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Clinical Presentation and Gene Expression of Acute Alcohol-Induced Microvesicular Steatosis Mimicking Alcoholic Hepatitis

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Acute alcoholic microvesicular steatosis (MIC) may complicate heavy alcohol intake and present as alcoholic hepatitis (AH) syndrome. However, detailed clinical, biological, and histologic data associated with MIC are scarce. We compared the clinical presentation, histologic features, and hepatic transcriptomic of patients presenting with AH due to either MIC or severe alcoholic steatohepatitis (ASH). In this case-control study, patients who drank heavily (>100 g/day) with the AH syndrome were included either in the MIC group (>50% severe microvesicular steatosis, no inflammation) or in the severe ASH group (polynuclear neutrophil infiltration, macrosteatosis, ballooned hepatocytes). All patients received standard supportive care plus steroids for those with severe ASH and were followed up for 3 months. Whole-liver transcriptome profiling was performed on liver snap-frozen biopsies. Compared to ASH (n = 24, mean age 49.3 years), patients in the MIC group (n = 12, mean age 49.1 years) had a higher reported alcohol intake ($P < 0.01$), lower Model for End-Stage Liver Disease score ($P < 0.05$), lower hepatic venous pressure gradient ($P < 0.01$), higher alanine aminotransferase ($P < 0.02$) and gamma-glutamyltransferase ($P < 0.001$), higher triglycerides ($P < 0.001$) and total cholesterol ($P < 0.002$), but similar bilirubin levels ($P = 0.54$). At histology, patients with MIC had a lower fibrotic stage compared to those with ASH ($P < 0.001$). A higher density of megamitochondria was seen in MIC compared to ASH ($P < 0.05$). During follow-up, death or transplantation occurred in 4/12 (33%) patients with MIC and 7/24 (29%) patients with severe ASH. Differential hepatic gene expression in MIC compared to ASH included down-regulation of genes related to inflammation and fibrosis and up-regulation of genes involved in lipid metabolism and mitochondrial function. **Conclusion:** MIC is an acute, noninflammatory, potentially severe alcoholic liver injury mimicking ASH, is associated with a lower fibrosis stage, and has a distinct gene expression profile. (*Hepatology Communications* 2021;5:618-628).

Excess alcohol consumption may be complicated by a rapid deterioration of liver function associated with jaundice, a clinical situation that is described under the name alcoholic hepatitis (AH) syndrome.⁽¹⁾ This clinical presentation includes a rapid onset of jaundice within weeks, which is clinical and biological evidence of hepatic decompensation in the setting of heavy ongoing alcohol

intake.^(2,3) The histologic expression of AH, under the name of alcoholic steatohepatitis (ASH), associates lobular infiltration with polynuclear neutrophils, steatosis, and ballooning degeneration of hepatocytes that may include Mallory-Denk bodies.⁽²⁾ However, AH syndrome may also result from other conditions, including biliary tract obstruction, drug-induced liver injury, metabolic liver disease, sepsis, or from

Abbreviations: AH, alcoholic hepatitis; ALD, alcoholic liver disease; ASH, alcoholic steatohepatitis; GGT, gamma-glutamyltransferase; GPAM, glycerol-3-phosphate acyltransferase, mitochondrial; HVPg, hepatic venous pressure gradient; MELD, Model for End-Stage Liver Disease; MIC, microvesicular steatosis; PLIN2, perilipin 2.

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severe alcoholic fatty liver with diffuse microvesicular steatosis (MIC).⁽⁴⁾ These coexisting confounding factors may account for up to 20% of patients with a clinical diagnosis of AH.^(1,3) According to both American Association for the Study of Liver Diseases and European Association for the Study of the Liver guidelines, a liver biopsy is recommended when the diagnosis is uncertain and when histologic confirmation of ASH is needed in clinical trials.^(2,3) In patients with severe AH, prednisolone improves short-term mortality⁽⁵⁾ and should be proposed in patients without contraindications to steroids.

Acute alcoholic MIC, also reported as “alcoholic foamy degeneration” or “spongiosis,” is less common than macrovesicular steatosis, may mimic ASH when severe, and is a poorly characterized manifestation of acute alcoholic liver injury.^(4,6) This condition differs histologically from ASH and typically demonstrates structural alterations of hepatic mitochondria or megamitochondria,⁽⁷⁾ numerous small intracytoplasmic fat droplets resulting from mitochondrial dysfunction,⁽⁸⁾ or no inflammation and varying degree of cholestasis.^(4,9) Thus, MIC shares some similarities with conditions demonstrating altered mitochondrial function,⁽¹⁰⁾ but the clinical outcome is uncertain⁽¹¹⁾ and no specific treatment is indicated. Therefore, the aim of this study was to explore this uncommon and often overlooked condition that is part of the AH syndrome by studying clinical and biological characteristics, histologic findings, and hepatic gene expression as well as follow-up of patients presenting with MIC compared to patients with severe ASH.

Patients and Methods

We carried out a case-control study as a substudy of an ongoing prospective cohort of patients with

alcoholic liver disease (ALD) performed in a single tertiary-care hospital with expertise in clinical hepatology and liver pathology.

