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Mitochondrial respiratory chain activity is associated with severity, corticosteroid response and prognosis of alcoholic hepatitis

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Summary

Background and Aims: Little is known about the extent of mitochondrial respiratory chain (MRC) activity dysfunction in patients with alcoholic hepatitis (AH). We aimed to assess the hepatic MRC activity in AH patients and its potential impact on the severity and prognosis of this life-threatening liver disease.

Methods: MRC complexes were measured in liver biopsies of 98 AH patients (non-severe, 17; severe, 81) and in 12 histologically normal livers (NL). Severity was assessed according to Maddrey's Index and MELD score. Corticosteroid response rate and cumulative mortality were also evaluated.

Results: The activity of the five MRC complexes was markedly decreased in the liver of AH patients compared with that of NL subjects, being significantly lower in patients

[Correction added on 09 March 2023, after first online publication: Affiliations have been added for Drs. Bataller and Solís-Herruzo].

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with severe AH than in those with non-severe AH. There was a negative correlation between the activity of all MRC complexes and the severity of AH. Interestingly, only complex I and III activities showed a significant positive correlation with the corticosteroid response rate and a significant negative correlation with the mortality rate at all-time points studied. In a multivariate regression analysis, besides the MELD score and the corticosteroid response rate, complex I activity was significantly associated with 3-month mortality (OR = 6.03; $p = 0.034$) and complex III activity with 6-month mortality (OR = 4.70; $p = 0.041$) in AH patients.

Conclusion: Our results indicate that MRC activity is markedly decreased in the liver of AH patients, and, particularly, the impairment of MRC complexes I and III activity appears to have a significant impact on the clinical outcomes of patients with AH.

1 | INTRODUCTION

Alcoholic hepatitis (AH) is the most severe consequence of alcohol-related liver disease (ALD) and is histologically featured by hepatic steatosis with neutrophil lobular infiltration, ballooning of hepatocytes and pericellular fibrosis.[1–3] Its main clinical characteristics are recent or worsening jaundice with a bilirubin level above 3 mg/dL and elevated AST with an AST/ALT ratio over 1.5 (with both values <400 IU/L), in patients with ongoing excessive intake of alcohol and <60 days of abstinence, as described by the NIAAA Alcoholic Hepatitis Consortia in their clinical criteria for the diagnosis of AH.[4] AH is a form of acute-on-chronic liver injury that may evolve into multiorgan failure and death, with up to 30%–50% 28-day mortality without treatment.[4]

Mitochondria have always been given a prominent role in the pathogenesis of ALD.[5–9] To better understand the significance of mitochondria in liver homeostasis, it is important to note that one of the main functions of mitochondria is the production of energy in the form of adenosine triphosphate, which is achieved via the electron transport chain, also known as mitochondrial respiratory chain (MRC), and the oxidative phosphorylation. Under normal conditions, the amount of reactive oxygen (ROS) and nitrogen species (RNS) yielded by the MRC activity is limited, and they are neutralised by the mitochondrial and cellular antioxidant and antiperoxynitrite mechanisms. However, when the MRC activity is altered, higher amounts of both ROS and RNS are formed,[10,11] inducing tumour necrosis factor (TNFa) production.[12] These molecules, in combination, can irreversibly damage the genes that codify the subunits of the five MRC complexes, limit the assembly of these subunits and are even capable of degrading fully assembled complexes.[13–15] Moreover, MRC dysfunction can also induce apoptosis, triggering the release of damage signals.[16] Collectively, the impairment of MRC activity could exacerbate the liver injury related to excessive alcohol consumption, thus contributing to liver disease progression.

Although previous studies suggest that MRC dysfunction plays a key role in the pathogenesis of ALD,[5–9] the scientific evidence of MRC dysfunction in humans is scarce, and the data in animal models of ALD show contradictory results.[17] Furthermore, the existing evidence of liver MRC dysfunction in AH is even weaker due to the lack of animal models and clinical studies in humans. In order to shed light on this issue, we conducted a prospective cohort study comparing the activity of MRC complexes in the liver tissue of patients with different degrees of severity of AH with respect to that of subjects with histologically normal livers and evaluating whether hepatic MRC activity is related to the severity and prognosis of AH patients.

2 | MATERIALS AND METHODS

2.1 | Study population and case selection

Patients with clinical suspicion of AH were initially screened. Clinical suspicion of AH was based on a history of alcohol abuse and timing of consumption, as well as biochemical characteristics, mainly AST, ALT and their ratio, and a recent elevation of serum bilirubin. Also, other liver diseases were ruled out by imaging and laboratory testing. Patients with contraindications for steroids on admission, like active infections or gastrointestinal bleeding, were not considered for the study. Patients with clinical suspicion of AH and no contraindications for steroids, or their legal surrogates, were offered to participate by having a transjugular liver biopsy to establish a definite diagnosis, to assess the stage and prognosis of the disease, and for the purposes of the study. Patients with liver biopsy results compatible with other diagnoses or not conclusive of AH were also excluded.

Histologically normal liver (NL) controls were patients to whom a liver biopsy was performed during an elective cholecystectomy. They had no history of alcohol abuse, and no signs of liver disease were detected both on ultrasound and in a complete biochemical assessment. This study was performed in agreement with the 1975 Declaration of Helsinki and with local and national laws. The study protocol was approved by the clinical research ethics committee of 12 de Octubre University Hospital (Madrid, Spain). Informed consent for the study was obtained from each patient or legal surrogate and control subject before enrolment after providing a detailed description of the study.

Demographic details of all patients studied were collected at admission. Venous blood samples were obtained at the time of the liver biopsy. Biochemical parameters, such as AST, ALT, bilirubin, albumin, INR, prothrombin time, creatinine and leucocytes, were collected.

2.2 | Severity assessment and response to steroid therapy

The clinical severity of AH was measured according to Maddrey's index and MELD score. A Maddrey's index value greater or equal to 32[18] or a MELD score value greater or equal to 21[19] was used to define severe AH.

