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Alcoholic Foamy Degeneration, an Entity Resembling Alcohol-Associated Hepatitis: Diagnosis, Prognosis, and Molecular Profiling

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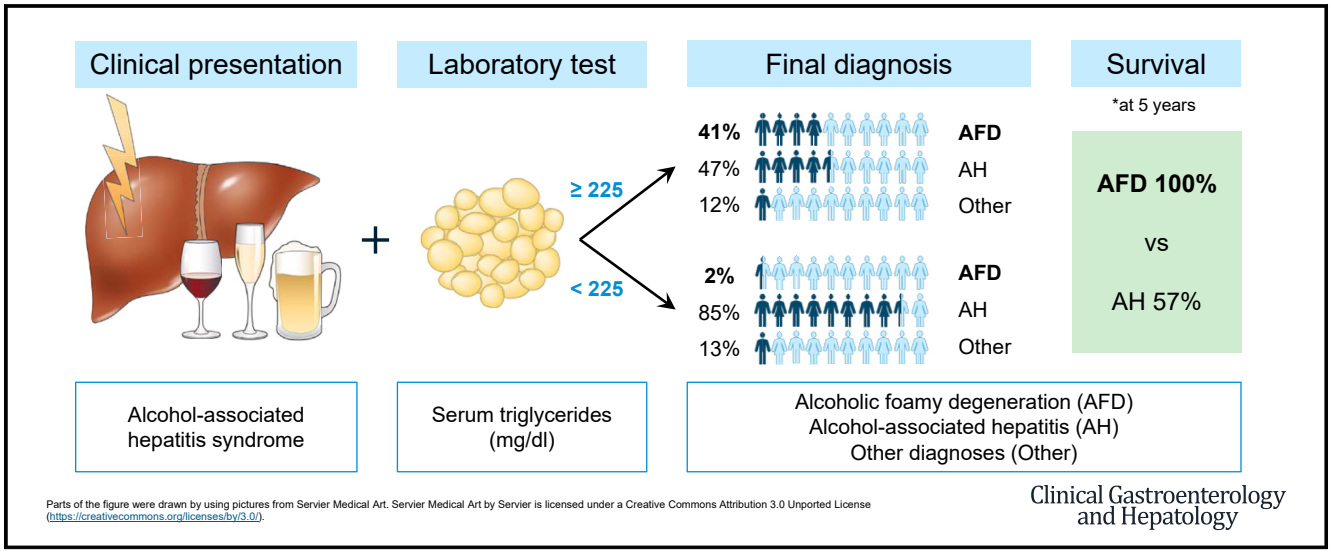

BACKGROUND & AIMS: **Alcoholic foamy degeneration (AFD) is a condition with similar clinical presentation to alcohol-associated hepatitis (AH), but with a specific histologic pattern. Information regarding the prevalence and prognosis of AFD is scarce and there are no tools for a noninvasive diagnosis.**

METHODS: **A cohort of patients admitted to the Hospital Clinic of Barcelona for clinical suspicion of AH who underwent liver biopsy was included. Patients were classified as AFD, AH, or other findings, according to histology. Clinical features, histology, and genetic expression of liver biopsy specimens were analyzed. The accuracy of National Institute on Alcohol Abuse and Alcoholism criteria and laboratory parameters for differential diagnosis were investigated.**

RESULTS: **Of 230 patients with a suspicion of AH, 18 (8%) met histologic criteria for AFD, 184 (80%) had definite AH, and 28 (12%) had other findings. In patients with AFD, massive steatosis was more frequent and the fibrosis stage was lower. AFD was characterized by down-regulation of liver fibrosis and**

Abbreviations used in this paper: AFD, alcoholic foamy degeneration; AH, alcohol-associated hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; IL, interleukin; MELD, model for end-stage liver disease; NIAAA, National Institute on Alcohol Abuse and Alcoholism.

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inflammation genes and up-regulation of lipid metabolism and mitochondrial function genes. Patients with AFD had markedly better long-term survival (100% vs 57% in AFD vs AH; $P = .002$) despite not receiving corticosteroid treatment, even in a model for end-stage liver disease–matched sensitivity analysis. Serum triglyceride levels had an area under the receiver operating characteristic of 0.886 (95% CI, 0.807–0.964) for the diagnosis of AFD, whereas the National Institute on Alcohol Abuse and Alcoholism criteria performed poorly. A 1-step algorithm using triglyceride levels of 225 mg/dL (sensitivity, 0.77; specificity, 0.90; and Youden index, 0.67) is proposed for differential diagnosis.

CONCLUSIONS: **AFD in the setting of suspicion of AH is not uncommon. A differential diagnosis is important because prognosis and treatment differ largely. Triglyceride levels successfully identify most patients with AFD and may be helpful in decision making.**

Keywords: Triglycerides; Biopsy; Histology; Survival.

Alcohol-associated hepatitis (AH) is a syndrome characterized by recent onset of jaundice that may be accompanied by liver decompensation in patients with ongoing alcohol abuse and frequently underlying liver disease.[1–4] Although the prevalence of AH is not well known, its incidence and impact on global health are probably increasing, especially in young adults.[5,6]

In clinical practice, the diagnosis of AH is most often made with clinical and laboratory criteria, following the recommendations of a panel of experts from the National Institute on Alcohol Abuse and Alcoholism (NIAAA).[7] Nevertheless, these criteria have shown moderate performance for noninvasive diagnosis of AH, with a non-negligible percentage of false-positive diagnoses.[8] Among entities resembling AH, alcoholic foamy degeneration (AFD) is a poorly known and underrecognized condition.

AFD is defined by a histologic pattern of microvesicular fatty degeneration with foamy appearance of hepatocytes in the absence of, or with minimal signs of, steatohepatitis.[9] The real prevalence of this entity in the context of suspicion of AH is not known. The few studies assessing the prognosis of AFD have reported contradictory results[10,11]; however, in a large series on the natural history of AFD this condition seems to have better short-term prognosis compared to AH, with rapid improvement of liver function in the absence of corticosteroid treatment.[10]

A differential diagnosis between AH and AFD seems clinically relevant because it may guide decisions on specific treatment with corticosteroids or even consideration for early liver transplant. However, identification of AFD remains challenging in cases of clinical suspicion of AH because a liver biopsy is rarely performed in this setting.[12]

In this context, the aims of this study were to assess the real prevalence and prognosis of AFD and provide new noninvasive tools for identification of this entity in clinical practice.

Patients and Methods

Study Design and Population

This study included consecutive patients with clinical suspicion of AH admitted to the Hospital Clinic of Barcelona from January 1, 2010, to December 31, 2020, and with clinical follow-up evaluation in our unit. Clinical suspicion of AH was defined based on the diagnostic coding in the patients' medical records. Codes in our center are assigned by data managers based on the clinical diagnosis made by the team responsible for the patient during hospitalization. All reports are reviewed internally to ensure a correct coding. Codes for AH during the study period were as follows: alcoholic hepatitis, with ascites; and alcoholic hepatitis, without ascites.

Exclusion criteria were as follows: absence of liver biopsy during hospitalization, insufficient sample size for histologic diagnosis (biopsy length <10 mm or <5 portal tracts), and lack of informed consent to be included in the study.

Liver Histology and Classification of Patients

The transjugular approach with measurement of hepatic venous pressure gradient was preferred in most cases to percutaneous biopsy. The main reasons to use a transjugular approach were impairment of coagulation tests and the presence of ascites. Liver biopsy specimens were formalin-fixed, paraffin-embedded, and stained by standard methods, including hematoxylin and eosin and Masson's trichrome staining in all cases.