Our cohort was divided into cases with a diagnosis of MIC and controls with a diagnosis of ASH who were matched for age and sex; the control-to-case ratio was 2:1. Inclusion criteria for all patients were as follows: heavy (>80 g/day) recent alcohol intake; clinical presentation with AH syndrome; Maddrey's discriminant function ≥ 32 ; no sepsis, no hepatitis B or C or human immunodeficiency virus infection, no exposure to drugs reported to be associated with MIC (i.e., valproic acid, tetracyclin, nucleoside reverse transcriptase inhibitors); and written informed consent to participate. All patients had a liver biopsy performed early (median, 3.3 days; range 1-7 days) after hospital admission by one investigator (L.S.) using the transjugular route, as described.⁽¹²⁾ The hepatic venous pressure gradient (HVPG) was measured, and the Model for End-Stage Liver Disease (MELD) score was calculated from all patients at the time of biopsy and at every follow-up visit. Laboratory testing included liver function tests as well as determination of total cholesterol and triglyceride in the serum by using a commercially available enzymatic method (Roche Cobas Analyzer 8000; Roche Diagnostics Inc., Rotkreuz, Switzerland). Clinical management included standard supportive care, management of alcohol use disorder, and Lille's score-guided steroid therapy in severe biopsy-confirmed ASH, as recommended.⁽³⁾ During a follow-up of 90 days, patients were listed as alive, dead, or having had a transplant, and the return or not to alcohol consumption was regularly assessed by the investigators during three outpatient visits.

The first part of the study aimed at describing patients' baseline characteristics and outcome. The second part was a genomic substudy in which the differential hepatic expression of genes was assessed in both groups.

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LIVER HISTOLOGY

For each patient, one part of the liver tissue specimen was fixed, processed, and stained using standard hematoxylin and eosin, Masson trichrome, and reticulin techniques, as described.⁽¹³⁾ Although the transjugular access for sampling liver tissue typically yields

fragmented material, it is accepted as suitable for a reliable assessment of histologic features.⁽¹⁴⁾ MIC was identified as numerous, small, intracytoplasmic vacuoles in an enlarged cell with a nucleus remaining in a central position, resulting in the hepatocyte having a foamy appearance^(4,6) (Fig. 1). In the absence of established diagnostic criteria of severe alcohol-induced