As mentioned, patients with contraindications for corticosteroid therapy were not included in the study, and therefore, all patients with severe AH were treated with corticosteroids.

Response to steroid treatment was measured using Lille's Model for alcoholic hepatitis on day 7, as previously described by Mathurin et al.[20,21] Patients with a Lille's score >0.45 were considered as corticosteroid non-responders. AH patients were clinically followed for 6 months, and mortality was assessed at 28 days as well as at 3 and 6 months.

2.3 | Liver samples and histology

All the patients included in the study had a transjugular liver biopsy to confirm the diagnosis, establish prognosis and obtain liver tissue for the MRC activity measurements. A single expert hepatopathologist who was blinded to the clinical data analysed each biopsy specimen and performed the histological assessment following current recommendations. The histological confirmation of alcoholic hepatitis was based on the presence of alcoholic steatohepatitis (ASH) in the liver biopsy.[22–24] The histological features of ASH include neutrophilic lobular inflammation, cellular ballooning and Mallory-Denk bodies as markers of hepatocyte degeneration, steatosis and pericellular fibrosis, frequently associated with advanced fibrosis or cirrhosis.

A portion of the liver tissue from both AH patients and NL controls was processed for routine histological examination. Another portion of tissue was snap-frozen in liquid nitrogen and stored at −80°C for later analysis.

2.4 | Measurement of the activity of the MRC complexes

Frozen liver tissues (14–24 mg) from both AH patients (17 non-severe AH and 81 severe AH) and NL controls (12 subjects) were homogenised with 15 vol of 20 mmol/L KP buffer, pH 7.4, and centrifuged at 800 ***g*** for 10 min. Because of the small amount of liver tissue obtained in each liver biopsy and given the tissue needed to perform the MRC activity assays, we made pools of liver tissue of patients with similar clinical severity according to Maddrey's index and MELD score. Each pool included liver tissue of 6–8 patients. Respiratory chain enzymes and citrate synthase (CS) activities were measured in a DU-650 spectrophotometer (Beckman Instruments) applying 10 ±30 mL of liver homogenate per 1 mL test volume. Incubation temperature for complex I (rotenone-sensitive reduced nicotinamide dinucleotide phosphate [NADH] coenzyme Q1 reductase), complex II (succinate dehydrogenase), complex III (antimycin A-sensitive ubiquinol-2 cytochrome c reductase), complex V (oligomycin-sensitive F1-adenosine triphosphate synthase) and CS was 30 and 38°C for complex IV (cytochrome c oxidase). Enzyme activities were obtained in supernatants as described elsewhere.[25] They were expressed in nanomoles of substrate used per minute per milligram of protein and, to correct for the hepatic content of mitochondria, indicated as a percentage of the specific activity of CS. All assays of MRC enzymatic activity were performed in triplicate. The final results are expressed as a percentage of activity in NL controls (100%).

2.5 | Statistical analysis

Qualitative variables are presented as absolute (number, n) and relative (percentage, %) frequencies. Quantitative variables are expressed as measures of central tendency (mean) and dispersion (SD). Kolmogorov–Smirnov test was applied to evaluate whether the variables were adjusted to a normal distribution. Qualitative data between groups were compared by Pearson's c2-test or Fisher exact test as appropriate. Quantitative variables were analysed using one-way ANOVA to calculate the difference of the means in the variables adjusted to a normal distribution, followed by Tukey's Honest Significant Difference test and Mann–Whitney *U* test to compare the variables without normal distribution or heteroscedasticity variances. Pearson's coefficient was used to evaluate correlations when appropriate. Logistic regression analysis, adjusted by age and gender, was performed to identify independent predictors of 28-day, 3- and 6-month cumulative mortality. Univariate and multivariate regression models were constructed, and parameters were selected by the likelihood ratio test. Box-Tidwell procedure was used for testing the linearity of logit. The goodness of fit of the model was evaluated using the Hosmer–Lemeshow statistic. Significance was set at a value of $p < 0.05$. Statistical analysis was performed using the SPSS statistical software version 26.0 (IBM SPSS Statistics).

3 | RESULTS

3.1 | Characteristics of the study population

Of the 194 patients screened, 71 (36.6%) had contraindications for steroids on admission. Of the remaining 123 patients, 16 (13%) refused to participate. In 9 of the patients that participated in the study (8.4%), the liver biopsy was compatible with other diagnoses, or the diagnosis was inconclusive.

Finally, 98 patients with biopsy-confirmed AH and 12 subjects with histologically normal liver (NL) as control group were included. In the group with AH, 17 patients had non-severe AH and 81 had severe AH, according to Maddrey's index and MELD score. A detailed flowchart of the study is presented in Figure S1.

Demographic and biochemical characteristics of the entire study population are detailed in Table 1. As expected, AH patients had significantly lower albumin and higher bilirubin levels, prothrombin time, and INR as well as higher Maddrey and MELD scores compared with NL controls. In addition, cumulative mortality was significantly higher in patients with severe AH than in those with non-severe AH and in subjects with NL at all-time points studied.

3.2 | MRC activity in patients with AH

The activity of all complexes was significantly reduced in patients with AH compared with that in NL controls ($p < 0.0001$ for all cases) (Figure 1). Moreover, the activity of all complexes was significantly lower in patients with severe AH group than in those with non-severe AH (CI: 19.19% vs. 44.36%, $p = 0.0022$; CII: 14.82% vs. 34.88%, $p = 0.0002$; CIII: 17.88% vs. 41.65%, $p = 0.0001$; CIV: 18.16% vs. 37.25%, $p = 0.0013$; CV: 14.43% vs. 31.53%, $p < 0.0001$) (Figure 1).

