Received: 20 January 2021 | Revised: 1 April 2021 | Accepted: 6 April 2021

DOI: 10.1111/liv.14898

ORIGINAL ARTICLE

Liver INTERNATIONAL WILEY

Definite and indeterminate nonalcoholic steatohepatitis share similar clinical features and prognosis: A longitudinal study of 1893 biopsy-proven nonalcoholic fatty liver disease subjects

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Abbreviations: AHT, arterial hypertension; BMI, body mass index; MAFLD, metabolic-associated fatty liver disease; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

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Funding information
This project has been partially funded by the 'Consejería de Salud de la Junta de Andalucía' (PI-0075-2014) and the 'Spanish Ministry of Economy, Innovation and Competition, *Instituto de Salud Carlos III*' (PI19/01404, PI16/01842, PI17/00535 and GLD19/00100). The funders have not had any role in the design, analysis, writing or interpretation of this project.

Handling Editor: Salvatore Petta

Abstract

Background and Aim: Histological score systems may not fully capture the essential nonalcoholic steatohepatitis (NASH) features, which is one of the leading causes of screening failure in clinical trials. We assessed the NASH distribution and its components across the fibrosis stages and their impact on the prognosis and their relationship with the concept of metabolic-associated fatty liver disease (MAFLD).

Methods: Spanish multicenter study including 1893 biopsy-proven nonalcoholic fatty liver disease (NAFLD) patients from HEPAmet registry. NASH was diagnosed by NAS score ≥4 (including steatosis, ballooning and lobular inflammation) and fibrosis by Kleiner score. The presence of MAFLD was determined. Progression to cirrhosis, first episode of decompensated cirrhosis and death were collected during the follow-up (4.7 ± 3.8 years).

Results: Fibrosis was *F*0 34.3% (649/1893), *F*1 27% (511/1893), *F*2 16.5% (312/1893), *F*3 15% (284/1893) and *F*4 7.2% (137/1893). NASH diagnosis 51.9% (982/1893), and its individual components (severe steatosis, ballooning and lobular inflammation), increased from *F*0 (33.6%) to *F*2 (68.6%), and decreased significantly in *F*4 patients (51.8%) ($P = .0001$). More than 70% of non-NASH patients showed some inflammatory activity (ballooning or lobular inflammation), showing a similar MAFLD rate than NASH (96.2% [945/982] vs. 95.2% [535/562]) and significantly higher than nonalcoholic fatty liver (NAFL) subjects (89.1% [311/349]) ($P < .0001$). Progression to cirrhosis was similar between NASH (9.5% [51/539]) and indeterminate NASH (7.9% [25/316]), and higher than steatosis (5% [14/263]) (logRank 8.417; $P = .015$). Death and decompensated cirrhosis were similar between these.

Conclusions: The prevalence of steatohepatitis decreased in advanced liver disease. However, most of these patients showed some inflammatory activity histologically and had metabolic disturbances. These findings should be considered in clinical trials whose main aim is to prevent cirrhosis progression and complications, liver transplant and death.

KEYWORDS
ballooning, fatty liver disease, inflammation, metabolic-associated fatty liver disease, natural coursesteatohepatitis, steatosis

Key points

- The prevalence of definite NASH histological criteria decreases in advanced liver disease, precluding these subjects from receiving new therapeutic options.
- Patients with NASH and not-NASH specific inflammatory activity share similar clinical features and prognosis, and they are different from those with simple steatosis.
- Most of the patients with significant fibrosis and cirrhosis without a well-defined NASH show metabolic disturbances.
- This is the first study in clinical practice reinforcing the renaming of MAFLD.

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of histological features that range from simple steatosis to nonalcoholic steatohepatitis (NASH) and, finally, cirrhosis.[1] Splitting NAFLD into three successive stages (steatosis, non-cirrhotic NASH and cirrhotic NASH) has provided a convenient conceptual framework for improving the diagnostic methods and identifying areas of potential future drug development.[2] However, the diagnosis of NAFLD is challenging sometimes, so we need to consider a more pragmatic approach to targeting these individuals. In this scenario, the term "metabolic-associated fatty liver disease" (MAFLD) has been recently proposed.[3] The diagnosis of MAFLD is based on recognizing underlying alterations in metabolism,[4] beyond the histological classification of NAFLD. MAFLD is defined by the presence of steatosis (by histology or imaging) and overweight or at least two metabolic risk factors. Notably, steatosis could be absent in the case of advanced liver disease.[5]

Subjects enrolled in NASH clinical trials are usually required to have a NAS ≥ 4 and a fibrosis stage from *F*1 to cirrhosis by liver biopsy. However, up to half of the screened individuals fail to meet these eligibility criteria.[6] We should consider that NAS score was developed as a tool to measure NAFLD changes during therapeutic trials instead of as a surrogate for the histologic diagnosis of NASH.[7] Notably, previous studies have reported that 20%–40% of the NAFLD population does not display a definite NASH histological diagnosis,[8,9] making these individuals ineligible for enrollment. Consequently, the large number of suboptimal biopsies adds significantly to the cost and duration of clinical trials. However, liver fibrosis is considered the strongest predictor of adverse clinical outcomes.[10] Besides, treatment goals for patients with advanced liver disease are to halt or slow fibrosis progression, prevent clinical decompensation, reduce the need for liver transplantation and improve survival.[11]

The liver biopsy must keep being essential in the management of NAFLD,[12] but we should avoid converting this tool into a barrier to diagnose, treat or identify at-risk patients. For instance, the development of non-invasive tests and imaging-based methods has allowed a wide expansion of liver fibrosis assessment in at-risk patients.[13-16] Thus, the liver biopsy should be relocated to maximize its advantages in selected patients. In a Spanish large cohort of individuals with biopsy-proven NAFLD followed-up during 4.7 years, we aimed to assess the prevalence of NASH and its components (steatosis, ballooning and lobular inflammation) across the fibrosis stages and link them with prognostic outcomes to determine if NAS score ≥4 reflects a reliable scenario in clinical practice as inclusion criterium in NAFLD clinical trials.