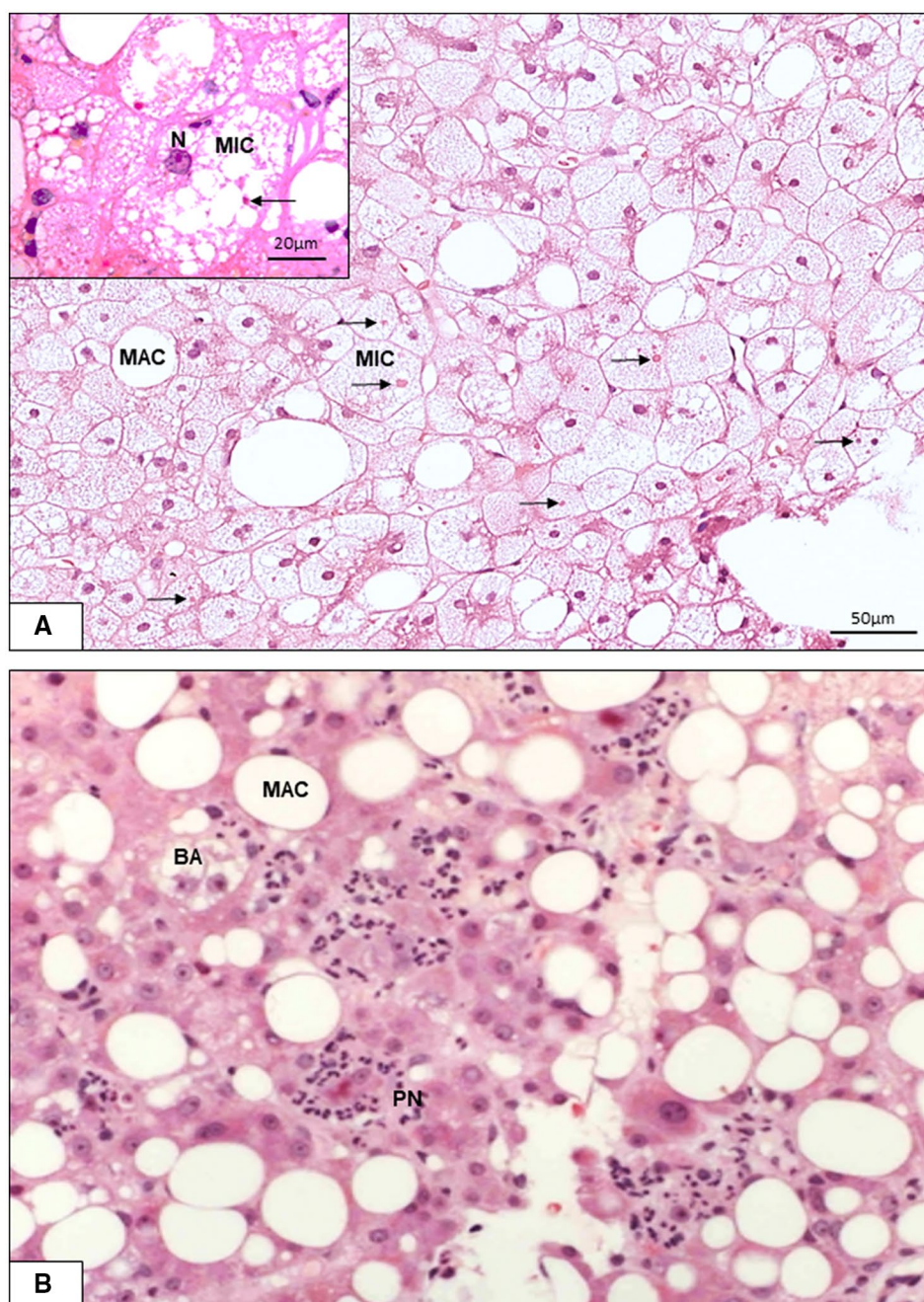


FIG. 1. Histologic aspect of typical lesions observed in MIC and ASH. (A) Severe MIC. (B) ASH. Arrows indicate megamitochondria. Inset shows a closer view of MIC and megamitochondria (hematoxylin and eosin stain; original magnification $\times 400$). Abbreviations: BA, ballooned hepatocyte; MAC, macrovesicular steatosis; N, nucleus; PN, polynuclear neutrophils.

MIC, we used the following histologic definition: >50% of MIC + variable degree of macrovesicular steatosis and fibrosis, no polynuclear infiltration. For the diagnosis of ASH, the presence of some degree of macrovesicular steatosis + fibrosis + polynuclear infiltration of the lobule + ballooned hepatocytes was required.^(3,15) The histologic aspects of both conditions are illustrated in Fig. 1.

We also performed a semiquantitative evaluation of several histologic features commonly observed in acute ALD by using a scoring method derived from a recent publication.⁽¹⁶⁾ This evaluation included steatosis, ballooned hepatocytes, lobular fibrosis, fibrosis stage, bilirubinostasis, neutrophilic infiltration, and megamitochondria. For the latter, the histologic analysis was as follows: 0, absent; 1, scarce; 2, numerous (>1 per high-power field). These features were assessed by an expert in liver pathology (L.R.B.) who was unaware of the patients' characteristics.

RNA EXTRACTION

The other part of the liver biopsy samples was immediately stored at -80°C in RNA_{later} stabilization reagent (Qiagen AG, Hombrechtikon, Switzerland) until analysis. Tissues were disrupted and homogenized in microtubes containing beads (BER0032; Labgene, Châtel Saint Denis, Switzerland) and 1 mL QIAzol (Qiagen AG) on a Minilys homogenizer (Berlin Technologies) according to the manufacturer's instructions. Briefly, after mixing at maximum speed 3 times during 20 seconds, lysates were spun, transferred to a new microtube, and incubated at room temperature for 5 minutes. Then, 200 μL of chloroform was added, and following vigorous shaking, microtubes were centrifuged at 12,000 g for 15 minutes at 4°C . The aqueous phase was mixed with 70% ethanol at a ratio 1:1 volume (vol)/vol and immediately loaded on a RNeasy mini spin column (Qiagen AG) in order to purify total RNA according to the manufacturer's instructions. RNAs were then eluted in 35 μL of ribonuclease-free water.

MICROARRAY STUDIES

An amount of 100 ng of total RNA was used as input for the preparation of single-strand complementary DNA, using the WT PLUS reagent kit

(Thermo Fisher Scientific, Reinach, Switzerland). Targets were then fragmented and labeled with the Affymetrix GeneChip WT Terminal Labeling Kit and hybridized on human Clariom S arrays according to the manufacturer's recommendations (Affymetrix Inc., Santa Clara, CA). The arrays were washed and stained on a GeneChip Fluidics Station 450 (protocol FS450_0001) and then scanned on a GS300 scanner with AGCC Scan Control software (Affymetrix Inc.). We used the Transcriptome Analysis Console (TAC) software (Affymetrix Inc.) to analyze hepatic gene expression in patients with MIC and in those with ASH. Only genes that showed at least a 2-fold increase or decrease were considered differentially expressed. The differentially up-regulated or down-regulated genes identified were then combined into groups with similar metabolic pathways and functions.^(17,18)

STATISTICAL ANALYSIS

Data were expressed as mean + SD or medians with range. All comparisons regarding demographic, biological, and histologic data were performed using the nonparametric Mann-Whitney or Wilcoxon's rank sum test and Fisher's exact test, as appropriate. For all statistical analyses, we used GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA). Differences were considered statistically significant at two-sided $P < 0.05$.

ETHICAL CONSIDERATIONS

This research protocol was in accordance with the relevant guidelines and Declaration of Helsinki and approved by our institutional review board (Commission Cantonale d'Ethique de la Recherche, N° 13-097). All patients gave written informed consent to participate.