3.3 | Relationship between MRC activity and the severity of AH

When stratifying the patients with severe AH into two groups with increasing severity (Maddrey's index between 32 and 50 and Maddrey's index >51), the activity of the 5 MRC complexes was lower in those patients with higher Maddrey's index. However, the difference was statistically significant only in complex I (CI, $p = 0.0036$) (Figure 2A). Furthermore, in patients with AH, MRC activity showed a negative correlation with both Maddrey's index (CI: $r = -0.8586$, $p < 0.0001$; CII: $r = -0.5410$, $p = 0.0018$; CIII: $r = -0.4668$, $p = 0.0050$; CIV: $r = -0.2583$, $p = 0.0530$; CV: $r = -0.4374$, $p = 0.0073$) (Figure 2B) and MELD score (CI: $r = -0.9025$, $p < 0.0001$; CII: $r = -0.6420$, $p = 0.0003$; CIII: $r = -0.5955$, $p = 0.0008$; CIV: $r = -0.3869$, $p = 0.0133$; CV: $r = -0.5152$, $p = 0.0026$) (Figure S2).

3.4 | Relationship between MRC activity and the response to corticosteroids in AH patients

Only activities of complex I and III significantly correlated with the therapeutic response to corticosteroids, as measured by Lille's score (CI: $r = 0.6729$, $p = 0.0006$; CII: $r = 0.1527$, $p = 0.1868$; CIII:

TABLE 1 Characteristics of the study population.

Feature	NL ($N = 12$)	Non-severe AH ($N = 17$)	Severe AH ($N = 81$)
Age (years)	50.50 ± 10.98	53.06 ± 11.13	50.30 ± 11.40
Gender			
Women, *n* (%)	5 (41.7)	4 (23.5)	17 (21)
Men, *n* (%)	7 (58.3)	13 (76.5)	64 (79)
Albumin (g/dL)	4.85 ± 0.43	3.10 ± 0.81**	2.77 ± 0.65**
Bilirubin (mg/dL)	0.76 ± 0.13	4.83 ± 1.41**	12.21 ± 6.77**,##
AST (U/L)	19.92 ± 2.68	156.00 ± 52.24***	145.91 ± 41.02***
ALT (U/L)	19.92 ± 4.93	87.59 ± 31.28***	80.31 ± 28.27***
INR	0.98 ± 0.12	1.49 ± 0.17**	2.14 ± 0.40**,##
Prothrombin time (s)	11.09 ± 1.32	15.95 ± 1.30**	20.56 ± 2.69**,##
Creatinine (mg/dL)	0.85 ± 0.16	0.73 ± 0.26	1.40 ± 1.12
Leucocytes (10^9/L)	6.65 ± 0.75	8.48 ± 5.16	13.14 ± 8.69*,#
Maddrey score	−0.22 ± 6.10	22.98 ± 6.43**	51.69 ± 13.50**,##
MELD score	6.75 ± 0.96	16.81 ± 2.04**	27.14 ± 5.91**,##
Fibrosis stage			
F0–F1, *n* (%)	12 (100)	0 (0)**	3 (3.7)**
F2, *n* (%)	0 (0)	3 (17.6)	7 (8.6)
F3, *n* (%)	0 (0)	7 (41.2)*	32 (39.5)**
F4, *n* (%)	0 (0)	7 (41.2)*	39 (48.1)**
Cumulative mortality			
28 days, *n* (%)	0 (0)	0 (0)	21 (25.9)#
3 months, *n* (%)	0 (0)	1 (5.9)	27 (33.3)*,#
6 months, *n* (%)	0 (0)	3 (17.6)	32 (39.5)**

Note: Data are shown as mean ± SD or as number of cases (%).

Abbreviations: NL, normal liver; AH, alcoholic hepatitis; AST, aspartate transaminase; ALT, alanine transaminase; INR, International Normalised Ratio.

*$p < 0.05$ vs. NL.

**$p < 0.01$ vs. NL.

***$p < 0.001$ vs. NL.

#$p < 0.05$ vs. non-severe AH.

##$p < 0.01$ vs. non-severe AH.