2 | METHODS

2.1 | Selection of patients

This is an observational study of 1893 patients with biopsy-confirmed NAFLD who had been enrolled and prospectively followed up from the Spanish HEPAmet Registry. This registry is governed by the Spanish Association for the Study of the Liver and the Network of Biomedical Research Centre for the Study of the Liver and Digestive Diseases (CIBERehd). Data monitoring is a fundamental element of the registry, ensuring data procurement accuracy and minimization of bias.

Patients underwent a liver biopsy according to the routine decisions in the clinical practice (eg, presence of fatty liver by imaging, increased aminotransferase levels, based on non-invasive tests and suspected advanced liver disease by imaging or laboratory tests) or at the time of bariatric surgery. The inclusion criterion was biopsy-proven NAFLD, irrespective of the existence of NASH or fibrosis stage. Exclusion criteria were significant alcohol intake (≥30 g daily for men and ≥20 g daily for women) and evidence of concomitant liver disease (ie, viral or autoimmune hepatitis, HIV, drug-induced fatty liver, hemochromatosis or Wilson's disease). The study was performed in agreement with the Declaration of Helsinki and approved by the Ethics and Clinical Research Committee of every centre. All patients were informed of the nature of the study and gave their written consent to participate.

2.2 | Clinical assessment

Demographic characteristics, anthropometric measurements and laboratory tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), triglycerides, cholesterol, HDL-c, LDL-c, fasting glucose, HbA1c, insulin, creatinine and albumin) were recorded at the same time of liver biopsy. An overnight (12-h) fasting blood sample was taken for routine biochemical analyses. Homeostatic model assessment (HOMA)

was calculated based on insulin and glucose (fasting insulin (mIU/ml) × fasting glucose (mg/ml)/405).

We defined the following metabolic risk factors at baseline[17]: (a) type 2 diabetes mellitus (T2DM), defined by fasting blood glucose ≥126 mg/dl, or Hb1Ac >6.5%, or use of blood glucose-lowering agents; (b) arterial hypertension (AHT), determined by blood pressure ≥130/85 mm Hg or use of blood pressure-lowering agents; (c) low HDL, defined by HDL-c < 40 mg/dl in men or <50 mg/dl in women or use of lipid-lowering agents; (d) hypertriglyceridemia, characterized by triglyceride levels ≥150 mg/dl or use of lipid-lowering agents; (e) overweight and obesity, determined by a body mass index (BMI) >25 and 30 kg/m^2, respectively; (f) insulin resistance, defined by HOMA > 2.5 in absence of T2DM. Metabolic syndrome was considered when at least three single components were present, while MAFLD was defined according to the recent consensus.[3]

Follow-up was defined, depending on the endpoint, as the time from the liver biopsy to the progression to cirrhosis (for non-cirrhotic patients), the first event of cirrhosis complication (ascites, hepatic encephalopathy and variceal bleeding) or death. They were collected whenever the investigator received notice about the event. In the case of no event, patients were censored at 10 years of follow-up or at the end of the study period (May 2020). In non-cirrhotic patients at baseline, the progression to cirrhosis was considered when typical findings of cirrhosis were observed during the follow-up in a second liver biopsy (3/90), abdominal ultrasound (38/90) or transient elastography (>16 kPa) (36/90), or when an episode of decompensated cirrhosis occurred (13/90) (TARGET-NASH criteria[18]).

2.3 | Histological assessment

The diagnosis of NAFLD was based on histological criteria. All liver biopsies were assessed by experienced hepato-pathologists (leaded by MJPM), associated with the LITMUS histopathologists group,[19] who were blinded regarding the patient's evaluation and clinical data. Samples of <15-mm length or <10 portal tracts were considered not suitable for diagnosis and were excluded. Several histological aspects were measured. Steatosis, lobular inflammation and hepatocyte balloon degeneration were systematically assessed according to the NASH CRN Scoring System: (a) Steatosis was rated as Grade 0 (<5%), Grade 1 (5%–33%), Grade 2 (33%–66%) and Grade 3 (>66%); (b) hepatocyte ballooning was considered as 0 (*none*), 1 (mild-*few*) and 2 (*moderate*-marked); (c) lobular inflammation was rated as 0 (*none*), 1 (*<2 foci/20 optical field*), 2 (*2-4 foci/20 optical field*) and 3 (*>4 foci/20 optical field*). Although NASH CRN does not define exactly NASH by using the NAS score,[20] we determined NASH according to NAS score ≥4 (with at least 1 point each in inflammation and ballooning).[7] This threshold was based on the inclusion criteria of most Food and Drug Administration (FDA)-approved clinical trials to develop NAFLD therapeutic drugs. The severity of fibrosis was staged from 0 to 4.

2.4 | Objectives

We assessed if NAS score ≥4 as inclusion criterion in NAFLD clinical trials reflects a reliable scenario in clinical practice, due to some cases with higher NAS score could not have findings of definite NASH and other cases of lower NAS score do.[20] To achieve this goal, secondary aims were (a) to analyze the presence and the distribution of NAS score ≥4 and of its single components (steatosis, inflammation and ballooning) across the fibrosis stages; (b) to analyze the histological features of patients with apparent lack of NASH (NAS < 4), who are usually excluded from clinical trials; (c) to perform an exploratory analysis to determine the prognosis of three histological patterns based on NAS score and liver inflammation (NASH vs. indeterminate NASH vs. NAFL) in terms of progression to cirrhosis, the appearance of the first episode of decompensated cirrhosis and death.