Results

PATIENT SELECTION

The algorithm of patient selection is provided in Fig. 2. Severe MIC represented 8% of all liver biopsies for AH over a 3-year period, consistent with published findings.⁽⁶⁾

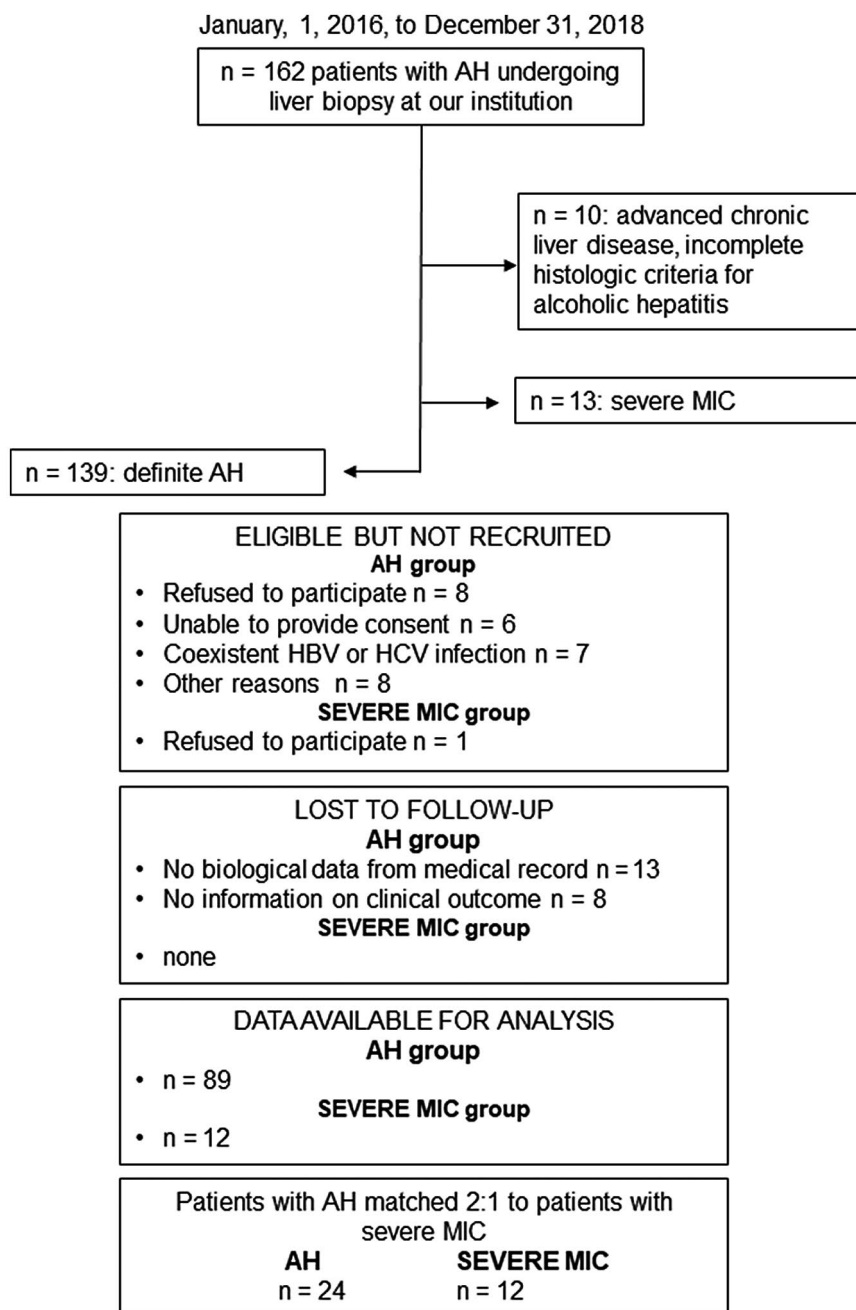


FIG. 2. Flowchart of patient selection. Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

CLINICAL STUDY

At hospital admission, in addition to jaundice, all patients presented with nonspecific symptoms, including fatigue and anorexia, but epigastric and/or right hypochondrium pain (presumably resulting from swelling of the steatotic liver and concomitant stretching of

the capsule) was more prevalent in patients with MIC than in those with ASH ($P < 0.03$). Patient characteristics at baseline are summarized in Table 1. The mean age was 49 years, with an equal number of men and women. Compared to patients with ASH, patients with MIC reported higher daily alcohol consumption, which was heavy continuous alcohol intoxication in all

TABLE 1. PATIENT CHARACTERISTICS

Parameter	MIC (n = 12)	ASH (n = 24)	P Value
Age (years)	49.1 ± 3.2	49.3 ± 1.7	0.85
Sex (male/female)	6/6	12/12	0.99
BMI (kg/m ²)	25.9 ± 4.1	25.3 ± 4.7	0.76
Reported alcohol intake (g/day)	190 ± 10.1	145 ± 11.6	<0.01
Time interval hospital admission to biopsy (days)	3.2 [1-7]	3.3 [1-6]	0.82
MELD score	16.9 ± 2.7	21.3 ± 1.3	<0.05
HVPG (mm Hg)	11 ± 1.4	17.3 ± 0.7	<0.001
WBC (G/L)	7.1 ± 1.9	11.6 ± 1.5	<0.05
AST (IU/L)	260 ± 98	115 ± 11	0.36
ALT (IU/L)	80 ± 15	39 ± 3.9	<0.02
GGT (IU/L)	1,331 ± 375	561 ± 131	<0.001
Alkaline phosphatase (IU/L)	261 ± 74	139 ± 16	<0.05
Serum bilirubin (μmol/L)	152.3 ± 3.8	201 ± 30	0.54
Serum triglycerides (mmol/L)	6.5 ± 1.8	1.48 ± 0.2	<0.01
Serum total cholesterol (mmol/L)	8.6 ± 1.8	3.3 ± 0.39	<0.001

Data show mean ± SD or median [range].