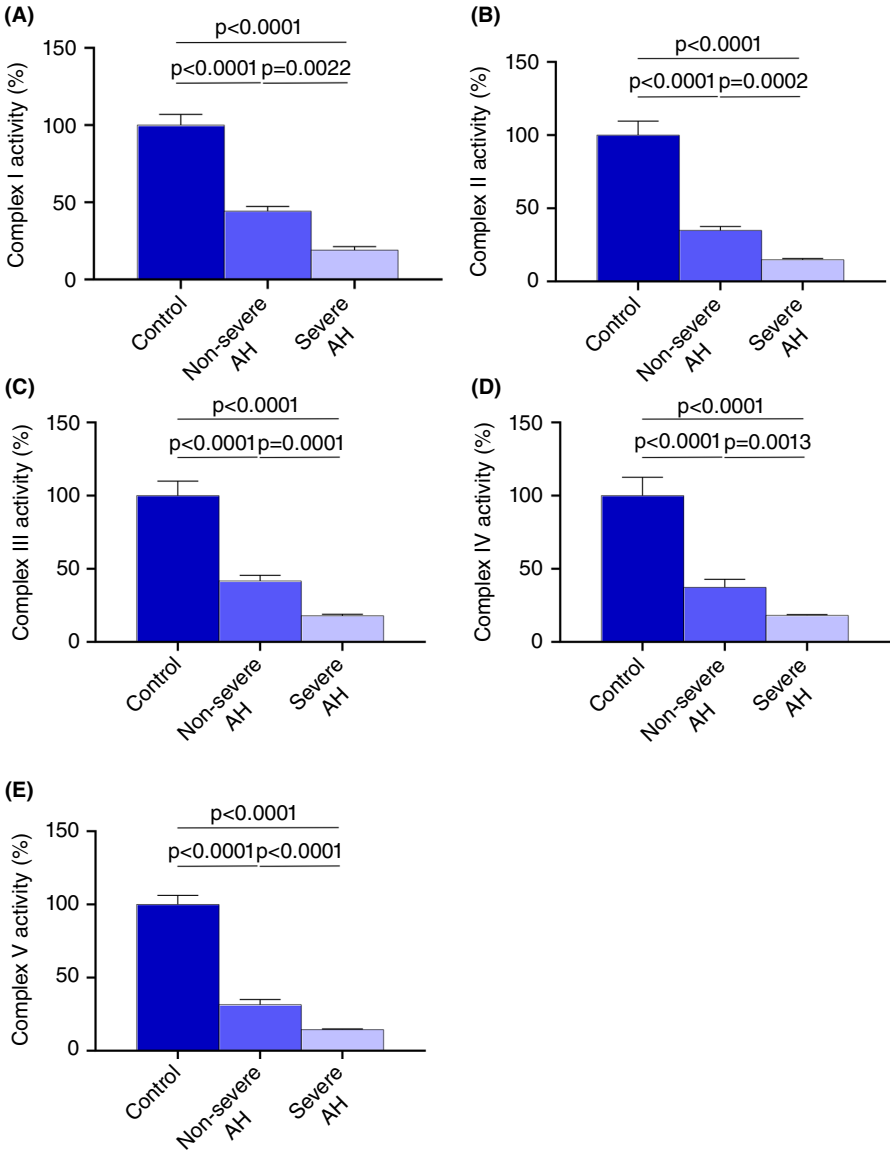

FIGURE 1 Hepatic MRC activity is decreased in patients with alcoholic hepatitis. (A–E) show complex I–V activity in control subjects ($n = 12$), non-severe ($n = 17$) and severe AH patients ($n = 81$). Bars represent mean ± SEM of the values. p values are shown on the top of the graphs.

$r = 0.3950$, $p = 0.0214$; CIV: $r = 0.0651$, $p = 0.4003$; CV: $r = 0.0847$, $p = 0.7832$) (Figure 3 and Figure S3). As expected, a negative correlation was observed between the therapeutic response to corticosteroids and mortality at all-time points studied (28-day: $r = -0.8014$, $p = 0.0010$; 3-month: $r = -0.8388$, $p = 0.0003$; 6-month: $r = -0.6648$, $p = 0.0007$) (Figure S4).

3.5 | Relationship between MRC activity and the prognosis of AH patients

Similar results were found regarding mortality: Only the activities of complex I and III significantly correlated with the mortality rate at all-time points studied (28-day: CI: $r = -0.8316$, $p = 0.0004$, and CIII: $r = -0.6064$, $p = 0.0171$; 3-month: CI: $r = -0.8343$, $p = 0.0004$, and CIII: $r = -0.6315$, $p = 0.0206$; 6-month: CI: $r = -0.7195$, $p = 0.0056$, and CIII: $r = -0.7170$, $p = 0.0058$) (Figures 4 and 5), while no correlation between the activities of complex II and IV and the mortality was found (Figure S5). Indeed, by using univariate and multivariate logistic regression analysis, we noted that MELD score (OR, 1.40; 95% CI: 1.15–1.71, $p = 0.001$), corticosteroids response (OR, 6.81; 95% CI: 1.46–31.82, $p = 0.015$) and complex I activity (OR, 6.03; 95% CI: 1.15–31.69, $p = 0.034$) were significantly associated with the 3-month mortality rate in patients with severe AH (Table 2). Interestingly, in addition to the MELD score (OR, 1.19; 95% CI: 1.02–1.39, $p = 0.027$) and corticosteroids response (OR, 20.35; 95% CI: 4.18–98.92, $p < 0.001$), complex III activity (OR, 4.70; 95% CI: 1.06–20.78, $p = 0.041$) was significantly associated with the 6-month mortality rate in patients with severe AH (Table 3).

4 | DISCUSSION

To our knowledge, this is the first prospective study determining the MRC enzymatic activity in the liver tissue of patients with