2.5 | Statistical analyses

Data were reported as the mean ± standard deviation for normal and median (interquartile range) for non-normal continuous variables, while frequency was used for discrete variables. In the univariate comparisons, we used the Student's t test and ANOVA with Bonferroni adjustments for continuous samples and chi-square test or Fisher's exact test for the qualitative ones. Non-parametric alternatives (Mann–Whitney U and Kruskal–Wallis tests) were used for non-normal distributions. Survival analysis was assessed by the Kaplan–Meyer method, and differences between patient subgroups were evaluated by the log-rank test. Values were supposed to be statistically significant when $P < .05$.

On the other hand, the annual rate of progression to cirrhosis and the appearance of the first episode of decompensated cirrhosis and death were computed by dividing the number of patients with the defined event by the number of person years of which patients were followed. We multiply rates by 100 to transform in cases per 100 person years.

The method used for missing data was complete-case analysis because statistical packages excluded individuals with any missing value. STATA (12.0, STATA Corporation, College Station, TX, USA) statistical package was used in all analyses and GraphPad Prism (version 6.0; GraphPad Software, Inc, La Jolla, CA) for graphics.

3 | RESULTS

3.1 | Baseline features of the study population

The baseline features of the study cohort are stated in Tables 1 and S1. Briefly, steatohepatitis (NAS ≥ 4) was present in 51.9% (982/1893) of the overall population. According to liver fibrosis: *F*0 34.3% (649/1893), *F*1 27% (511/1893), *F*2 16.5% (312/1893), *F*3 15% (284/1893) and *F*4 7.2% (137/1893). The 41.6% (788/1893), 36.8% (696/1893) and 21.6% (409/1893) showed Grades 1, 2 and

3 of steatosis, respectively. On the other hand, lobular inflammation was seen in 73.5% (1391/1893) of patients (50.5% had mild and 23% moderate-to-severe inflammation), while ballooning was present in 67% (1269/1893) of subjects (47.6% showed mild-few and 19.4% moderate-marked). The histological activity in patients, stratified by fibrosis stage, is shown in Table 2.

TABLE 1 Baseline features of the overall population

Characteristic	Overall cohort (n = 1893)
Male sex	48% (909/1893)
Age; years ± SD	51.8 ± 12.3
BMI ± SD (kg/m^2)	35.3 ± 8.8
Obesity (BMI > 30 kg/m^2)	61.5% (1164/1893)
Arterial hypertension	48.7% (922/1893)
Type 2 diabetes mellitus	36.7% (695/1893)
Glucose ± SD (mg/dl)	118 ± 43
HOMA-IR ± SD	5.5 ± 5.2
HOMA > 2.5 (in absence of T2DM)	14.4% (273/1893)
Total cholesterol ± SD (mg/dl)	194 ± 45
HDL-c ± SD (mg/dl)	49 ± 15
LDL-c ± SD (mg/dl)	117 ± 38
Triglycerides ± SD (mg/dl)	166 ± 116
Hypertriglyceridemia	48.1% (910/1893)
AST ± SD (IU/L)	43 ± 38
ALT ± SD (IU/L)	60 ± 53
GGT ± SD (IU/L)	102 ± 57
Phosphatase alkaline ±SD (IU/L)	95 ± 53
Bilirubin ± SD (mg/dl)	0.68 ± 0.4
Albumin ± SD (g/dl)	4.39 ± 0.4
Creatinine ± SD (mg/dl)	0.83 ± 0.2
Platelet count ± SD (×10^9/L)	234 ± 71
INR ± SD	1.03 ± 0.1

Abbreviations: BMI, body mass index; SD, standard deviation.

3.2 | Histological features depending on the fibrosis stage

We analyzed the distribution of severe steatosis (Grade 3), ballooning and lobular inflammation according to the fibrosis stage (Figure 1A). All these three single components of the histological definition of steatohepatitis were less frequent in patients with *F*0. The prevalence of these features, according to the fibrosis stage, showed an inverted U-shaped curve. Particularly, severe steatosis, ballooning and lobular inflammation increased from *F*1 to *F*2, while their percentage was similar in *F*3 and decreased in *F*4 patients. According to the NAS score, the same trend was observed for the distribution of NASH-specific inflammation, which was significantly lower in cirrhotic than in *F*2 and *F*3 patients (Figure 1B). Besides, we analyzed the distribution of the following histological patterns by fibrosis stage: steatohepatitis (NAS ≥ 4) versus indeterminate NASH (at least, presence of ballooning or lobular inflammation) versus NAFL (lack of ballooning and lobular inflammation); in the overall cohort (Figure 2A) and depending on the presence of diabetes mellitus (Figure 2B), obesity (BMI > 30) (Figure 2C), the indication of the liver biopsy (Figure 2D) and the sex (Figure 2E).

On the other hand, the distribution of steatohepatitis, as well as their individual components, is shown in Table S2, according to the presence of MAFLD clinical criteria. Briefly, the presence of metabolic derangement influenced a higher inflammatory activity in the liver only in patients with *F*0: ballooning 51.6% versus 22.5%, lobular inflammation 57.5% versus 32.5% and NASH 35% versus 12.5% ($P < .05$ for all comparisons).