AFD was diagnosed on liver pathology when a pattern of microvesicular steatosis was present, with an absence of, or minimal signs of, steatohepatitis.[9] Microvesicular steatosis was defined as the infiltration of the hepatocyte's cytoplasm by numerous small fat droplets of uniform size, causing an enlargement of the cell, without nuclear displacement (Figure 1). Patterns of steatosis not meeting these criteria were not considered as microvesicular steatosis and, thus, were not classified as AFD.[13] A histologic diagnosis of AH was defined by the presence of any type of steatosis, associated with hepatocyte degenerative changes (hepatocellular ballooning and/or Mallory–Denk bodies) and lobular inflammatory infiltration[14] (Figure 1). When signs of AFD and AH were present in the same specimen, it was classified as one or the other depending on the predominant pattern, defined

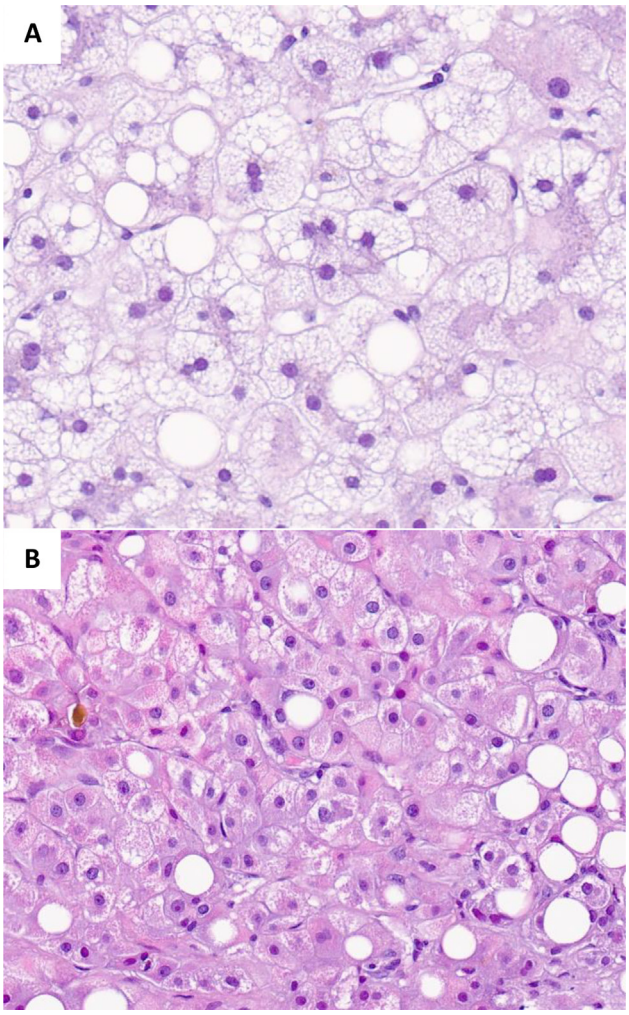

Figure 1. (*A*) Liver pathology examination in patients with alcoholic foamy degeneration (AFD). AFD is characterized by a pattern of microvesicular steatosis, which is defined by an infiltration of the hepatocytes' cytoplasm by small lipid droplets, uniform in size, that do not displace the cell nucleus. The infiltrated hepatocytes are enlarged and have a foamy appearance. (*B*) Liver pathology examination in patients with alcohol-associated hepatitis (AH). AH is characterized by steatohepatitis, defined by any degree of steatosis (any type of steatosis, although macrovesicular steatosis is by far the most common pattern), hepatocyte degenerative changes (hepatocellular ballooning and/or Mallory–Denk bodies), and lobular inflammatory infiltration, predominantly neutrophilic.[13]

as the one occupying more than 50% of the sample's area. Patients whose liver biopsy assessment did not meet the criteria for the diagnosis of AFD or AH were classified as *other findings* and excluded from the main analysis. Liver biopsy specimens from all patients were reviewed by 2 expert pathologists (A.D. and C.M.) who were blinded to the patients' characteristics and outcomes. The agreement between both pathologists was 97% (196 of 202 cases). In the few cases of disagreement, final consensus was reached after a joint revision of the slide using a multihead microscope.

What You Need to Know

Background

Patients with a clinical syndrome of alcohol-associated hepatitis (AH) in fact may have other clinical entities, such as alcoholic foamy degeneration (AFD), which do not benefit from corticosteroids.

Findings

AFD is a differentiated entity from AH and has an excellent long-term prognosis. Levels of triglycerides help to identify patients with AFD.

Implications for patient care

The results of this study may be important for clinicians to avoid unnecessary treatments and for patients for a correct knowledge of their prognosis. They also may interest researchers when considering patients with AH for clinical trials.

Patients were categorized into 2 groups based on the pathology diagnosis: AFD, which corresponded to the study group, and AH, which was established as the control group.

In regard to fibrosis, it was evaluated using both Meta-analysis of Histological Data in Viral Hepatitis[15] and Study of Alcohol-related LiVer disease in Europe[16] staging systems.

Data Collection

The inclusion date was set as the date that the liver biopsy was performed. Demographic, clinical, and biochemical data at the time of liver biopsy were collected carefully, including previous alcohol consumption quantified in standard units/day,[17] comorbidities (ie, cardiovascular diseases and metabolic risk factors), and previous or current episodes of decompensation of cirrhosis. Prognostic scores including model for end-stage liver disease (MELD), Maddrey's discriminant function, and Child–Pugh scores were also calculated. Fulfillment of the clinical criteria for probable AH of both the NIAAA[7] and the modified NIAAA criteria with the addition of C-reactive protein levels[8] was analyzed (Supplementary Table 1). Furthermore, other variables were reviewed and registered, such as the treatment that the patients received, episodes of hepatic decompensation after discharge, and time of alcohol abstinence. Per protocol, all patients were evaluated by an addiction specialist during hospitalization and were referred to the addiction unit after discharge. Alcohol use was assessed by patient self-reporting and by urine ethyl glucuronide, when available. Finally, we assessed survival based on the patients' clinical status on the date of the last follow-up evaluation.

RNA Extraction and Sequencing Analysis

Paraffin-embedded liver biopsy specimens from patients with AFD with sufficient tissue sample available were used for genetic expression analysis. A randomly selected group of 20 patients with AH was used for comparison. Further details on RNA extraction and sequencing analysis are shown in the Supplementary Methods.

Statistical Analysis

Quantitative variables with a normal distribution were expressed as means and SD and those with a non-normal distribution were expressed as median and interquartile range. Categoric variables were expressed as absolute count and percentages. Differences between groups were studied with the chi square test, *t* test, or Mann–Whitney test. Factors associated with the presence of AFD on liver histology were studied with a univariate and multivariate logistic regression analysis. Variables included in the multivariate analysis were those with a *P* value in the univariate analysis <.05 and those that were clinically relevant (ie, sex). Due to the relatively low number of AFD cases, a single multivariate analysis including all relevant variables would not be statistically acceptable; therefore, we performed

Table 1. Baseline Characteristics of Patients Included in the Study Classified According to the Diagnosis of AFD or AH

	Patients with AFD (n = 18)	Patients with AH (n = 184)	*P* value
Age, *y*	47 (38–57)	52 (45–59)	.074
Sex, female	6 (33)	54 (29)	.724
Obesity	3 (17)	46 (25)	.620
Diabetes mellitus	3 (17)	43 (23)	.419
Alcohol use, *SU/d*	10 (6–16)	10 (7–20)	.294
Duration of alcohol use, *y*	20 (14–22)	30 (23–38)	**<.001**
Decompensation at inclusion	5 (28)	138 (75)	**<.001**
Ascites	4 (22)	120 (65)	**<.001**
Overt hepatic encephalopathy	1 (6)	61 (33)	.053
Variceal bleeding	0 (0)	13 (7)	.244
Spontaneous bacterial peritonitis	0 (0)	10 (5)	.310
HVPG, *mm Hg*	10.5 (5.0–19.0)	18 (14.4–22.0)	**.012**
C-reactive protein level, *mg/dL*	1.3 (0.0–3.0)	3.2 (1.42–5.17)	**.019**
Serum creatinine level, *mg/dL*	0.9 (0.7–1.1)	0.8 (0.6–1.2)	.603
Total cholesterol level, *mg/dL*	264 (181–440)	124 (98–171)	**<.001**
Triglyceride level, *mg/dL*	273 (197–661)	124 (93–170)	**<.001**
AST level, *IU/L*	203 (136–382)	122 (85–165)	**<.001**
ALT level, *IU/L*	107 (72–151)	48 (32–77)	**<.001**
GGT level, *IU/L*	960 (568–1804)	251 (124–613)	**<.001**
AP level, *IU/L*	344 (156–541)	216 (143–343)	**.020**
Total bilirubin level, *mg/dL*	7.9 (2.2–14.1)	10.8 (3.8–21.2)	.201
Albumin level, *g/L*	29 (24–34)	26 (24–31)	.169
Leukocytes, $\times 10^9$/L	5.7 (3.6–7.3)	8.5 (6.1–13.2)	**.002**
Platelets, $\times 10^9$/L	160 (101–232)	112 (75–186)	.056
INR	1.1 (1.1–1.4)	1.7 (1.4–2.1)	**<.001**
MELD score	17 (10–20)	22 (17–27)	**.002**
Maddrey's discriminant function	18 (12–34)	54 (34–76)	**<.001**
Child–Pugh score	8 (7–10)	11 (9–12)	**.001**