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; G/L, gigagram per liter; IU, international units; WBC, white blood cell.

patients and not a binge drinking pattern. Compared to ASH, patients in the MIC group presented with a lower MELD score and HVPG value. Laboratory tests of patients with MIC showed lower white blood cell count, significantly elevated values of the transaminases gamma-glutamyltransferase (GGT) and alkaline phosphatase, but similar values of total bilirubin compared to those with severe ASH. Strikingly, serum values of both total cholesterol and triglycerides were higher in MIC compared to ASH (Table 1), but the body mass index was similar between groups.

All patients received standard supportive care that was supplemented by steroid therapy in patients with severe ASH; all but 7 responded to the therapy based on the Lille score.⁽¹⁹⁾ During the 3-month follow-up period, death or liver transplantation occurred in 11 patients in total. Four patients died in the MIC group after a median time of 34.5 days (range, 5-55 days) due to infections (n = 2), cardiovascular disease (n = 1), and renal cause (n = 1). In the ASH group, 1 patient received early liver transplantation and 6 patients died after a median time of 28.5 days (range, 10-62 days) due to liver-related complications (n = 3), infections (n = 2), and cardiovascular disease (n = 1) (Fig. 3). Clinical episodes of hepatic encephalopathy

were observed in 1 and 3 patients from the MIC and ASH groups, respectively. Available data at 4 weeks in survivors showed a significant reduction in the MELD score compared to baseline values in both MIC ([mean + SD] 16.9 + 2.7 to 11.8 + 2; *P* < 0.03) and ASH (21.3 + 1.3 to 16.3 + 2; *P* < 0.03) groups.

In spite of systematic in-hospital management of alcohol use disorder, 4 patients (25%) and 5 patients (17%) in the MIC and ASH groups, respectively, returned to harmful alcohol consumption. However, none presented with recurrence of AH syndrome during follow-up.

HISTOLOGIC CHARACTERIZATION

A detailed examination and semiquantitative evaluation of histologic lesions on liver biopsy were obtained from all patients. By definition,⁽³⁾ all patients with ASH showed macrovesicular steatosis of varying intensity, polynuclear neutrophilic infiltration, and ballooned hepatocytes. Ballooning was scant or not observed in patients with MIC. A comparison of histologic features between groups is illustrated in Fig. 4. Compared to ASH, patients in the MIC group had significantly more steatosis (both macrovesicular and microvesicular) but less hepatic fibrosis. All patients with ASH had advanced underlying fibrosis at a stage of cirrhosis except for 1 patient with extensive fibrosis. The intensity of bilirubinostasis was similar, being canalicular or ductular alone or associated with a hepatocellular pattern in 50% of patients with MIC and 33% of those with ASH (*P* = 0.47). Megamitochondria were significantly more frequent in patients with MIC compared to ASH and occasionally colocalized to areas of MIC. Concordance was excellent between both liver pathologists, with a low interobserver variation (kappa value, 0.9). Hence, relevant differences between MIC and ASH included microvesicular and macrovesicular steatosis, inflammation, stage of liver fibrosis density, and density of megamitochondria.

HEPATIC GENE EXPRESSION ANALYSIS

The genomic substudy was restricted to liver biopsy specimens from 7 and 14 patients with MIC and ASH, respectively. High-quality RNA obtained

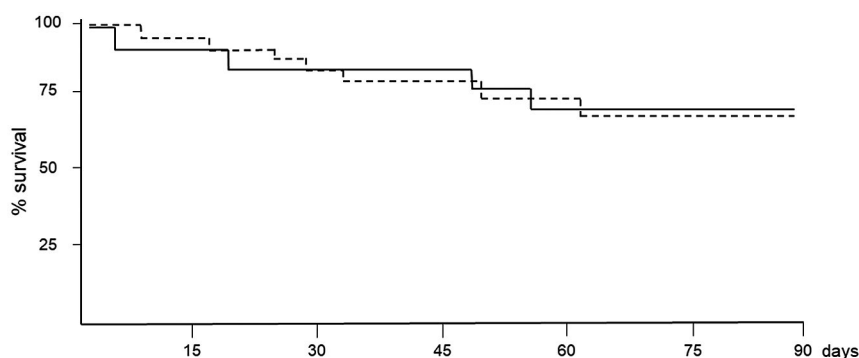


FIG. 3. Patient survival at 3 months of follow-up. The solid line is MIC, the dotted line is severe ASH. *P* value is nonsignificant.

from all samples allowed a comprehensive hybridization and gene expression profile study. Four hundred genes demonstrated a differential expression of 2-fold or greater and a false discovery rate ≤ 0.05 (Fig. 5). Thus, compared to patients with ASH, we identified in the MIC group several up- and down-regulated genes associated with the following biological pathways or functions: cellular proliferation and repair (cyclin A2 [*CCNA2*], marker of proliferation Ki-67 [*MKI67*], cluster of differentiation 34 [*CD34*], hepatocyte growth factor [*HGF*]), inflammation (arachidonate 5-lipoxygenase activating protein [*ALOX5AP*], C-C motif chemokine receptor 2 [*CCR2*], C-C motif chemokine ligand 21 [*CCL21*], neutrophil cytosolic factor 1 [*NCF1*], tumor necrosis factor superfamily member 14 [*TNFSF14*]), lipid metabolism (thyroid hormone responsive [*THRSP*], perilipin 2 [*PLIN2*], sterol-C5-desaturase [*SC5D*]), mitochondrial activity (glycerol-3-phosphate acyltransferase, mitochondrial [*GPAM*]), liver fibrosis (fibroblast growth factor 7 [*FGF7*], collagen type V alpha 1 chain [*COL5A1*], lysyl oxidase like 1 [*LOXL1*]), and detoxification (cytochrome P450 family 4 subfamily F member 22 [*CYP4F22*]). The most relevant differentially expressed genes in patients with MIC compared to ASH are summarized in Fig. 5.