TABLE 2 Histological activity, including steatohepatitis, steatosis, ballooning and lobular inflammation, stratified by fibrosis stage

Fibrosis stage	*F*0	*F*1	*F*2	*F*3	*F*4
NASH (NAS > 4)	33.6% (218/649)	56.2% (287/511)	68.6% (214/312)	67.6% (192/284)	51.8% (71/137)
Steatosis					
Grade 1	50.5% (328/649)	37.4% (191/511)	34.3% (107/312)	34.9% (99/284)	46% (63/137)
Grade 2	34.4% (191/649)	38.7% (198/511)	36.2% (113/312)	39.8% (113/284)	35.8% (49/137)
Grade 3	15.1% (98/649)	23.9% (122/511)	29.5% (92/312)	25.4% (72/284)	18.2% (25/137)
Ballooning					
0	50.2% (326/649)	25.2% (129/511)	18.6% (58/312)	23.2% (66/284)	32.8% (45/137)
1	39% (253/649)	54% (276/511)	51% (159/312)	52.5% (149/284)	46.7% (64/137)
2	10.8% (73/649)	20.7% (106/511)	30.4% (95/312)	24.3% (69/284)	20.4% (28/137)
Lobular inflammation					
0	44.1% (286/649)	20.9% (107/511)	11.9% (37/312)	15.1% (43/284)	21.2% (29/137)
1	43.8% (284/649)	56.2% (287/511)	53.5% (167/312)	51.4% (146/284)	52.6% (72/137)
2	10.3% (67/649)	18.6% (95/511)	28.8% (90/312)	30.3% (86/284)	22.6% (31/137)
3	1.8% (12/649)	4.3% (22/511)	5.8% (18/312)	3.2% (9/284)	3.6% (5/137)

Abbreviation: NASH, nonalcoholic steatohepatitis.

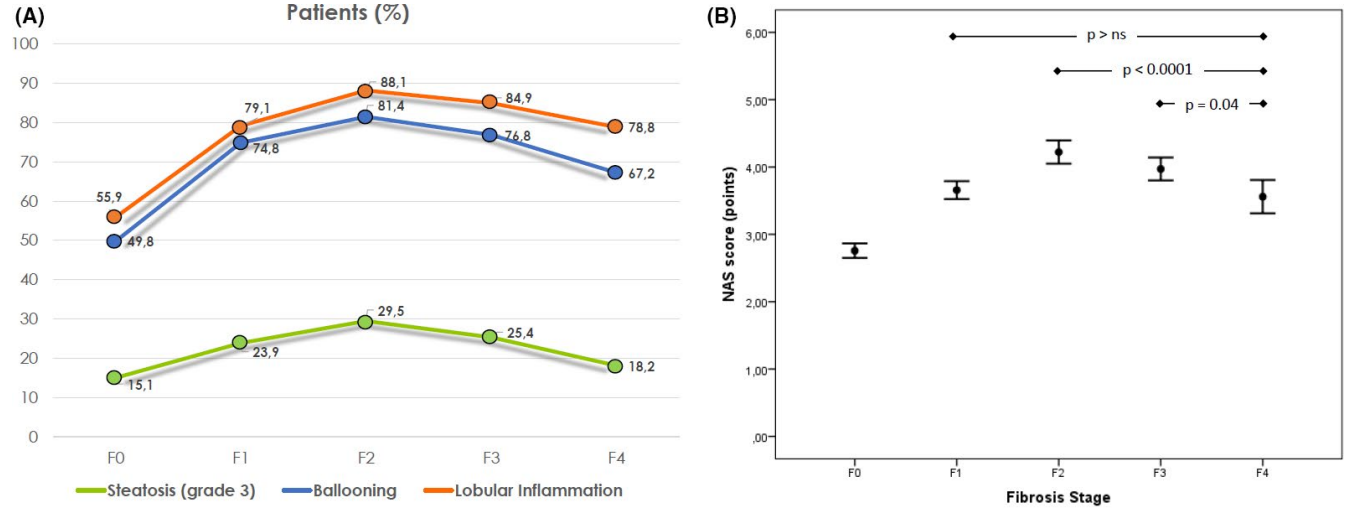

FIGURE 1 Histological features depending on the fibrosis stage. A, Prevalence of severe steatosis (Grade 3)*, ballooning (>1)**, and lobular inflammation (>1)***. B, NAS score. **P values for steatosis. F0 versus F4 0.356; F1 versus F4 0.162; F2 versus F4 0.012; F3 versus F4 0.104. **P values for ballooning. F0 versus F4 0.0002; F1 versus F4 0.074; F2 versus F4 0.0009; F3 versus F4 0.036. ***P values for inflammation. F0 versus F4 <0.0001; F1 versus F4 0.953; F2 versus F4 0.010; F3 versus F4 0.036*