NOTE. Values are median (±interquartile range) or absolute count (percentage). Bolded values are those with *P* value <.05.
AFD, alcoholic foamy degeneration; AH, alcohol-associated hepatitis; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HVPG, hepatic venous pressure gradient; INR, international normalized ratio; MELD, model for end-stage liver disease; SU, standard units.

numerous models of logistic regression including up to 4 variables. Considering the variables associated independently with the presence of AFD in the multivariate analysis, a receiver operating characteristic curve analysis was performed for each variable and the Youden index was calculated to identify the cut-off value with the best performance for a noninvasive diagnosis of AFD. Survival curves were calculated with the Kaplan–Meier method and compared with the log-rank test in the overall cohort and in a randomly MELD-matched cohort at a 2:1 ratio (2 cases of AH per 1 case of AFD). The significance level for all statistical tests was set at .05 two-tailed. All statistical analysis were performed using SPSS version 25.0.0.1.

Ethical Aspects

All research was conducted in accordance with both the Declaration of Helsinki and Istanbul. The protocol was approved by the institutional review board of the hospital and all patients provided written informed consent to participate in the study.

Results

Prevalence and Clinical Characteristics

A total of 317 patients with clinical suspicion of AH were hospitalized in the Liver Unit of the Hospital Clinic of Barcelona during the study period and 230 patients were included in the study (Supplementary Figure 1). Eighteen patients met the histologic criteria of AFD, with a prevalence of 8% in the study cohort.

Baseline characteristics of patients with AFD and AH are shown in Table 1. Patients with AFD presented a less severe impairment of liver function tests as shown by lower MELD values, Maddrey's discriminant function, and Child–Pugh scores, and a lower prevalence of decompensation of liver disease. Higher levels of aminotransferases, γ-glutamyl transferase (GGT), cholesterol, and triglycerides, and lower values of the international normalized ratio were found in patients with AFD. Of note, total bilirubin levels were not significantly different when comparing both entities. In regard to treatment, 109 (59%) patients with AH received corticosteroids compared with only 3 (17%) patients with AFD ($P < .001$).

Evolution and Survival

All patients with AFD survived the index hospitalization. After a median of 7 days from admission, patients with AFD presented a characteristic clinical pattern of rapid reduction in aminotransferase levels (median aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels decreased from 203 IU/L to 77 IU/L and from 107 IU/L to 44 IU/L, respectively) and serum bilirubin levels (median levels decreased from 7.9 mg/dL to 3.1 mg/dL), as well as MELD score (median MELD score decreased from 17 to 11). Moreover, a trend toward normalization of lipid profile was evident, with median triglyceride levels decreasing from 273 mg/dL to 153 mg/dL, and median cholesterol levels decreasing from 264 mg/dL to 187 mg/dL (Supplementary Figure 2).

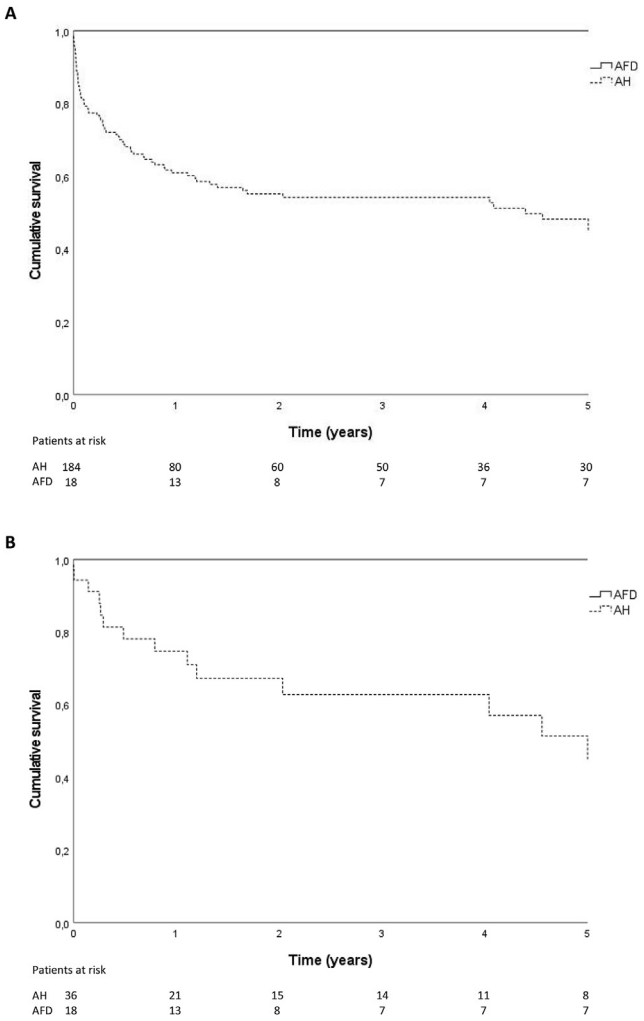

Figure 2. Kaplan–Meier curves showing the long-term survival of patients with alcoholic foamy degeneration (AFD) and alcohol-associated hepatitis (AH) in (*A*) the total cohort ($P = .002$, log-rank test), and (*B*) the MELD-matched cohort ($P = .005$, log-rank test).

In the long term, after 5 years of follow-up evaluation, patients with AFD had a survival rate of 100% (median follow-up period, 618 days [range, 375–2753]). Only 1 patient had recurrent hospitalizations for decompensation of liver disease in the context of persistent alcohol consumption. The excellent prognosis of patients with AFD contrasted with the poor survival of patients with AH: 57% survival in AH (median follow-up period, 347 days [range, 64–1234]) (Figure 2*A*). In the MELD-matched cohort, the 5-year survival rate in patients with AH remained significantly lower compared with that of patients with AFD (100% in AFD vs 60% in AH; $P = .005$) (Figure 2*B*).

Table 2. Histologic Findings on Liver Biopsy Examination of Patients With AFD and AH

	Patients with AFD (n = 18)	Patients with AH (n = 184)	*P* value
METAVIR fibrosis stage (F)			**<.001**
F0	5 (28)	1 (1)	
F1	2 (11)	13 (7)	
F2	4 (22)	13 (7)	
F3	5 (28)	26 (14)	
F4	2 (11)	129 (71)	
SALVE fibrosis stage (SFS)			**<.001**
SFS 0	1 (6)	0 (0)	
SFS 1	3 (17)	3 (2)	
SFS 2	7 (39)	24 (13)	
SFS 3	5 (28)	26 (14)	
SFS 4	2 (11)	129 (71)	
Perisinusoidal fibrosis	13 (72)	139 (76)	.755
Massive steatosis (>2/3 of the sample)	16 (89)	54 (29)	**<.001**
Microvesicular steatosis (any degree)	18 (100)	46 (25)	**<.001**
Portal inflammatory infiltrate	2 (11)	93 (50)	**.001**
Lobular inflammatory infiltrate	6 (33)	130 (76)	**<.001**
Neutrophilic infiltration	2 (11)	124 (67)	**<.001**
Steatohepatitis	2 (11)	184 (100)	**<.001**
Ductular reaction	4 (22)	75 (41)	.124
Canalicular cholestasis	9 (50)	102 (55)	.658
Ductular cholestasis	4 (22)	45 (25)	.833
Hepatocyte ballooning	3 (17)	161 (88)	**<.001**
Mallory–Denk bodies	2 (11)	169 (92)	**<.001**
Apoptotic bodies	1 (6)	6 (3)	.611
Megamitochondria	4 (22)	31 (17)	.565

NOTE. Values are absolute count (percentage). Bolded values are those with *P* value <.05.
AFD, alcoholic foamy degeneration; AH, alcohol-associated hepatitis; F, Meta-analysis of Histological Data in Viral Hepatitis fibrosis stage; METAVIR, Meta-analysis of Histological Data in Viral Hepatitis; SALVE, Study of Alcohol-related LiVer disease in Europe; SFS, Study of Alcohol-related LiVer disease in Europe fibrosis stage.