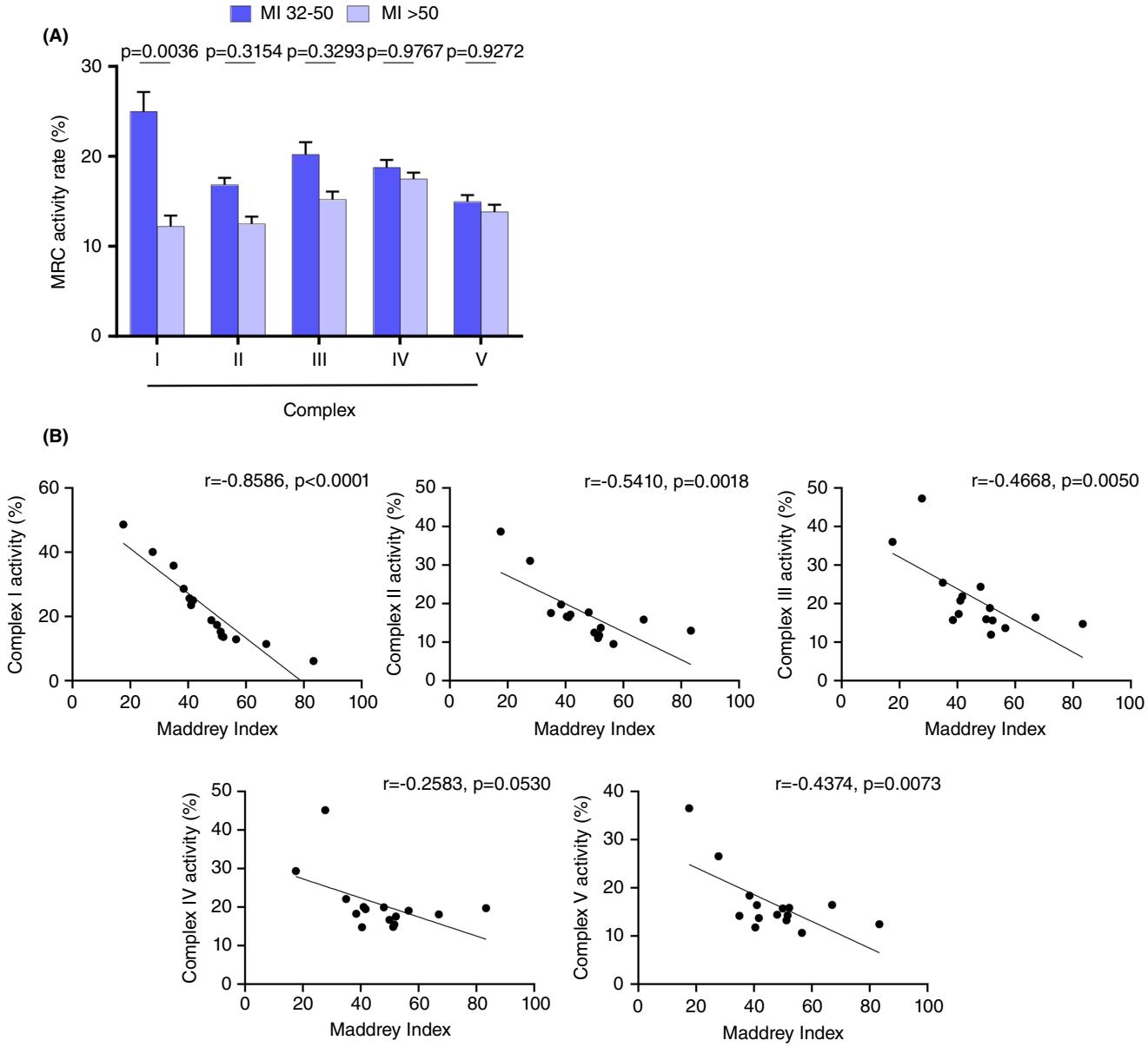

FIGURE 2 MRC complexes activity was lower in patients with higher Maddrey's index ($n = 98$). (A) Complex I–V activities in AH patients stratified according to Maddrey Index (MI). (B) Correlations between Maddrey Index and MRC activity. Bars represent mean ± SEM of the values. *p* values are shown on the top of the graphs.

biopsy-proven AH. We show herein that the activity of the different complexes of the MRC was significantly decreased in patients with AH compared with that observed in subjects with NL. Moreover, we found that a lower activity of each MRC complex correlated significantly with a higher severity of AH, measured by both Maddrey's index and MELD score, and with a poorer therapeutic response to corticosteroids. In addition, our study shows that a decreased activity of complexes I and III was significantly associated with an elevated mortality rate at all-time points.

It has been previously reported that mitochondrial morphology and function are affected in different ways in distinct chronic liver diseases, such as ALD[8,26–28] and non-alcoholic fatty liver disease (NAFLD).[29,30] Regarding ALD, only a few studies have directly measured the activity of the MRC enzymatic complexes in the liver tissue of ALD animal models[31–33] and indirectly in humans by measuring the decarboxylation of ketoisocaproate by a breath test.[34] Noteworthy, MRC dysfunction has been reported in NAFLD patients as well,[35] but the impairment of the activity of MRC complexes was less dramatic than we observed in the present study. Notably, the direct assessment of MRC activity in liver samples from animal models of ALD showed contradictory results, with opposite findings in mice and rats.[32,33] These discrepancies could be related to the animal species used, to the way alcohol-related liver damage was induced in the model and even to the different histologic forms of ALD studied. In this regard, the ALD animal models used in these studies resemble the histological features of chronic forms of alcohol-related liver injury rather than AH. One of the major strengths of the present study is that the study population comprised a large cohort of patients

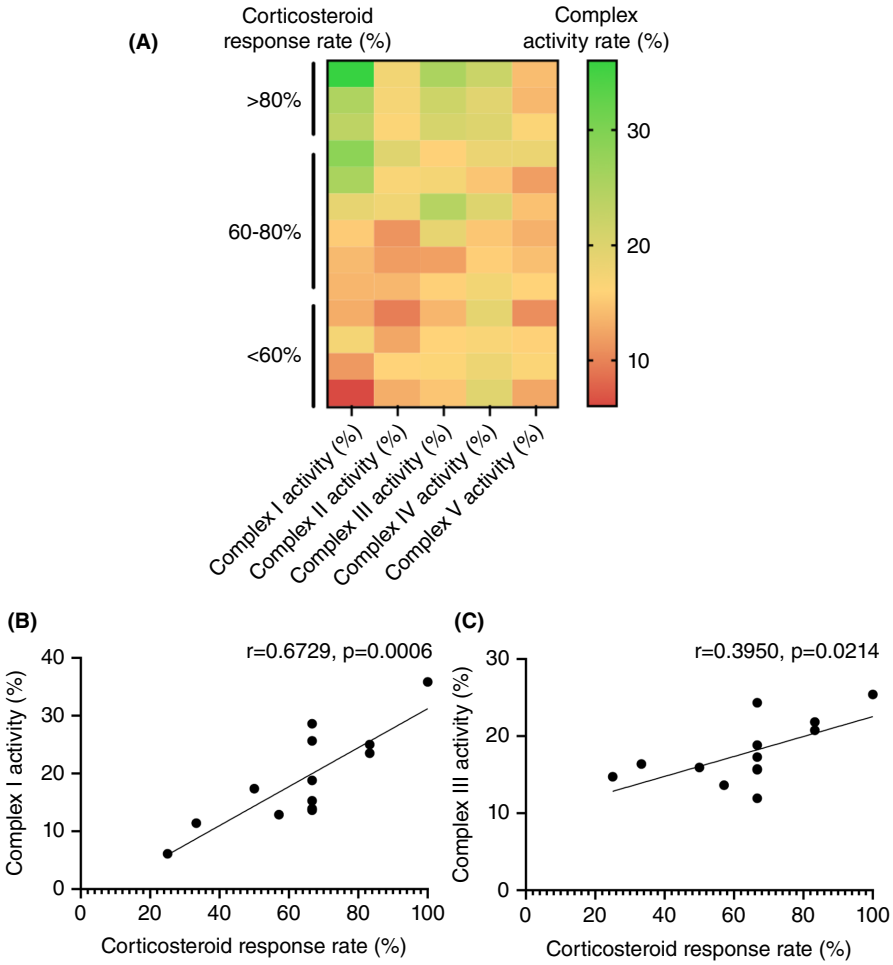

FIGURE 3 Activities of complex I and III correlated positively with the therapeutic response to corticosteroids in severe AH patients ($n = 81$).
(A) Heatmap of MRC activity according to the corticosteroids response rate. Red colour shows lower complex activity, while green shows higher.
(B, C) Correlations between the corticosteroids response rate and mitochondrial complex I and III activities.