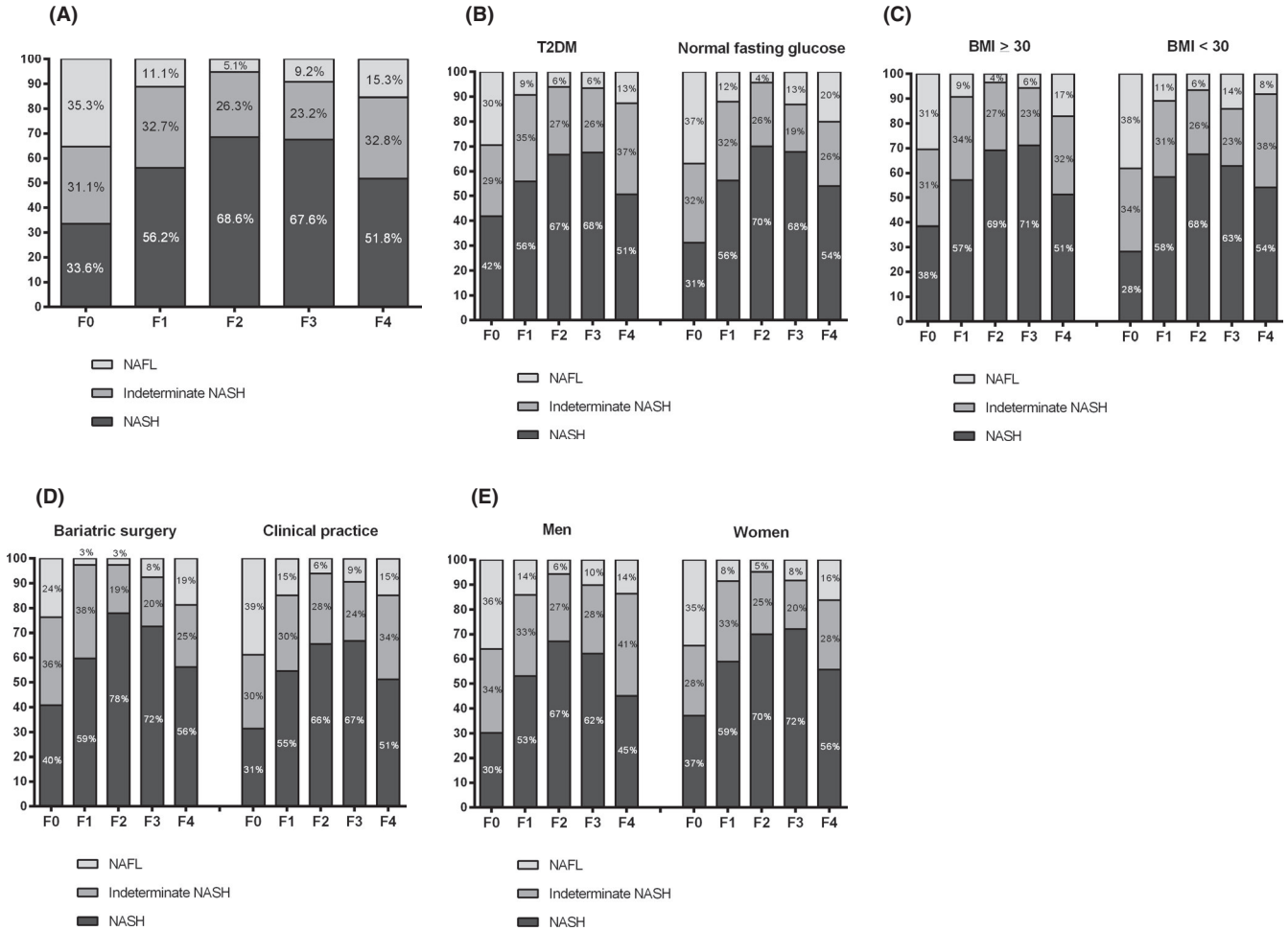

FIGURE 2 Distribution of definite nonalcoholic steatohepatitis (NASH), indeterminate NASH, and nonalcoholic fatty liver (NAFL). A, Overall cohort. B, Depending on the presence of type 2 diabetes mellitus (T2DM). C, Depending on the presence of obesity. D, Indication of the liver biopsy. E, Comparison between men and women

3.3 | Histological features in patients without NASH

Despite 31.4% of patients showing *F2* had no NASH, 83.7% of these patients showed some inflammatory component (ballooning or lobular inflammation) in the liver biopsy (Figure 3A). Also, a third of patients with advanced fibrosis were not diagnosed with NASH. However, up to 71.7% of them showed some inflammatory degree in the liver (Figure 3B). In cirrhotic patients, the percentage of patients without steatohepatitis was 48.2%, although up to 68.2% of them showed at least ballooning or lobular inflammation (Figure 3C).

3.4 | Baseline features according to the histological pattern

According to the histological pattern, the baseline features of the overall population are shown in Table 3. Briefly, both NASH and indeterminate NASH groups showed similar rates for metabolic factors such as obesity, diabetes mellitus and arterial hypertension, as well as they were not significantly different for liver tests like bilirubin, albumin or platelet levels. Instead, NASH patients showed significantly higher AST and ALT levels. Also, MAFLD criteria were present in 96.2% (945/982) of NASH patients and 95.2% (535/562) in indeterminate NASH, while it was 89.1% (311/349) in patients with NAFL ($P < .0001$). On the other hand, the distribution of MAFLD by histological pattern and fibrosis stage is shown in Figure S1.

3.5 | Prognosis according to the histological pattern

After the exclusion of patients who underwent liver biopsy during bariatric surgery (n = 539) and were enrolled in clinical trials (n = 120), 1234 subjects (65.2% of the overall cohort) were included in the survival analysis. During the follow-up (mean time 4.7 ± 3.8 years), 2.8% (34/1234) of patients died, 1.9% (24/1234) suffered from a first decompensation (10.3% (12/116) among baseline cirrhotic patients), 8.1% (90/1118) of non-cirrhotics suffered progression to cirrhosis and 1.1% (13/1234) developed hepatocellular carcinoma.

Fibrosis stage was the main histological determinant on the prognosis of the overall cohort (mortality was *F0* 1.8% [7/392] vs. *F1* 1.6% [5/315] vs. *F2* 3.1% [6/196] vs. *F3* 2.8% [6/215] vs. F4 9.6% [10/116]; logRank 22.369, $P = .0001$). On the other hand, despite having more patients with advanced liver disease, NASH patients had not a worse survival (2.7% [16/598]) than those with indeterminate NASH (1.4% [5/355]) or NAFL (4.6% [13/281]) (logRank 3.175; $P = .204$). Also, the three groups suffered a similar rate of first cirrhosis decompensation during the study (2% [12/598] vs. 1.7% [6/355] vs. 2.1% [6/281]; $P = .806$). Nevertheless, the incidence of progression to cirrhosis was significantly higher for both NASH patients (9.5% [51/539]; HR 2.35 [95% CI 1.30-4.26]) and those with indeterminate NASH (7.9% [25/316]; HR 1.90 [95% CI 1.01-3.66] [p = ns between them]) compared to subjects with NAFL (5% [14/263]; HR 1.00 [reference]) (logRank 8.417; $P = .015$) (Figure 4A). Consequently, the annual incidence rate of