Alcohol consumption was also assessed during follow-up evaluation. A similar proportion of patients underwent clinical follow-up evaluation in our center's Addiction Unit after their index hospitalization (62% in AFD vs 68% in AH; $P = .768$). The percentage of patients who remained abstinent from alcohol at the last follow-up visit was similar between both groups (42% in AFD vs 49% in AH; $P = .634$).

Histologic Features

Histologic features from liver biopsy specimens of patients with AFD were compared with those of patients with AH (Table 2). Patients with AFD had massive steatosis (>2/3 of the sample) in a higher proportion when compared with patients with AH (89% vs 30%; $P < .05$). Advanced fibrosis defined by a Meta-analysis of Histological Data in Viral Hepatitis stage >F2 or a Study of Alcohol-related LiVer disease in Europe fibrosis stage >2 was significantly less common in patients with AFD (39% vs 85%; $P < .001$), whereas perisinusoidal fibrosis had a similar prevalence in the 2 groups (72% in AFD vs 76% in AH; $P = .755$). Of note, 48 patients had findings compatible with both AFD and AH. However, only 2 patients had a predominant pattern of microvesicular steatosis; therefore, only these 2 patients were classified as AFD.

Transcriptomic Analysis

A transcriptomic analysis of 17 of 18 liver biopsy specimens from patients in the AFD cohort was performed and compared with that of 20 randomly selected liver biopsy specimens from patients in the AH cohort. Baseline characteristics of both groups were similar (data not shown). RNA sequencing analysis showed that patients with AFD and AH have different gene expression patterns (Supplementary Figure 3). On the principal component analysis, patients with AFD clustered apart

from patients with AH, although a moderate overlap was seen between both groups (Supplementary Figure 4). Furthermore, the functional analysis of the deregulated genes using Ingenuity Pathway Analysis (Qiagen) showed that when compared with patients with AH, patients with AFD had different expression of genes and functional pathways that have been related to the pathogenesis of AH (Supplementary Figure 5). Pathways associated with liver fibrosis, hepatic stellate cell activation, wound healing, and mesenchymal cell activation were down-regulated in AFD. We also found down-regulation of genes involved in inflammatory pathways related to the role of macrophages, fibroblasts, and endothelial cells in rheumatoid arthritis; interleukin (IL)1, IL6, IL8, IL17, and IL22 signaling pathways; or C-X-C chemokine receptor type 4 signaling pathway, among others. By contrast, a significant up-regulation was seen in AFD in functional pathways associated with mitochondrial function and lipid metabolism, cholesterol, and triglyceride biosynthesis, such as pyridoxal-5-phosphate or adipogenesis pathways.

Differential Diagnosis With Alcohol-Associated Hepatitis

On univariate regression analysis, variables associated with the presence of AFD were younger age; shorter duration of alcohol use; absence of ascites; lower hepatic venous pressure gradient; higher levels of AST, ALT, GGT, alkaline phosphatase, cholesterol, and triglycerides; and lower leukocyte count, international normalized ratio, and MELD score. Interestingly, NIAAA criteria were not associated with the presence of AH in the univariate analysis (Table 3). Several models of multivariate analysis were performed including variables related to alcohol use, liver enzyme levels, liver function scores, and lipid profile. Notably, when included together in a multivariate model, duration of alcohol use, AST level, and triglyceride level, but not MELD score, were independently associated with the presence of AFD (Table 3).

Because NIAAA criteria are currently the most widely used clinical criteria for the diagnosis of AH, we investigated the performance of these criteria for the differential diagnosis of AH and AFD. NIAAA criteria showed moderate sensitivity (70%) with low specificity (44%) and a diagnostic accuracy of 65% for the differential diagnosis between AH and AFD. Overall performance of the modified NIAAA C-reactive protein criteria was better, but not optimal (sensitivity, 73%; specificity, 45%; and diagnostic accuracy, 68%) (Supplementary Table 2).

Because the precision of these criteria was suboptimal, we investigated other noninvasive tools for the differential diagnosis. Four analytical parameters, ALT, AST, cholesterol, and triglyceride levels, were associated with high diagnostic accuracy. Of those, serum triglyceride levels had the best diagnostic performance for the diagnosis of AFD, with an area under the receiver operating characteristic curve of 0.886 (95% CI, 0.807–0.964), and 225 mg/dL was the value with the best diagnostic performance (sensitivity, 0.77; specificity, 0.90; and Youden index, 0.67) (Supplementary Figures 6 and 7). Using this threshold, we generated a 1-step, easy-to-use algorithm that identifies a subpopulation of patients with clinical suspicion of AH in whom the diagnosis of AFD is notably prevalent (Figure 3).

Table 3. Univariate and Multivariate Analysis of Factors Associated With AFD

Variable	OR	*P*	95% CI
Univariate analysis			
Age, *y*	0.951	.047	0.905–0.999
Sex, male	1.204	.724	0.430–3.372
Alcohol use, *SU/d*	0.951	.146	0.890–1.018
Duration of alcohol use, *y*	0.904	.001	0.853–0.957
Ascites at inclusion	0.152	.001	0.048–0.482
HVPG, *mm Hg*	0.897	.015	0.822–0.979
C-reactive protein level, *mg/dL*	0.723	.066	0.511–1.022
Total cholesterol level, *mg/dL*	1.016	<.001	1.010–1.023
Triglyceride level, *mg/dL*	1.005	.002	1.002–1.008
AST level, *IU/L*	1.002	.030	1.000–1.004
ALT level, *IU/L*	1.003	.009	1.001–1.006
GGT level, *IU/L*	1.001	<.001	1.000–1.001
AP level, *IU/L*	1.003	.009	1.001–1.004
Total bilirubin level, *mg/dL*	0.963	.186	0.911–1.018
Leukocytes, $\times 10^6$/L	0.803	.012	0.676–0.953
INR	0.031	<.001	0.005–0.201
MELD score	0.903	.004	0.842–0.967
NIAAA criteria for probable AH	0.533	.209	0.200–1.423
Multivariate analysis			
Duration of alcohol use, *y*	**0.893**	**.001**	**0.833–0.987**
Triglyceride level, *mg/dL*	**1.003**	**.005**	**1.001–1.005**
AST level, *IU/L*	**1.004**	**.020**	**1.000–1.007**
MELD score	0.938	.286	0.834–1.055

NOTE. Different models of multivariate analysis were created including a maximum of 4 variables. Models were generated by combining 1 variable from each of the following: duration of alcohol use, liver function (MELD or INR), liver enzymes (AST, ALT, AP, or GGT), and lipid profile (cholesterol or triglycerides). The model shown includes the variables that were associated most consistently to AFD in all models generated. Bolded values are those with *P* value <.05.
AFD, alcoholic foamy degeneration; AH, alcohol-associated hepatitis; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; HVPG, hepatic venous pressure gradient; INR, international normalized ratio; MELD, model for end-stage liver disease; NIAAA, National Institute of Alcohol Abuse and Alcoholism; OR, odds ratio; SU, standard unit.

Discussion

In this study, we report the actual prevalence of AFD, a poorly known entity frequently misdiagnosed as AH, with differentiated histologic features and genetic signature, and a drastically different prognosis. In addition, we provide a simplified, clinically actionable algorithm based on triglyceride levels for the differential diagnosis between AFD and AH.