fulfilling most clinical, biochemical and histologic features of AH and a control group of subjects with histologically normal livers. In addition, a relevant novelty of our study is that measurements of the activity of MRC complexes were carried out in pooled liver tissue samples from well-characterised AH patients stratified on the basis of validated scores such as Maddrey's index and MELD score.

The present study has, however, some limitations. Causal interpretations of the role of mitochondrial dysfunction in the pathogenesis and outcome of AH cannot be drawn from a clinical cross-sectional study. Since this is not a longitudinal study, we could not detect dynamic changes in MRC activity at different points in time during the clinical course of the disease. We could clinically justify performing one liver biopsy to establish the diagnosis and for prognostic reasons. However, a second biopsy would not be appropriate from an ethical standpoint due to the associated risk of bleeding. Also, the scarce amount of liver tissue obtained in each biopsy and the need to generate pools of patients for the determination of MRC activity did not allow for more detailed mechanistic bioenergetic or metabolomic studies. Therefore, further experimental studies with patients and using validated animal models of AH would be needed to elucidate the molecular mechanisms underlying the impairment of the activity of MRC complexes linked to AH and to determine the potential impact of mitochondrial dysfunction in the clinical progression of AH to severe forms of liver failure and death.

An interesting finding of our study is that the decreased activity of the different MRC complexes correlated significantly with the severity of AH. Notably, a significant negative correlation between MRC activity and disease severity was also described in an animal model of diet-induced NAFLD.[36] It seems logical that since the histopathologic findings of ALD and NAFLD are quite similar, these liver diseases could share molecular pathophysiological mechanisms, including MRC dysfunction, which leads to lipid peroxidation promoting hepatocyte death, inflammation and fibrogenesis.[37] It is also conceivable to hypothesise that the activity level of each MRC complex is dynamic and could decrease over time during AH, thus contributing to fatal outcomes. However, since we only performed liver biopsies at baseline, we cannot determine whether dynamic changes in the activity of the MRC complexes actually exist during the course of an alcoholic hepatitis episode.

The data of our study also provide convincing evidence showing a significant correlation between a lower MRC complex I and III activity and a poorer response to corticosteroids. Further studies, however, are required to assess whether this positive correlation between MRC dysfunction and lack of response to corticosteroids is actually due to a direct effect of MRC modulating the pharmacologic actions of steroids or is merely an epiphenomenon related to the severe clinical hallmark featuring those AH patients with lower MRC activity. A recent study assessed the phenotypic profile of