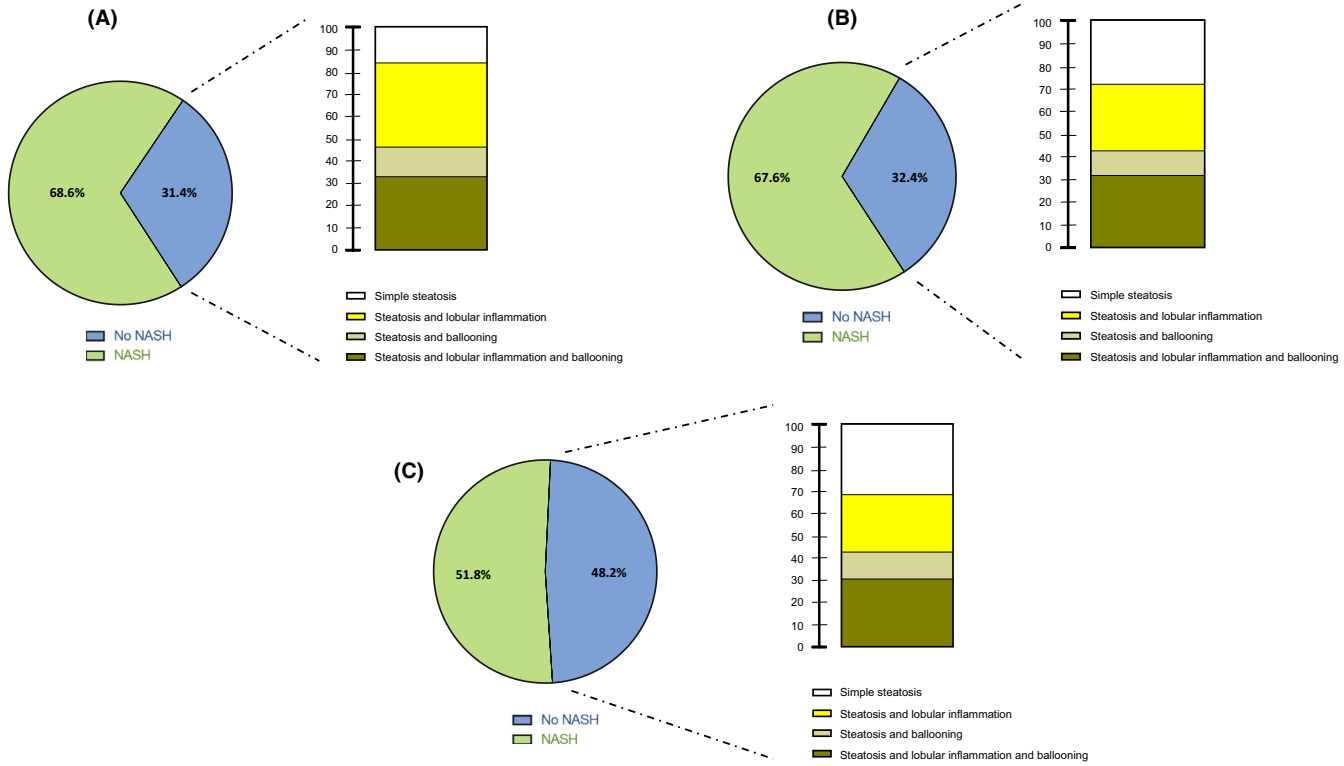

FIGURE 3 Histological features in patients without a definite nonalcoholic steatohepatitis (NASH) diagnosis. A, Significant fibrosis. B, Advanced fibrosis. C, Cirrhosis

TABLE 3 Baseline features of the overall cohort according to the histological pattern

Characteristic	NASH	Indeterminate NASH	NAFL	*P* value for the trend
Male sex	44.6% (438/982)[a,b]	51.6% (290/562)	51.9% (181/349)	.008
Age; years ± SD	51.9 ± 12.4	52.1 ± 12.4	50.8 ± 11.9	.243
BMI ± SD (kg/m^2)	35.9 ± 8.7[a]	35.2 ± 8.7	34 ± 9	.005
Obesity (BMI ≥30 kg/m^2)	70.9% (640/903)[a]	67.5% (349/517)[c]	59.7% (175/293)	.002
Arterial hypertension	51.8% (515/982)[a]	48.2% (271/562)[c]	40.7% (142/349)	.002
Type 2 diabetes mellitus	40.4% (397/982)[a]	37.4% (210/562)[c]	25.2% (88/349)	.0001
Glucose ± SD (mg/dl)	121 ± 44[a]	117 ± 46	112 ± 37	.005
HbA1c (%)	6.5 ± 1.5[a]	6.4 ± 1.4[c]	6 ± 1.3	.017
Total cholesterol ± SD (mg/dl)	194 ± 47	193 ± 44	194 ± 43	.913
HDL-c ± SD (mg/dl)	48 ± 15[b]	51 ± 17	49 ± 13	.003
LDL-c ± SD (mg/dl)	116 ± 39	118 ± 37	120 ± 36	.346
Triglycerides ± SD (mg/dl)	177 ± 120[a,b]	160 ± 125	147 ± 75	.0001
Hypertriglyceridemia	53% (520/982)[a,b]	46.3% (260/562)[c]	37.2% (130/349)	.0001
AST ± SD (IU/L)	48 ± 46[a,b]	38 ± 25	36 ± 28	.0001
ALT ± SD (IU/L)	67 ± 61[a,b]	53 ± 40	54 ± 46	.0001
Bilirubin ± SD (mg/dl)	0.67 ± 0.4	0.70 ± 0.4	0.70 ± 0.4	.252
Albumin ± SD (g/dl)	4.39 ± 0.4	4.37 ± 0.5	4.41 ± 0.4	.520
Creatinine ± SD (mg/dl)	0.82 ± 0.3	0.83 ± 0.2	0.83 ± 0.2	.682
Platelet count ± SD (×10^9/L)	233 ± 70	231 ± 71	240 ± 74	.209
INR ± SD	1.03 ± 0.1	1.04 ± 0.2	1.03 ± 0.2	.830
MAFLD criteria	96.2% (945/982)[a]	95.2% (535/562)[c]	89.1% (311/349)	.0001
Cirrhosis	7.2% (71/982)	8% (45/562)	6% (21/349)	.530

Abbreviations: BMI, body mass index; MAFLD, metabolic-associated fatty liver disease; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; SD, standard deviation.