Discussion

The present study provides detailed characteristics of patients with acute alcohol-induced severe MIC of the liver mimicking ASH, a condition also known as alcoholic foamy degeneration.⁽⁶⁾ Compared to an

age- and sex-matched population with histologically confirmed severe ASH, we show that patients who drink heavily and present with the AH syndrome and a diagnosis of MIC demonstrate striking differences in biochemical profile, histologic alterations, and differentially expressed genes in liver biopsy specimens. Being aware of this particular form of acute alcoholic liver injury is important and clinically relevant and underlines the heterogeneity of histologic lesions that may be observed in patients with clinical AH. In our tertiary-care hospital with approximately 60 patients admitted annually with the AH syndrome and a liver biopsy performed early and systematically according to our local guidelines, we report an actual frequency of 8% for severe MIC, which is consistent with the limited reported data.⁽⁶⁾ Except for supportive care and promotion of alcohol abstinence, no specific therapy is indicated in this situation.⁽⁴⁾ Contrasting with severe ASH with high short-term mortality even when steroids are administered,⁽⁵⁾ as in our population, most patients with MIC reported in the literature have a favorable clinical course and show improved biological tests under supportive treatment.⁽²⁰⁾ However, it has to be stressed that reported cases are relatively rare, the severity of MIC infiltration is not systematically detailed,⁽⁴⁾ and causes of death when provided are in relation to serious metabolic alterations⁽¹¹⁾ consistent with mitochondrial dysfunction.⁽²¹⁾

The biochemical profile of the patients in our study presenting with MIC shows differences compared to patients with severe ASH. The absence of leucocytosis is consistent with a noninflammatory condition. A typically mild elevation in transaminases in ASH⁽²²⁾ contrasts with the higher value observed