We used multiple approaches to provide evidence that AFD is a differentiated entity from AH. From a clinical perspective, we found that some clinical features

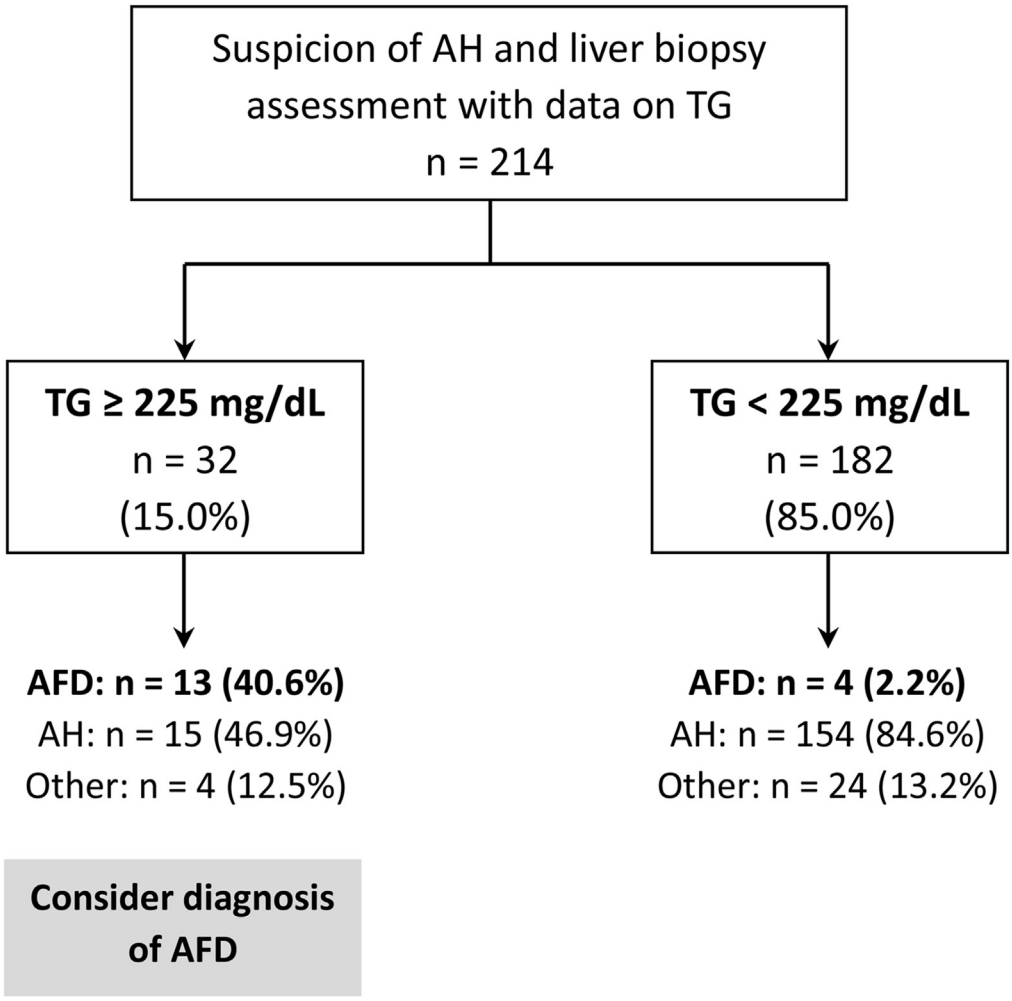

Figure 3. Simplified algorithm for identification of patients with alcoholic foamy degeneration (AFD) according to serum triglyceride levels. AH, alcohol-associated hepatitis; TG, triglyceride.

of patients with AFD differ from those of patients with AH. Notably, these patients present less frequently with clinical decompensation of liver disease. Moreover, impairment of liver function is less severe when compared with that of patients with AH, as shown by a lower MELD score. In contrast, levels of aminotransferases and GGT are markedly higher in patients with AFD.

Regarding the pathogenesis of AFD, lipid metabolism seems to play a key role in this condition. Massive fat infiltration is the most characteristic histologic feature in the liver pathology analysis of these patients. This finding is accompanied by a marked increase in circulatory triglyceride and cholesterol levels. In parallel with this, lipid metabolism–related genes were overexpressed in the transcriptomic analysis of liver biopsy specimens of patients with AFD when compared with AH.

One of the most relevant findings of this study is the excellent prognosis of patients with AFD, which is drastically different from that of patients with AH.[18,19] Published data on long term prognosis of patients with AFD is lacking and the few studies that have assessed short and midterm prognosis have yielded contradictory results.[10,11] Our study clearly shows an excellent prognosis of this population, both short and long term, with neither deaths nor liver transplants occurring during follow-up evaluation. Furthermore, significant differences in survival were also shown when matching patients with AFD to patients with AH based on MELD score at admission. Of note, AFD patients improved spontaneously despite not receiving corticosteroids. This finding, together with the absence of hepatic and systemic inflammation, should discourage the use of steroids in AFD.

To date, an AFD diagnosis has relied only on liver biopsy assessment. However, current clinical practice guidelines recommend using the NIAAA noninvasive criteria for the diagnosis of probable AH, restricting the liver biopsy to a limited number of cases of diagnostic uncertainty or coexistence of confounding factors.[3,4] We provide a 1-step algorithm based on serum triglyceride levels, which have shown the best accuracy for identifying patients with AFD (area under the receiver operating characteristic, 0.886; 95% CI, 0.807–0.964). Given the wide availability and low cost of serum triglyceride measurements, the provided algorithm may be useful to guide decision making in clinical practice.

This study had some limitations that should be mentioned. First, it is possible that patients with confounding factors for the diagnosis of AH were more prone to have a liver biopsy proposed and this may have affected the cohort composition. However, this is unlikely

because a liver biopsy is performed in the majority of patients with suspicion of AH in routine clinical practice in our unit, considering the possibility of erroneous diagnoses using only clinical criteria.[8] Second, misclassification owing to sampling error or misinterpretation of histologic features is possible, especially in patients with mixed features of AH and AFD. The fact that 2 independent pathologists evaluated each sample nuances this potential limitation. Finally, despite being a large series of patients with AFD, the number of patients included was relatively low; ideally, the diagnostic capacity of serum triglyceride levels for the identification of AFD should be explored further in future studies.

In conclusion, AFD is a previously neglected entity differentiated from AH, with excellent prognosis and no need for steroid treatment. Serum triglyceride levels are a valuable tool for the identification of this condition.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://doi.org/10.1016/j.cgh.2023.11.031.

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Elisa Pose Méndez (Conceptualization: Lead; Funding acquisition: Equal; Investigation: Equal; Methodology: Equal; Project administration: Lead; Writing – original draft: Supporting; Writing – review & editing: Equal)

Conflicts of interest

These authors disclose the following: Isabel Graupera has received a grant from Pfizer (77145101). Ramón Bataller is on the speakers bureau of AbbVie and Gilead. Pere Ginès has received funding from Gilead and Grífols, speaking fees from Pfizer and consulted or attended advisory boards for Gilead, RallyBio, SeaBeLife, Merck, Sharp and Dohme (MSD), Ocelot Bio, Behring, Roche Diagnostics International and Boehringer Ingelheim. The remaining author discloses no conflicts.