[a]p<0.05 for comparison between NASH and indetermined NASH.

[b]p<0.05 for comparison between NASH and NAFL.

[c]p<0.05 for comparison between indetermined NASH and NAFL.

progression to cirrhosis was higher for patients with NASH and indeterminate NASH than those with NAFL (Figure 4B).

On the other hand, the impact of the histological pattern on both mortality and decompensated cirrhosis was not influenced by MAFLD despite showing higher percentages of these outcomes in dysmetabolic patients ($P > .05$). According to progression to cirrhosis, MAFLD also did not modify the impact of patients with NASH or indeterminate NASH. By contrast, patients showing NAFL and MAFLD showed higher progression to cirrhosis than those metabolically healthy patients (6.1% [14/231] vs. 0% [0/32]; logRank 3.677, $P = .049$) (Figure 4C).

4 | DISCUSSION

Our study observed that the prevalence of NASH, defined by NAS ≥ 4 (with at least 1 point each in inflammation and ballooning), decreased in advanced liver disease, particularly in cirrhotic patients. Also, more than 70% of patients with NAS < 4 showed some inflammatory degree in the liver biopsy. Both NASH and indeterminate NASH patients shared a similar rate of metabolic risk factors (including obesity, diabetes, arterial hypertension and dyslipidemia) and liver tests (albumin, bilirubin and platelets), although those with NAS ≥ 4 presented higher levels of AST and ALT. Besides, these subgroups showed a significantly higher proportion of MAFLD criterion than simple steatosis, focusing this term on patients with a higher likelihood of some hepatic inflammatory grade. Interestingly, both NASH and indeterminate NASH showed similar progression rates to cirrhosis, decompensated cirrhosis and mortality. Therefore, our findings cast doubt on current selection criteria for clinical trials because many subjects, who would benefit from an experimental treatment to halt the progression of the liver disease, could be excluded from clinical trials due to the absence of one single component of definite NASH.

A relatively high proportion of our patients did not show findings related to definite NASH (notably, half of the cirrhotic patients and a third of those with advanced fibrosis could not be diagnosed from NASH). A recent Italian study demonstrated that up to 33% of patients with NAFLD-related significant fibrosis did not show NASH (up to 10% had no inflammation), although the authors did not assess

