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ORIGINAL RESEARCH—CLINICAL

Low Baseline but Not Delta Cortisol Relates to 28-Day Transplant-Free Survival in Acute and Acute-on-Chronic Liver Failure

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BACKGROUND AND AIMS: The clinical, prognostic, and therapeutic impact of adrenal insufficiency in acute liver failure (ALF) and acute-on-chronic liver failure (ACLF) remains controversial and exact diagnostic criteria are lacking. We sought to determine the diagnostic and therapeutic value of cortisol measurement and glucocorticoid (GC) treatment in ALF and ACLF. **METHODS:** 28-day transplant-free survival (TFS) was studied in relation to absolute cortisol concentrations and to GC treatment in ALF (n = 30) and ACLF (n = 34) patients. Cortisol concentrations and short synacthen test were assessed by chemiluminescence immunoassay and liquid chromatography-mass spectrometry. Clinicians decided independently on GC treatment. In relation, phenotypic and functional characteristics of circulating monocytes were assessed. **RESULTS:** In ALF, baseline cortisol concentrations <387 nmol/L predicted TFS (sensitivity 83%, specificity 53%). In ACLF, baseline cortisol <392 nmol/L correlated with TFS (sensitivity 80%, specificity 61%). In both, ALF and ACLF, GC treatment did not influence 28-day TFS in patients with low baseline cortisol. However, in patients with baseline cortisol exceeding 387 and 392 nmol/L, respectively, TFS was higher if they had been treated with GC. High baseline cortisol was associated with low HLA-DR expression on monocytes. **CONCLUSION:** Our data suggest a prognostic value of baseline cortisol measurement in ALF and ACLF. Overall, strong activation of the hypothalamic–pituitary–adrenal axis indicated poor prognosis. Furthermore, baseline cortisol deserves prospective evaluation as a guide for GC treatment decision-making.

Keywords: Critical illness-related corticosteroid insufficiency (CIRCI); Adrenal insufficiency (AI); Acute-on-chronic liver failure (ACLF); HLA-DR

Introduction

Acute liver failure syndromes, consisting of acute liver failure (ALF) and acute-on-chronic liver failure (ACLF), are characterized by rapidly progressive hepatic dysfunction with subsequent multiorgan failure and high short-term mortality.[1,2] Pathophysiologically, increased concentrations of circulating damage- and pathogen-associated molecular patterns, inflammatory cytokines and altered immune cell differentiation and function are found in septic shock as well as in acute liver failure syndromes.[3,4]

In acute illness, activation of the hypothalamic–pituitary–adrenal (HPA) axis leads to increased circulating cortisol concentrations, which is vital for maintaining cellular and organ homeostasis.[5] Loss of the circadian rhythm of cortisol secretion is observed[6] and the degree of HPA axis activation relates to the degree of stress.[7,8] Adrenal insufficiency (AI) in this setting is referred to as critical illness-related corticosteroid insufficiency (CIRCI) and is associated with increased morbidity and mortality.[9] Its estimated overall prevalence ranges from 10% to 20% in critically ill medical patients to 60% in septic shock.[6] Among the spectrum of liver diseases, AI has been described with remarkable frequency.[4,10,11] However, previous data provided conflicting results on the mechanisms,

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Abbreviations used in this paper: ACLF, acute-on-chronic liver failure; AI, adrenal insufficiency; ALF, acute liver failure; APACHE II, Acute Physiology and Chronic Health Evaluation II; CBG, cortisol binding globulin; CIRCI, critical illness-related corticosteroid insufficiency; CLIA, chemiluminescence immunoassay; CLIF-SOFA, Chronic Liver Failure–Sequential Organ Failure Assessment; DILI, drug-induced liver injuries; GC, glucocorticoid; HPA, hypothalamic–pituitary–adrenal; LC-MS, liquid chromatography mass spectrometry; SAPS II, Simplified Acute Physiology Score II; SST, short synacthen test; TFS, transplant-free survival.

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diagnosis, prevalence, clinical and prognostic implication, and therapeutic management of AI in patients with liver diseases.

