Male | 55 (36.7%) | 28 (38.9%) |
| Race, n (%) | | |
| n | 145 | 71 |
| White | 124 (85.5%) | 60 (84.5%) |
| Black or African American | 11 (7.6%) | 3 (4.2%) |
| Asian | 7 (4.8%) | 7 (9.9%) |
| Other | 3 (2.1%) | 1 (1.4%) |
| Not Available | 5 | 1 |
| Ethnicity, n (%) | | |
| n | 149 | 70 |
| Hispanic or Latino | 28 (18.8%) | 7 (10.0%) |
| Not Hispanic or Latino | 120 (80.5%) | 63 (90.0%) |
| Other | 1 (0.7%) | |
| Not Available | 1 | 2 |
| Site Type, n (%) | | |
| n | 150 | 72 |
| Academic | 112 (74.7%) | 45 (62.5%) |
| Community | 38 (25.3%) | 27 (37.5%) |
| Pathology Type, n (%) | | |
| n | 150 | 72 |
| Central | 150 (100.0%) | --- |
| Local | --- | 72 (100.0%) |

**Table 3. Kappa statistics for concordance of histological interpretation of NASH for central vs local read of biopsies**

| Histological Characteristic | Number of Pathology Reports Compared | Weighted Kappa Statistic (95% CI) | Concordance Interpretation* |
|---|---|---|---|
| Steatosis | 66 | 0.31 (0.15, 0.46) | Fair |
| Lobular Inflammation | 37 | 0.01 (-0.19, 0.22) | Slight |
| Portal Inflammation | 35 | 0.18 (-0.05, 0.40) | Slight |
| Hepatocyte Ballooning | 34 | 0.20 (0.02, 0.38) | Slight |
| Fibrosis Stage | 109 | 0.56 (0.46, 0.65) | Moderate |
| NAFLD Activity Score | 77 | 0.35 (0.23, 0.46) | Fair |
| Brunt Grade (Inflammation) | 27 | 0.34 (0.11, 0.58) | Fair |
| Brunt Stage (Fibrosis) | 112 | 0.57 (0.48, 0.66) | Moderate |

NAFLD = nonalcoholic fatty liver disease

*Concordance: 0-0.20 as slight; 0.21-0.40 as fair; 0.41-0.60 as moderate; 0.61-0.80 as substantial; 0.81-1 as almost perfect (Landis, JR, Koch, GG. “The measurement of observer agreement for categorical data.” *Biometrics*. 1977;33(1):159-174.)

**Table 4. Histological and clinical characteristics of patients with possible NASH by central pathologist**

| | Histologic Features of NASH | | | | | Clinical Features of NASH | | | |
|---|---|---|---|---|---|---|---|---|---|
| Subject | Steatosis | Ballooning | Inflammation | | Fibrosis | Steatosis | Elevated ALT* | BMI ≥30 kg/m² | Diabetes and/or dyslipidemia** |
| | | | Portal | Lobular | | | | | |
| 1 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 2 | Yes | No | Yes | Yes | No | Yes | Yes | Yes | No |
| 3 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 5 | Yes | No | Yes | Yes | No | Yes | Yes | Yes | Yes |
| 6 | Yes | No | No | Yes | No | Yes | Yes | Yes | No |
| 7 | Yes | No | Yes | Yes | No | Yes | Yes | Yes | Yes |
| 8 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | Yes | No | Yes | Yes | No | Yes | Yes | Yes | Yes |
| 10 | Yes | No | Yes | Yes | No | Yes | Yes | No | Yes |
| 11 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | Yes | No | Yes | Yes | No | Yes | Yes | Yes | Yes |
| 13 | Yes | No | Yes | Yes | N/A | Yes | Yes | Yes | Yes |
| 14 | Yes | No | No | Yes | Yes | Yes | Yes | Yes | Yes |
| 15 | Yes | No | Yes | Yes | Yes | Yes | Yes | No | Yes |
| 16 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 17 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 19 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 20 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 21 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 22 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | Yes | No | Yes | Yes | Yes | Yes | Yes | No | Yes |

*Adults: ALT>19 U/L for female, >30 U/L for male at any time.

**The present of type 2 diabetes was defined by HbA1c >6.5% or medical history; dyslipidemia identified by review of the patient's medical history.

**Table 5. Sensitivity and specificity of the TARGET-NASH clinical definition of NASH according to biopsy-defined NASH categories**

| Biopsy-defined NASH | Sensitivity (95% CI) | Specificity (95% CI) | PPV | NPV |
|---|---|---|---|---|
| Definite NASH | 94.2 (87.8, 97.8) | 34.0 (20.9, 49.3) | 75.8 | 72.7 |
| Definite or Possible NASH | 95.2 (89.9, 98.2) | 66.7 (44.7, 84.4) | 93.8 | 72.7 |

NASH = nonalcoholic steatohepatitis; PPV = positive predictive value; NPV = negative predictive value

**Appendix**

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