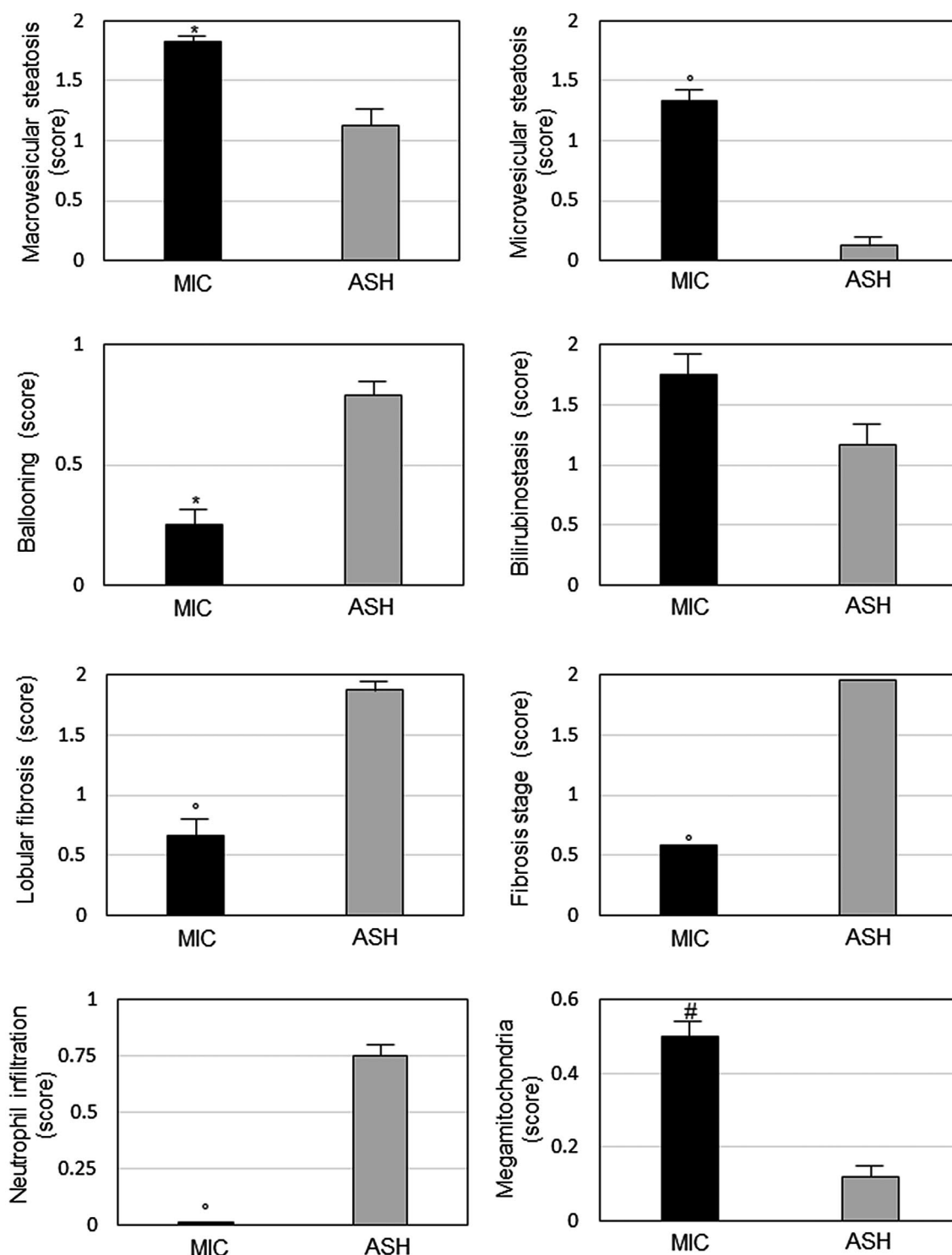


FIG. 4. Graphical illustration of histologic features measured semiquantitatively in liver biopsies of patients with MIC or severe ASH. Data show mean +SEM. ° $P < 0.001$ versus ASH; * $P < 0.01$ versus ASH; # $P < 0.05$ versus ASH.

in MIC, while the high GGT value could be related to the more important reported daily alcohol intake. The finding of high alkaline phosphatase and total

serum bilirubin in all patients is consistent with published studies both in severe ASH^(23,24) and MIC^(4,6) and is associated with the severity of this acute liver

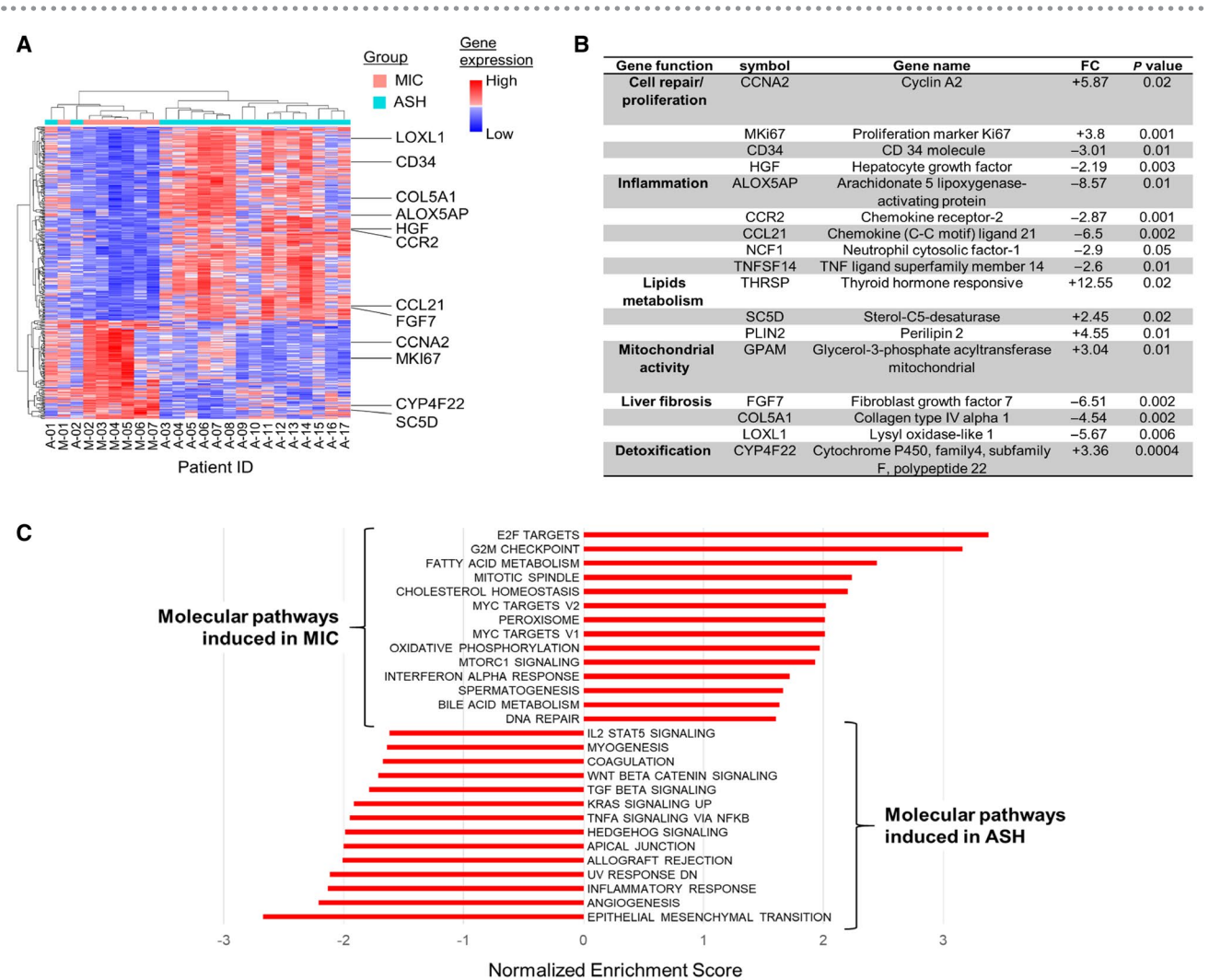


FIG. 5. Molecular comparison of MIC and severe ASH. (A) Microarray data with hierarchical clustering analysis of most differentially expressed hepatic genes (false discovery rate, ≤ 0.05) in patients with MIC compared to patients with ASH. The patient's number appears in columns, and the expressed genes are listed in rows. Key selected genes are highlighted. Gene names are shown in (B). (B) Table summarizing a selection of differentially expressed genes in MIC and severe ASH. (C) Molecular pathways expressed in MIC and severe ASH. Abbreviations: DN, down; E2F, transcription factor E2F; IL2, interleukin 2; MTORC1, mammalian target of rapamycin complex 1; NFKB, nuclear factor kappa B; STAT5, signal transducer and activator of transcription 5; TGF, transforming growth factor; TNF, tumor necrosis factor; UV, ultraviolet.