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Supplementary Methods

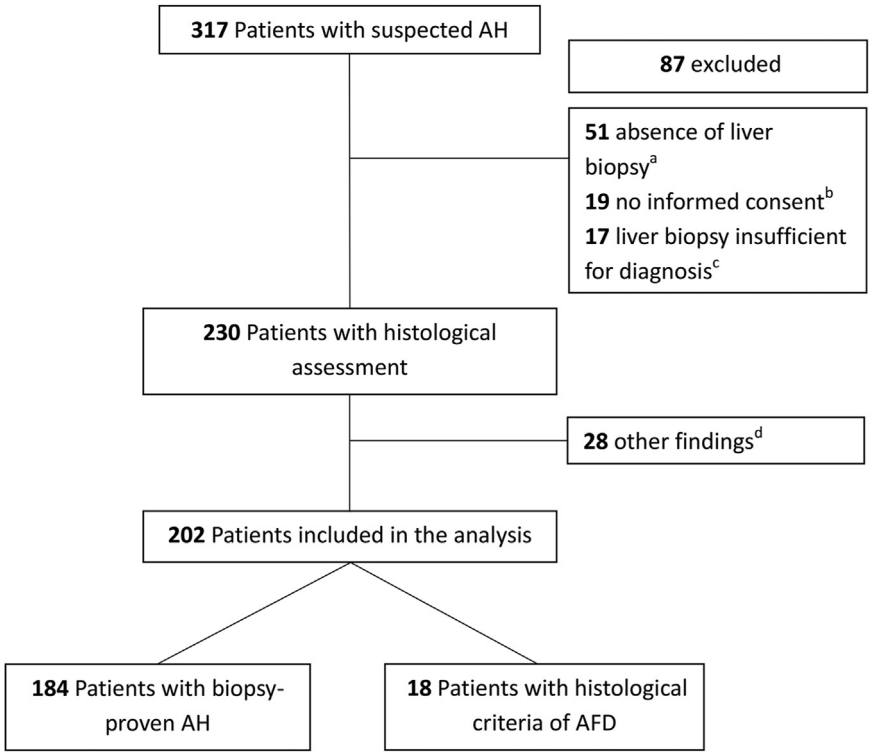
RNA Extraction and Sequencing Analysis

RNA extraction was performed using the RNeasy formalin-fixed, paraffin-embedded kit (Qiagen) following the manufacturer's protocol. RNA concentration was assessed using Nanodrop and Agilent RNA 6000 Nano and Pico Chips (cat# 5067-1511 and 5067-1513; Agilent Technologies).

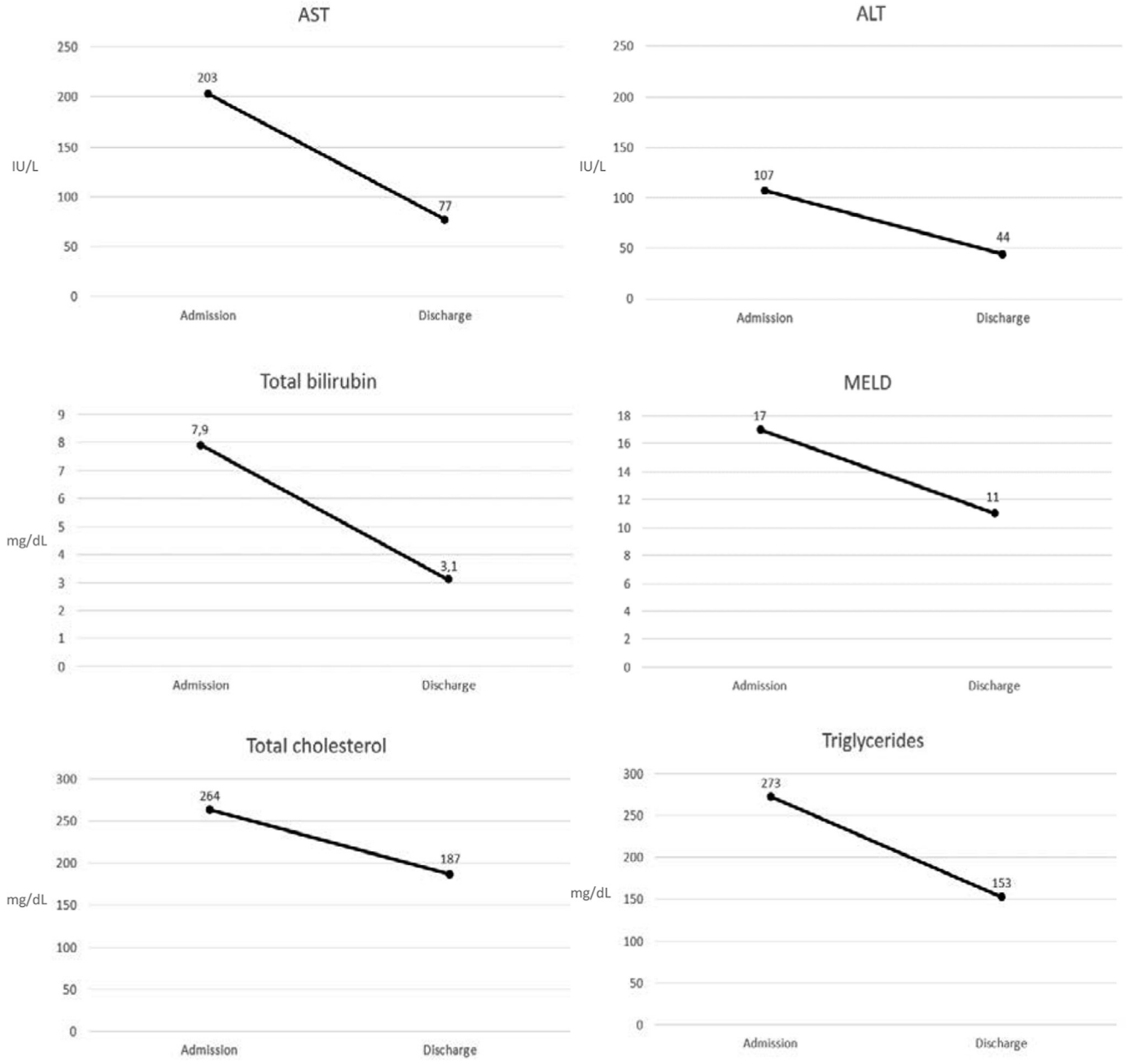
Sequencing libraries were prepared using the SMARTer Stranded Total RNA-seq Kit v2 – Pico Input Mammalian kit (cat# 634411; Takara Bio USA), following the kit user manual (revision 050619). In summary, starting from 50 ng formalin-fixed, paraffin-embedded RNA samples, and without fragmentation before first-strand complementary DNA synthesis, the first-strand complementary DNA synthesis was performed using SMARTScribe reverse transcriptase, for 90 minutes at 42°C, 10 minutes at 70°C, and paused at 4°C. Afterwards, Illumina Adapters and Indexes were added, performing a preamplification polymerase chain reaction (60 seconds at 94°C, 5 cycles of 15 seconds at 98°C, 15 seconds at 55°C, 30 seconds at 68°C, and paused at 4°C). Then, ribosomal complementary DNA was depleted with ZapR v2 and R-Probes v2 (Takara Bio). Finally, enrichment of libraries was achieved by polymerase chain reaction (60 seconds at 94°C; 13–17 cycles of 15 seconds at 98°C, 15 seconds at 55°C, 30 seconds at 68°C, and paused at 4°C). Final libraries were visualized on an Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA kit (cat# 5067-4626; Agilent Technologies), quantified using the Qubit dsDNA HS DNA Kit (cat# Q32854; Thermo Fisher Scientific), and sequenced in a NovaSeq 6000 (Illumina, Inc) with 100-nucleotide paired-end reads.

Unique mapped reads (Novoalign software v3.02.08) were summarized as counts representing the gene expression levels for more than 20,800 different genes present in the AmpliSeq Human Gene Expression panel. Low expressed genes were not considered from the differential expression phase if the sum of counts was less than 100. Linear modeling and differential expression were calculated by means of limma Rpackage (Smyth GK, 2015). Fold changes, moderated P values, and their adjusted P values for multiple testing were calculated using the Benjamini–Hochberg procedure to estimate the false-discovery rate. RNA concentration and quality were determined with a Pico Bioanalyzer.