In nonstressed subjects, AI is defined by baseline cortisol <140 nmol/L or peak cortisol after the short synacthen test (SST, 250 μg Synacthen) <500–550 nmol/L.[12,13] According to The Society of Critical Care Medicine and European Society of Intensive Medicine CIRCI can either be diagnosed by delta cortisol <250 nmol/L or random plasma cortisol <275 nmol/L.[9] Normally, 90%–95% of circulating cortisol is bound to cortisol binding globulin (CBG) and albumin, synthesized in the liver. The remaining free cortisol exerts physiologic activity.[6] In liver failure, AI might be overestimated when measuring total cortisol concentrations, while free cortisol remains within the normal range.[14] Therefore, some experts suggest measuring free instead of total cortisol in patients with hepatic dysfunction.

For decades, chemiluminescence immunoassay (CLIA) has been the standard method for cortisol assessment due to low costs and technical ease.[15] However, this method is limited by variable and unpredictable cross reactivity with structurally related steroids. Liquid chromatography mass spectrometry (LC-MS) is an analytical technique characterized by superior specificity and is nowadays widely recommended as the method of choice for assessment of steroid hormones.[15]

Here, we sought to assess the diagnostic value of cortisol measurement using CLIA and LC-MS, and the therapeutic effect of glucocorticoid (GC) treatment, in patients with ALF and ACLF.

Material and Methods

Objectives

We primarily evaluated 28-day transplant-free survival (TFS) and secondarily the development of secondary infectious complications and 3 months TFS.

Patients

Between January 2013 and November 2014, we recruited 64 patients with acute liver failure syndromes within 24 hours after admission to Liver Intensive Therapy Unit at King's College Hospital. Patients were categorized into ALF and ACLF groups. ACLF was defined using the Chronic Liver Failure–Sequential Organ Failure Assessment (CLIF-SOFA) classification.[16] Compensated cirrhotic patients served as a control group for ACLF patients. Exclusion criteria were age <18 years, known disorder of the HPA axis, neoplasia, and immunosuppressive therapy. Patients with poor prognostic criteria were identified for emergency transplantation after multidisciplinary team discussion. Candidates were followed until hospital discharge, liver transplantation, or death. The study was approved by the King's College Hospital Ethics Committee (12/LO/0167). Assent was obtained by the nominated next of kin if patients were unable to provide informed consent themselves.

Clinical, Hematologic, and Biochemical Parameters

Vital signs, sedation requirements, mechanical ventilation, renal replacement, medication, evidence of infections, underlying liver disease, cause of acute hepatic injury, concomitant diseases, emerging complications, blood count, INR, biochemistry, and blood gas analysis were prospectively entered into a database. Child–Pugh, model of end-stage liver disease, CLIF-SOFA,[16] North American Consortium for Study of End-stage Liver Disease,[17] Acute Physiology and Chronic Health Evaluation II (APACHE II), Simplified Acute Physiology Score II (SAPS II), and SOFA were calculated.

Assessment of Corticosteroid Hormones

Baseline serum cortisol was assessed by immunoassay (CLIA, Siemens Centaur XPi) and LC-MS as previously described.[18] In a subset of patients, additional steroid hormones were measured by LC-MS: cortisone, 11-deoxycortisol, 17-hydroxyprogesterone, androstenedione, and corticosterone. CBG and albumin concentrations (Siemens Advia 2400) were analyzed.

SST using 250 μg adrenocorticotrophic hormone intravenously (Synacthen, Atnahs Pharma Ltd, Basildon, UK) was performed within 24 hours of admission with total cortisol measurement before, after 30 and 60 minutes by CLIA. Peak cortisol was defined as the maximum cortisol concentration within 60 minutes and delta cortisol as the difference between peak and baseline cortisol.

Absolute baseline cortisol concentrations were related to 28-day TFS rates in the ALF, ACLF, and the whole liver failure (LF) cohort, respectively. Criteria to predict TFS were calculated using area under the receiver operating characteristic curves. Additionally, we applied 5 different previously published and commonly used definitions for AI (Table A2): baseline cortisol <275 nmol/L[9]; baseline cortisol <414 nmol/L[5]; peak cortisol <550 nmol/L[19]; delta cortisol <250 nmol/L and baseline cortisol >275 nmol/L[9,20,21]; delta cortisol <250 nmol/L and baseline cortisol >414 nmol/L.[9,20,21]

Glucocorticoid Treatment

Baseline cortisol and SST results could be retrieved, yet the responsible clinicians decided independently, whether to administer glucocorticoids (GCs) or not. Treatment consisted of intravenous hydrocortisone 50 mg TDS or QDS or oral prednisolone 40 mg daily.