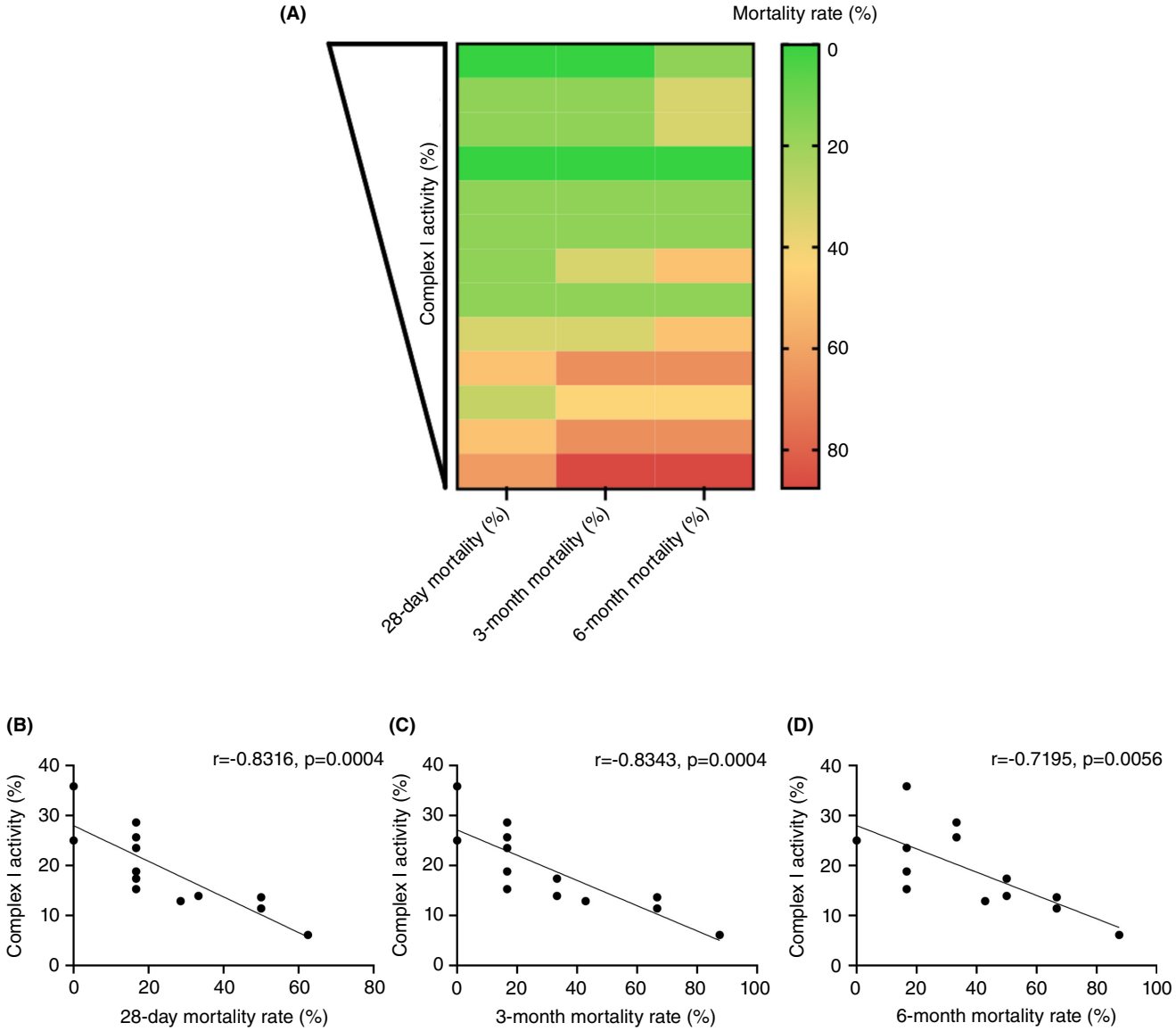

FIGURE 4 Complex I activity correlated negatively with the mortality rate in severe AH patients ($n = 81$). (A) Heatmap of 28-day, 3- and 6-month mortality according to complex I activity. Red colour shows higher mortality rate while green shows lower. (B–D) Correlations between mortality rate at different time-points and MRC complex I activity.

peripheral blood mononuclear cells in severe AH patients, detecting overexpression of MRC genes in corticosteroid non-responders.[38] These results are in line with ours. A compensatory up-regulation of MRC genes in corticosteroid non-responders is plausible and could be due to the impaired MRC activity we found in AH patients who do not respond to this therapy.

Using multivariate logistic regression analysis, we found that the only MRC complexes capable of predicting mortality at 3 and 6 months were complexes I and III, respectively. The significant negative correlation between the activity of these two specific MRC complexes and the mortality rate of AH patients studied might be explained by the effect of TNFa, which damages these two MRC complexes preferentially and can release cytochrome c from mitochondria, promoting hepatocyte apoptosis.[37,39] Moreover, it has been described in ALD animal models that electrons can predominantly accumulate in complexes I and III due to the imbalance between a high electron input and a restricted outflow. These electrons react with oxygen to form the superoxide anion radical, a highly reactive oxygen species leading to further mitochondrial dysfunction and cell death. Superoxide anion and other reactive oxygen species can lead to progressive mitochondrial injury through different mechanisms, including mitochondrial DNA damage, the enhancement of lipid peroxidation-related mitochondrial damage, increased hepatic TNFα formation and depletion of mitochondrial and cellular antioxidants. MRC dysfunction leads to a vicious cycle in which more reactive species are formed. Increased amounts of TNFα and Fas ligand can also induce apoptosis mediated by mitochondrial membrane permeability.[37] Despite our interesting findings, a limitation of these measurements in clinical practice is the amount of liver tissue needed for the experiments, as previously mentioned. Other methods for estimating