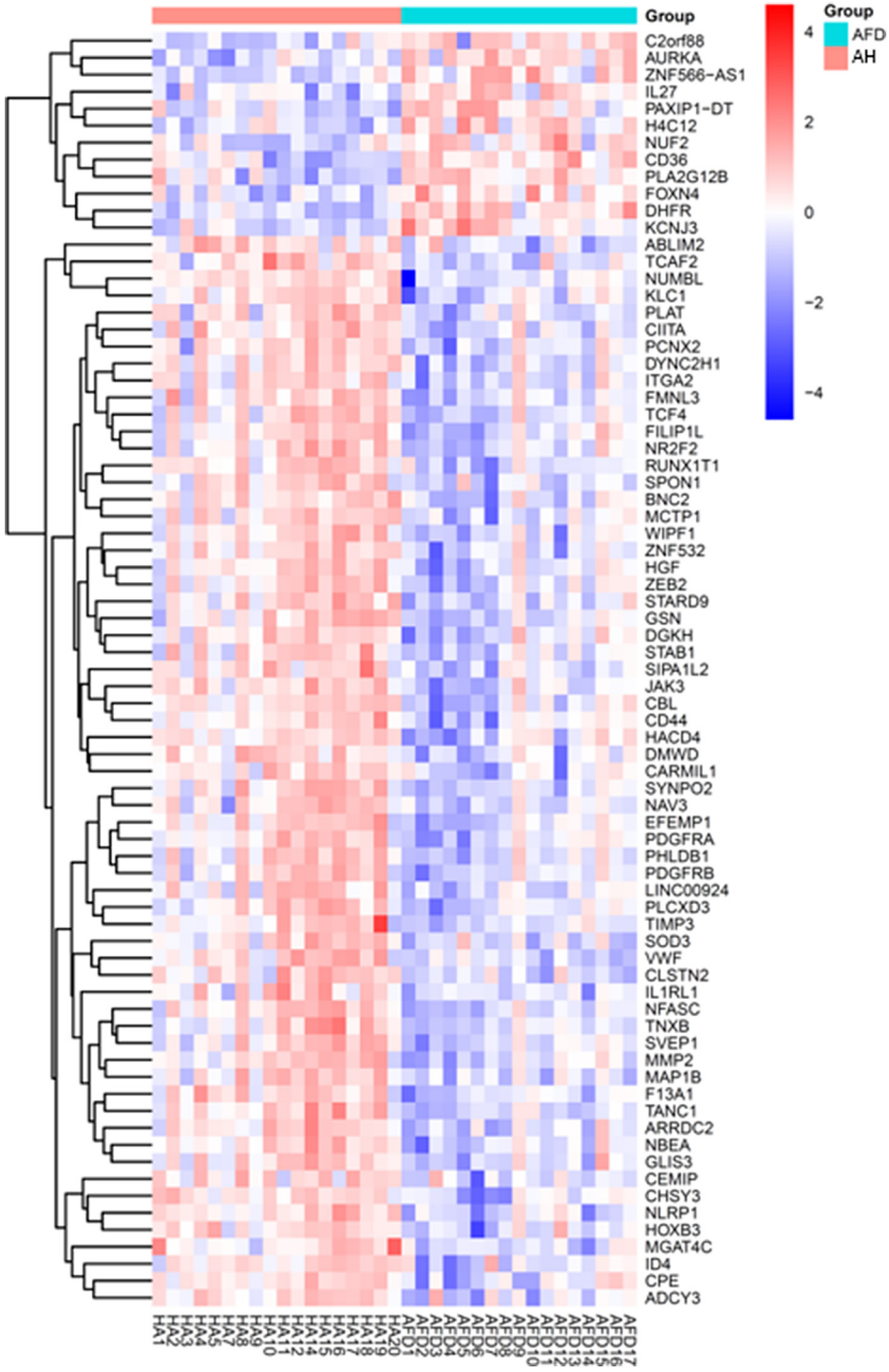
Unsupervised principal component analysis was performed by princomp function using R statistical software (v3.4.3).



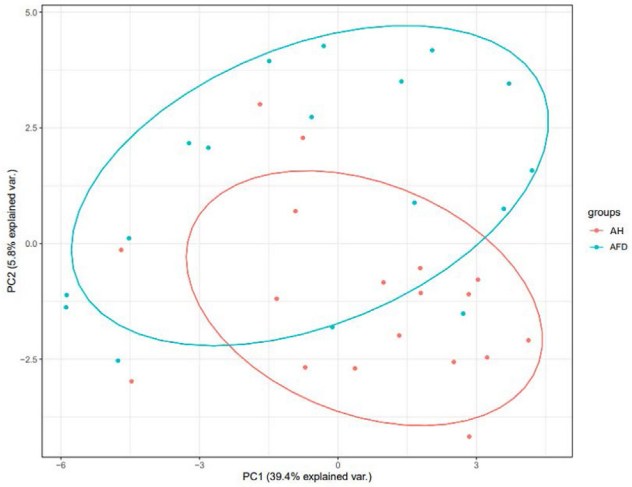
Supplementary Figure 1. Study flowchart. [a]Patients diagnosed with alcohol-associated hepatitis (AH) based on the National Institute on Alcohol Abuse and Alcoholism clinical criteria. [b]Patients signed the informed consent for liver biopsy but did not sign the informed consent to be included in the study. [c]Biopsy specimens less than 10 mm in length or with fewer than 5 portal tracts were considered invalid. [d]Patients with histologic features different from AH and AFD: advanced fibrosis with minimal or no steatosis (n = 17), predominant macrovesicular steatosis (n = 10), and isolated perisinusoidal fibrosis (n = 1). AFD, alcoholic foamy degeneration.



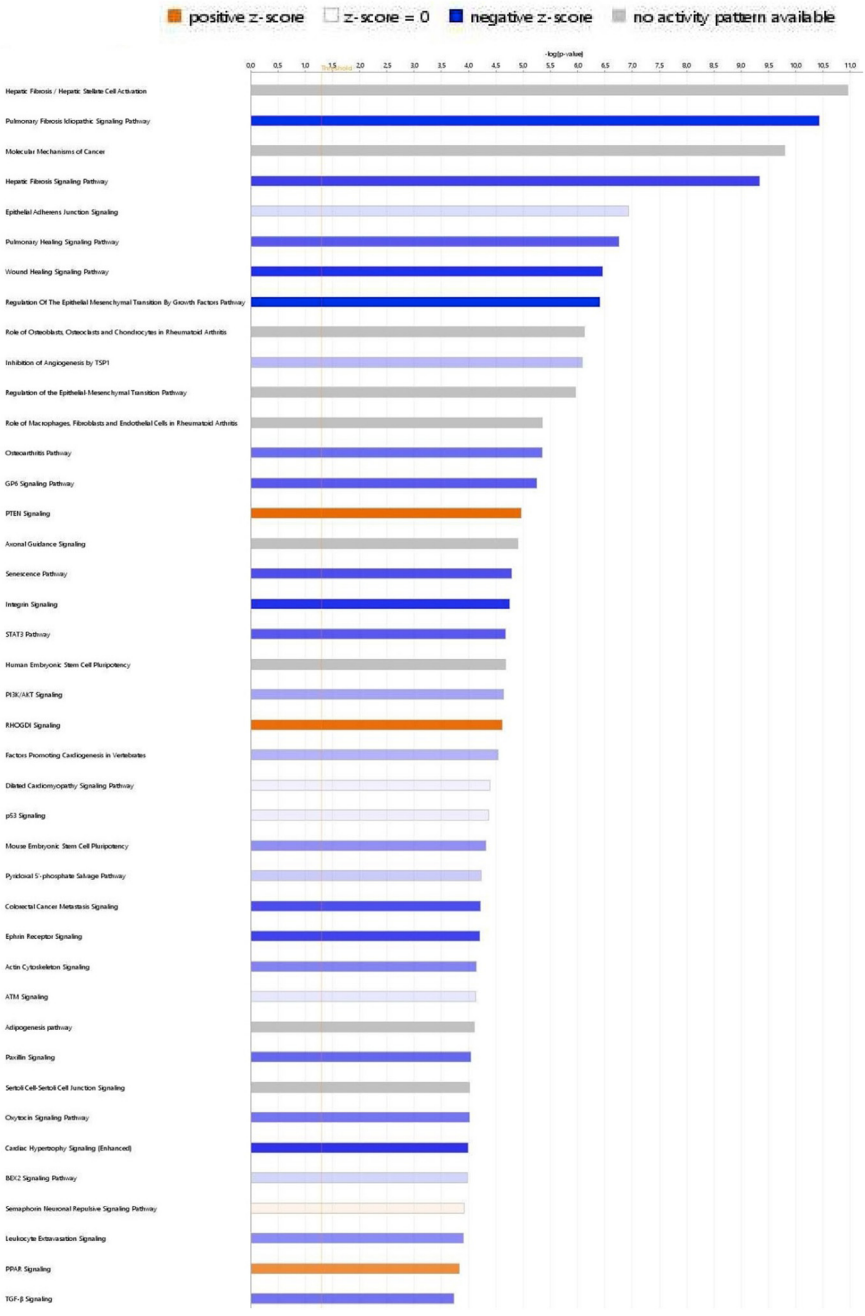
Supplementary Figure 2. Changes in laboratory tests in patients with alcoholic foamy degeneration. The median time between tests was 7 days. ALT, alanine aminotransferase; AST, aspartate aminotransferase; MELD, model for end-stage liver disease.