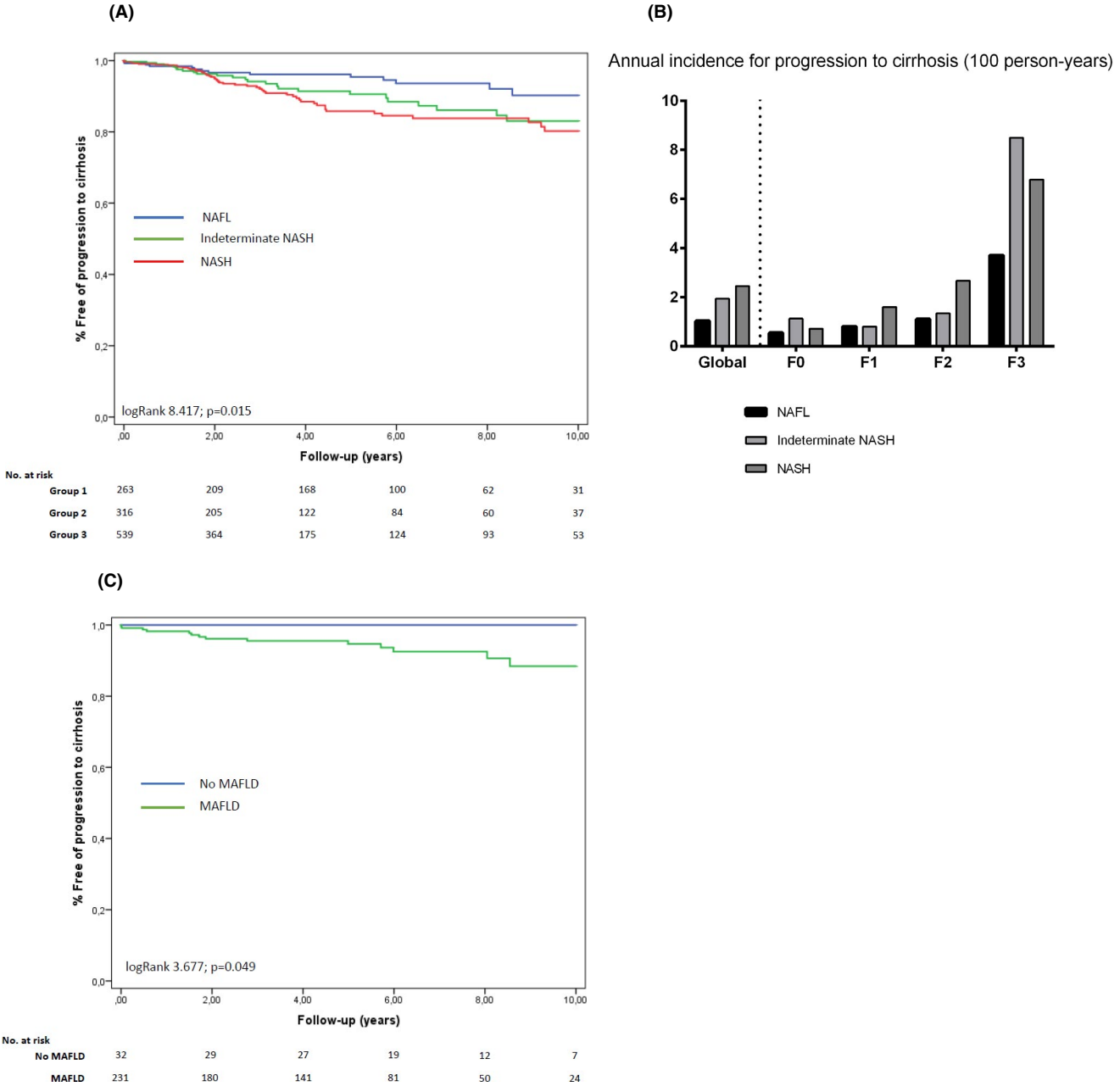

FIGURE 4 A, Kaplan–Meier curve to show the impact of the histological pattern on the progression to cirrhosis. B, Annual incidence rate of progression to cirrhosis depending on histological pattern and fibrosis stage (cases per 100 person-years). C, Kaplan–Meier curve to show the impact of metabolic-associated fatty liver disease (MAFLD) on the progression to cirrhosis in patients with nonalcoholic fatty liver (NAFL)

the distribution of NAS single components across the different fibrosis stages.[21] Another French study defined histologically severe disease as a SAF activity score ≥3 or advanced fibrosis (not requiring steatohepatitis), assuming that patients with NAFLD-related advanced liver disease could not have some definite elements of NASH.[22] Besides, steatofibrosis demonstrated to have higher overall mortality than NASH in NAFLD patients.[23]

Interestingly, despite the lack of a definite NASH diagnosis, most of the patients included in our study showed some inflammatory degree in the liver biopsy, irrespective of the fibrosis stage. Indeterminate NASH, a subclassification recognized by the NASH CRN system, is characterized by steatosis with inflammation and hepatocyte injury but atypical of definite steatohepatitis, and it can be associated with varying amounts of fibrosis.[5] It has been documented that fibrosis can progress even in patients without baseline steatohepatitis features, due to ballooning and lobular inflammation predicted fibrosis progression.[24] Consequently, these patients would more likely develop complications and should be cautiously monitored. In this scenario, we observed that both patients with NASH and indeterminate NASH showed similar

progression rates to cirrhosis, decompensated cirrhosis and mortality. These findings would support the concept of burn-out in NASH-related cirrhosis.[25]

Indeterminate NASH, irrespective of fibrosis stage, belongs to the subpopulation of non-NASH, similar to NAFL, according to the Case Definitions Working Group.[5] Consequently, some scenarios could not contemplate this entity as the main etiology of liver disease. We observed a similar clinical phenotype between NASH and indeterminate NASH patients, including the presence of metabolic factors (MAFLD was shown by more than 95% for the two groups) and liver tests (eg, albumin and bilirubin). An integral diagnosis, including histological criteria and metabolic risk factors, could avoid the diagnosis of cryptogenic liver disease in a significant number of individuals.[26] Most importantly, it could help to decrease screening failures in clinical trials and avoid wasting the opportunity of useful therapy in patients at risk of progression.[27] Besides, considering the clinical phenotype to define the origin of liver cirrhosis in some circumstances would have two significant benefits. First, knowing the etiology of cirrhosis is mandatory for personalized counselling and management. For instance, in the liver transplant, immunosuppressive approach, monitoring of extrahepatic events and the likelihood of recurrence could change depending on the etiology. Second, patients suffer from anxiety and frustration and have many future uncertainties when they are diagnosed with a leading cause of mortality, such as cirrhosis, but they have no explanations about how the disease appeared and how they have to proceed.[28,29] For instance, primary biliary cirrhosis has been renamed to cholangitis owing to these reasons.[30]

The study has some limitations usually observed when real-world data are evaluated. Ideally, the biopsies of all patients should be assessed by one single pathologist to avoid introducing some interobserver variability. Besides, the sampling variability of the biopsies might explain part of the findings. Despite these concerns, the results were consistent and robust after analyzing the data centre by centre (in fact, almost half of the cohort was reviewed by a single central pathologist -MJPM-). Probably, the fact of having a very selected group of expert hepato-pathologists who review all the biopsies after an initial discussion to homogenizes the interpretation of histologic criteria[19] and that patients whose samples are not suitable for diagnosis are excluded from Hepamet Registry could mitigate the worries. Also, a mean follow-up time of 4.7 years could not be enough to observe a high number of prognostic outcomes (eg, hepatocellular carcinoma). However, we performed an exploratory analysis in which we found statistically significant differences for cirrhosis progression, probably because there were fast progressors, including the use of annual incidence rate that considers for subjects the actual time at risk and does not require to complete the total follow-up time.

In summary, steatohepatitis features seem to be absent in half of the patients with significant fibrosis and cirrhosis, which could increase the screening failure rates and preclude to receive new therapeutic options in a subset of patients at high risk of progression and extrahepatic and/or liver-related outcomes. Future studies should individualize the most appropriate criteria for diagnosing and including patients in clinical trials according to the integration of clinical and histological features due to the NAS score limitation for such purpose.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Guarantor of the article: JA and MRG. Study design: JA. Drafting the manuscript: JA and MRG. Statistical analyses and interpretation: JA and MRG. Data acquisition and critical review of the manuscript: All authors. All authors approved the final version of the article, including the authorship list.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Ampuero J, Aller R, Gallego-Durán R, et al; HEPAmet Registry. Definite and indeterminate nonalcoholic steatohepatitis share similar clinical features and prognosis: A longitudinal study of 1893 biopsy-proven nonalcoholic fatty liver disease subjects. *Liver Int*. 2021;41:2076–2086. https://doi.org/10.1111/liv.14898