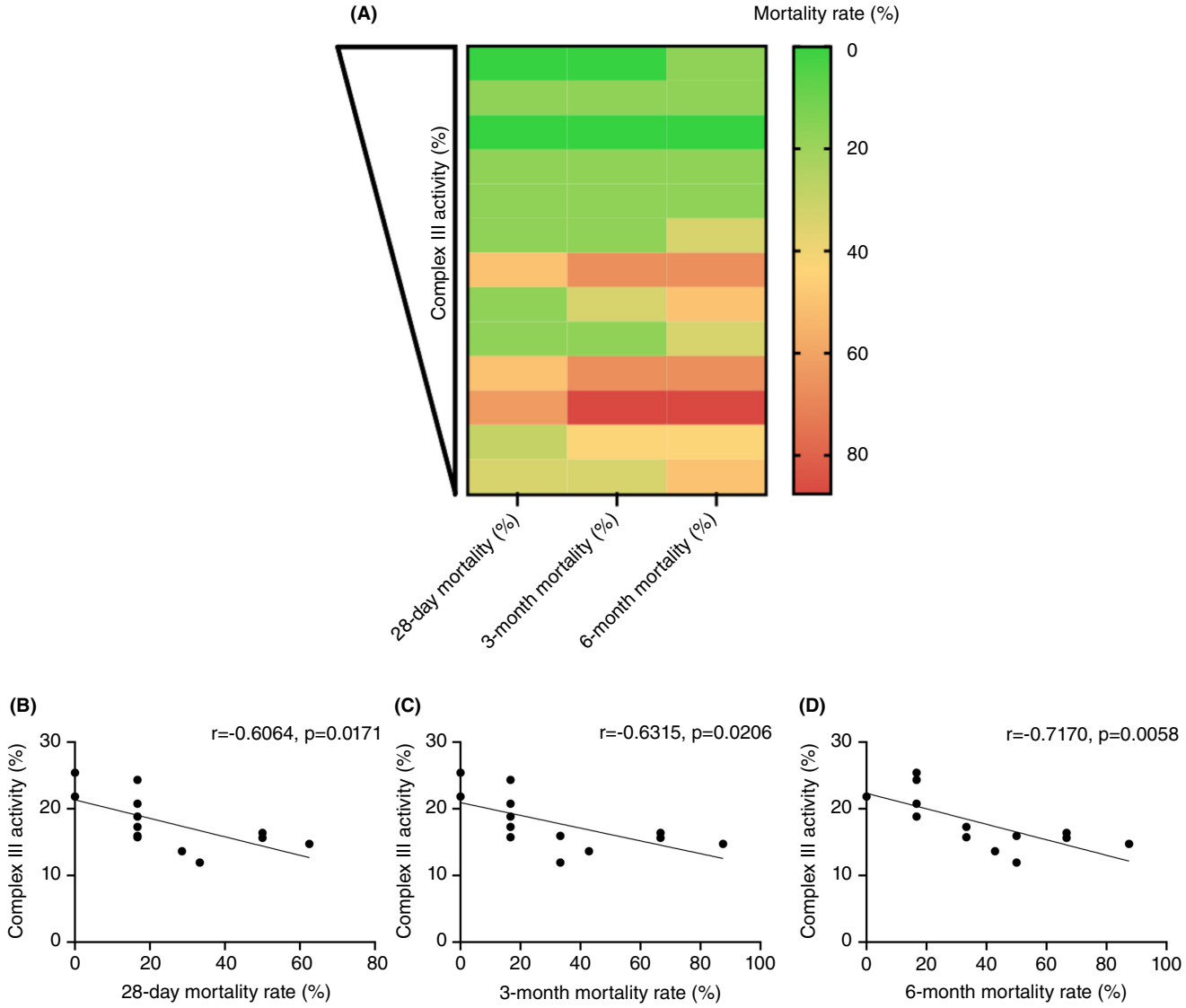

FIGURE 5 Complex III activity correlated negatively with the mortality rate in severe AH patients ($n = 81$). (A) Heatmap of 28-day, 3- and 6-month mortality according to complex III activity. Red colour shows higher mortality rate, while green shows lower. (B–D) Correlations between mortality rate at different time-points and MRC complex III activity.

TABLE 2 Univariate and multivariate logistic regression analysis of the independent variables associated with 3-month mortality in patients with severe AH ($n = 81$).

	Univariate analysis		Multivariate analysis	
Independent variables	**OR (95% CI)**	***p* value**	**OR (95% CI)**	***p* value**
Age (years)	0.97 (0.93–1.02)	0.216		
Sex (male/female)	0.55 (0.16–1.88)	0.339		
MELD score	1.48 (1.25–1.75)	<0.001	1.40 (1.15–1.71)	0.001**
Corticosteroid response (n/y)	17.50 (5.51–55.58)	<0.001	6.81 (1.46–31.82)	0.015*
Complex I activity (below/above median)	7.00 (2.40–20.39)	<0.001	6.03 (1.15–31.69)	0.034*

Abbreviations: CI, confidence interval; OR, odds ratio.

*$p < 0.05$.

**$p < 0.01$.

MRC activity need less tissue but do not provide the direct activity of the five mitochondrial respiratory complexes we wanted to obtain. However, comparing our results with those obtained with other methods of assessing MRC activity is an exciting idea. Depending on those results, estimating MRC activity with smaller amounts of liver tissue in selected patients might be feasible.

TABLE 3 Univariate and multivariate logistic regression analysis of the independent variables associated with 6-month mortality in patients with severe AH ($n = 81$).

Independent variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age (years)	0.96 (0.92–1.00)	0.071	0.96 (0.90–1.03)	0.225
Sex (male/female)	0.57 (0.18–1.81)	0.342		
MELD score	1.34 (1.17–1.53)	<0.001	1.19 (1.02–1.39)	0.027*
Corticosteroid response (n/y)	31.43 (9.02–109.50)	<0.001	20.35 (4.18–98.92)	<0.001***
Complex III activity (below/above median)	4.14 (1.60–10.72)	0.003	4.70 (1.06–20.78)	0.041*

Abbreviations: CI, confidence interval; OR, odds ratio.

*$p < 0.05$.

***$p < 0.001$.

Also, future studies should investigate MRC activity in more accessible cells. For example, assessing a possible correlation between complex I activity in hepatocytes and leucocytes or keratinocytes would be interesting since measuring MRC activity in these easily obtainable cells could be useful in clinical practice.

Finally, finding non-invasive markers of mitochondrial respiratory chain dysfunction in the blood is also interesting. The simplest option would be determining levels of lipid peroxidation products like malondialdehyde, cellular antioxidants like glutathione, markers of DNA oxidative damage like 8-hydroxy-2′-deoxyguanosine (8-OHdG) or ROS in the patient's serum. However, these markers are abnormal in many liver diseases and do not correlate directly with MRC dysfunction. Other markers of mitochondrial function, like lactate, pyruvic acid, fibroblast growth factor-21, growth differentiation factor-15 or creatine kinase, are also non-specific. According to our study's results, novel non-invasive markers specific to MRC dysfunction are warranted for alcoholic hepatitis.

Despite important efforts made in the past decade, there are insufficient advances in the study of mitochondrial dysfunction in ALD and even fewer in AH. The striking findings shown herein prompt us to perform further investigations searching for factors affecting mitochondrial function in patients with AH. In addition, future research will need to elucidate whether factors modifying MRC activity could be potentially used as non-invasive diagnostic biomarkers or therapeutic targets. In conclusion, the results of the present study indicate that the activity of the MRC complexes is markedly decreased in patients with AH, and, particularly, the activity of complexes I and III is significantly associated with the severity and prognosis of these patients.

AUTHOR CONTRIBUTIONS

Pablo Solís-Muñoz: Conceptualization (equal); formal analysis (equal); resources (equal); software (equal); writing – original draft (lead); writing – review and editing (lead). **María de la Flor-Robledo:** Formal analysis (supporting); resources (lead); writing – review and editing (supporting). **Inmaculada García-Ruíz:** Formal analysis (supporting); resources (lead); writing – review and editing (supporting). **Carlos Ernesto Fernández-García:** Formal analysis (lead); software (lead); writing – review and editing (equal). **Águeda González-Rodríguez:** Formal analysis (equal); writing – original draft (supporting); writing – review and editing (lead). **Naina Shah:** Formal analysis (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Ramon Bataller:** Formal analysis (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Michael Heneghan:** Formal analysis (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Carmelo Garcia-Monzon:** Writing – original draft (supporting); writing – review and editing (lead). **José Antonio Solís-Herruzo:** Conceptualization (equal); formal analysis (supporting); resources (equal); writing – original draft (equal). All the authors have actively discussed the manuscript and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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