injury⁽⁴⁾ and poor prognosis.⁽²³⁾ The disturbed lipid profile, which is often described in heavy alcohol consumption,⁽²⁵⁾ showed particularly elevated blood values of triglycerides and total cholesterol in patients with MIC, as reported.^(4,6) This alcohol-induced hyperlipidemia results from an altered lipoprotein and cholesterol metabolism⁽²⁶⁾ with a possible role of associated mitochondrial dysfunction. Accordingly, binge alcohol-related MIC in mice induces epigenetic modifications and hepatic triglyceride accumulation.⁽²⁷⁾ Regarding hematologic manifestations, we

did not observe concomitant hemolytic anemia, such as described under the name of Zieve's syndrome.⁽²⁸⁾ Except for the importance of MIC, major differences at histology observed in MIC compared to ASH include minimal liver fibrosis, absence of inflammation, few ballooned hepatocytes, and increased megamitochondria density. The markedly increased density of megamitochondria in the liver biopsy of patients with MIC confirms previous observations.^(4,29) The combination of megamitochondria and low fibrosis stage has been

described in ALD and associated with few liver-related complications and good outcome.⁽³⁰⁾ From a pathogenic point of view, it has been proposed that megamitochondria formation results from an adaptive survival strategy for the cell in an unfavorable environment,⁽⁷⁾ as in a situation of heavy alcohol consumption.⁽²⁹⁾

Our microarray-based approach provides original data consistent with biological and histologic findings in MIC compared to ASH, showing up- or down-regulated hepatic genes and metabolic pathways. Of note, genes encoding for biological functions, such as inflammation, fibrosis, and lipid metabolism, were differentially expressed in MIC and were able to truly discriminate these two entities. *PLIN2* (or adipophilin) is increased in MIC from other causes, such as metabolic disorders (including glycogenosis and toxic liver damage), and *PLIN2* immunohistochemistry could thus be a marker of small lipid droplets.⁽³¹⁾ Another noteworthy difference between the two diseases points to a possible role of acquired mitochondriopathy with increased megamitochondrial density and overexpression of a gene encoding for a key enzyme in fatty acid oxidation.^(32,33)

The underexpression of genes involved in liver fibrosis and inflammation in patients with MIC is consistent with the histologic description of the lesions. These ultrastructural changes are believed to result from severe alcohol-induced oxidative stress affecting both the mitochondrial membrane structure and energy production. The marked overexpression of the *GPAM* gene modulating an important enzymatic activity in fatty acid oxidation⁽³³⁾ is consistent with impaired mitochondrial function due to alcohol⁽¹⁰⁾ and may participate in the accumulation of intracellular lipids.

Thus, how do we conciliate development of the AH syndrome with major mitochondrial alterations, severe microvesicular changes, but no inflammation? It is well accepted that heavy alcohol consumption generates major oxidative stress⁽³⁴⁾ that affects a number of intracytoplasmic structures, including the mitochondria.⁽³⁵⁾ As a result, mitochondrial β -oxidation and energy production are severely depressed,⁽¹⁰⁾ promoting MIC, dyslipidemic changes, and hepatocellular damage that may be associated with apoptosis or necrosis.⁽³⁶⁾ The mechanisms of hyperbilirubinemia and bilirubinostasis in MIC have not been elucidated to date. We speculate that factors, including hepatocellular damage, intracytoplasmic mechanical stress due to numerous lipid droplets, or

mitochondriopathy-associated energy failure affecting bile transport and flow, such as reported in drug-induced liver injury,⁽³⁷⁾ could participate in intrahepatic cholestasis of severe MIC.

We acknowledge that our study suffers from some limitations. First, choosing a cut-off value of 50% or more on liver biopsy prevents generalization of our results to steatosis of minor intensity, as MIC has to be regarded as a continuum in histologic alterations seen in ALD. Second, we speculate that MIC-associated mitochondriopathy could have played a role in those patients with poor outcome, although this should be confirmed in future translational approaches. Third, in spite of some differentially expressed genes involved in lipid metabolism and mitochondrial function, our genomic analysis simply corroborates histologic and biological findings observed in MIC and ASH. The possible use of these data as therapeutic targets or as biomarkers could be future subjects of research. Finally, the clinical implications and changes over time of serum lipid alterations observed in MIC deserve further investigations.

To conclude, this clinically relevant and original study describes the characteristics of severe MIC as an acute ALD that comprises 8% of patients presenting with AH and that clinicians need to be aware of. No specific treatment has proved beneficial and clinical outcome is variable^(6,11); thus, further investigations are necessary.

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