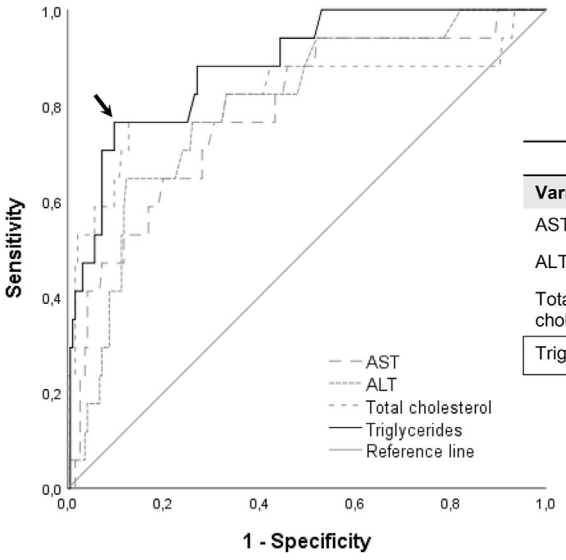
Supplementary Figure 3. Heatmap of the most up-regulated (red) and down-regulated (blue) genes in liver biopsy specimens of patients with alcoholic foamy degeneration (AFD) (*right*) and alcohol-associated hepatitis (AH) (*left*).



Supplementary Figure 4. Principal components analysis plot of the transcriptomics of patients with alcoholic foamy degeneration (AFD) and alcohol-associated hepatitis (AH). PC1, principal component 1; PC2, principal component 2.

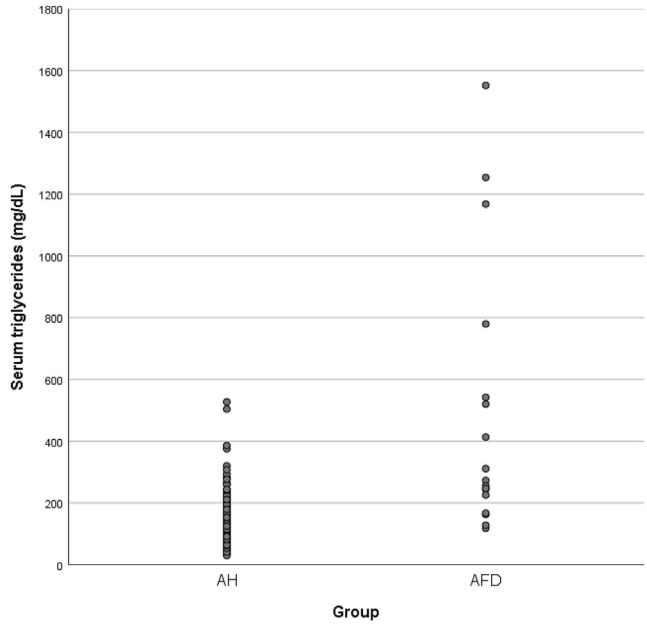


Supplementary Figure 5. Functional enrichment analysis of canonical pathways in patients with alcoholic foamy degeneration (AFD) compared with patients with alcohol-associated hepatitis (AH), using Ingenuity Pathway Analysis. Pathways in blue are down-regulated; pathways in orange are up-regulated.



Area under the ROC curve		
Variables	**Area**	**CI 95%**
AST	0.786	0.669-0.903
ALT	0.796	0.691-0.902
Total cholesterol	0.821	0.680-0.961
Triglycerides	0.886	0.807-0.964

Supplementary Figure 6. Area under the receiver operating characteristic curve representing the performance of different variables for the diagnosis of alcoholic foamy degeneration. The best cut-off value for serum triglycerides (*arrow*) was 225 mg/dL (sensitivity, 0.77; specificity, 0.90; Youden index, 0.67). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ROC, receiver operating characteristic.



Supplementary Figure 7. Individual values of serum triglycerides in patients with alcohol-associated hepatitis (AH) and alcoholic foamy degeneration (AFD).

Supplementary Table 1. NIAAA and NIAAAm-CRP Clinical Criteria for the Diagnosis of Probable Alcohol-Associated Hepatitis

NIAAA clinical criteria
1. Onset of jaundice within prior 8 weeks.
2. Ongoing consumption of >40 g (female) or >60 g (males) alcohol/d for ≥6 months, with <60 days of abstinence before the onset of jaundice
3. Aspartate aminotransferase level >50 IU/L, aspartate aminotransferase/alanine aminotransferase ratio >1.5, and both values <400 IU/L
4. Total serum bilirubin level >3.0 mg/dL
5. Absence of potential confounding factors[a]
NIAAAm-CRP clinical criteria
1. Onset of jaundice within prior 8 weeks
2. Ongoing consumption of >40 g (female) or 60 g (males) alcohol/day for ≥6 months, with <120 days of abstinence before the onset of jaundice
3. Aspartate aminotransferase level ≥50 IU/L, aspartate aminotransferase > alanine aminotransferase
4. Total serum bilirubin level ≥2.5 mg/dL
5. C-reactive protein ≥1 mg/dL

NIAAA, National Institute of Alcohol Abuse and Alcoholism; NIAAAm-CRP, modified National Institute of Alcohol Abuse and Alcoholism–C-reactive protein.
[a]Confounding factors included the following: possible ischemic hepatitis (ie, severe upper gastrointestinal bleeding, hypotension, or cocaine use within 7 days), possible drug-induced liver injury, uncertain alcohol use assessment, and atypical laboratory tests such as antinuclear antibody >1:160 or smooth-muscle antibodies >1:80.

Supplementary Table 2. Performance of NIAAA Criteria and NIAAAm-CRP Criteria for the Differential Diagnosis of Patients With AFD and AH

	AH	AFD	Total		AH	AFD	Total
NIAAA+	129 (70)	10 (56)	139 (69)	NIAAAm-CRP+	123 (73)	6 (55)	129 (72)
NIAAA-	55 (30)	8 (44)	63 (31)	NIAAAm-CRP-	45 (27)	5 (45)	50 (28)
Total	184	18	202	Total	168	11	179

	Value, %	95% CI		Value, %	95% CI
Sensitivity	70	63–77	Sensitivity	73	66–80
Specificity	44	22–69	Specificity	45	17–77
PPV[a]	83	77–89	PPV[a]	84	76–90
NPV[a]	27	17–39	NPV[a]	30	17–46
Diagnostic accuracy[a]	65	58–72	Diagnostic accuracy[a]	68	60–74

NOTE. Neither NIAAA (odds ratio, 1.88; 95% CI, 0.70–5.01) nor NIAAAm-CRP (odds ratio, 2.28; 95% CI, 0.66–7.83) criteria were able to differentiate alcoholic foamy degeneration from alcohol-associated hepatitis. Values shown are the absolute count (percentage) for the top half of the table and the percentage for the bottom half of the table.
AFD, alcoholic foamy degeneration; AH, alcohol-associated hepatitis; NIAAA, National Institute on Alcohol Abuse and Alcoholism; NIAAAm-CRP, modified National Institute on Alcohol Abuse and Alcoholism–C-reactive protein; NPV, negative predictive value; PPV, positive predictive value.
[a]Values shown are considering the prevalence of alcohol-associated hepatitis in this cohort (80%).