Monocyte Differentiation

Monocyte phenotyping, TNF-α/IL-6 production upon lipopolysaccharide treatment, phagocytosis, and oxidative burst capacity were determined by flow cytometry as previously described.[22,23] Flow cytometry data were analyzed using Flowlogic software (Inivai Technologies, Mentone, Australia).

Statistical Analysis

For data analysis and graphing, we used Prism 9 (GraphPad Software, La Jolla, CA) or MedCalc Statistical Software version 19.1.5 (MedCalc Software bv, Ostend, Belgium). For data that

Table 1. Clinical Baseline Characteristics of Patients With Acute Liver Failure, Acute-on-Chronic Liver Failure, or Stable Cirrhosis

Variables (median, range)	ALF (n = 30)	ACLF (n = 34)	Stable cirrhosis (n = 8)
Age (y)	34.31 (18–57)***	51 (19–70)	54.27 (36–65)
Sex (m:f)	10:20**	22:12	7:1
Underlying liver disease(s) [%]	NA	ALD 15 [44.1] NAFLD4 [11.8] Viral hepatitis (HCV)1 [2.9] Other12 [35.3] Unknown 2 [5.9]	ALD 6 [75] Viral hepatitis (HCV) 1 [12.5] M. Wilson 1 [12.5]
Cause of acute liver failure [%]	APAP induced DILI 18 [60] DILI 5 [16.7] Budd Chiari 1 [3.3] Acute HBV 1 [3.3] Acute M. Wilson 1 [3.3] DKA 1 [3.3] Acute fatty liver of pregnancy 1 [3.3] HELLP 1 [3.3] Unknown 1 [3.3]	Gastrointestinal bleeding 14 [41.2] Infection 7 [20.6] Alcohol consumption 2 [5.9] Ischemic hit 2 [5.9] Bile duct obstruction 1 [2.9] New onset of AIH 1 [2.9] Intraabdominal bleeding 1 [2.9] Unknown 6 [17.6]	NA
Death (28 d)	4*	12	0#
Death (3 mo)	5*	14	0#
OLT (3 mo)	8	8	1
MAP (mmHg)	78 (64–102)	73 (56–109)	79 (73–113)
Use of vasoactive agents [%]	16 [53.3]	22 [64.7]	0###
Mechanical ventilation [%]	17 [56.7]	15 [44.1]	0
Bilirubin (μmol/L)	76.5 (11–786)	112 (23–603)	58 (13–121)##
AST (U/L)	5352 (210–15,560)***	110 (28–8896)	49 (31–368)
GGT (U/L)	81 (18–451)	47 (11–592)	83 (19–2423)
AP (U/L)	107 (16–235)	114 (29–335)	114 (55–765)
Albumin (g/L)	26 (14–33)	24 (14–36)	32 (26–39)
CBG (g/L)	20	25	-
INR	5.12 (1.08–15.0)***	1.97 (1.25–3.78)	1.44 (1.01–1.95)##
Ammonia (μmol/L)	70 (22–188)	65 (20–124)	89 (88–90)
Grade III-IV HE	9	7	0##
Creatinin (μmol/L)	144 (42–462)	110 (20–331)	61 (50–116)
Sodium (mmol/L)	139 (121–150)	138 (119–147)	138 (134–143)
Renal support (cvvHDF) [%]	23 [76.7]	17 [50]	0#
Fasting glucose (mmol/L)	6.3 (2.2–10)	6.4 (3.5–12.6)	5.7 (4.7–6.7)
PaO2/FiO2	321 (176–467)*	261 (138–480)	-
WBC (G/L)	11.69 (3.2–23.06)*	8.265 (1.3–22.03)	5.95 (3.22–13.5)
Neutrophils (G/L)	8.97 (2.61–21.87)*	6.595 (0.92–18.17)	3.455 (2.02–10.96)
Monocytes (G/L)	0.29 (0.08–1.96)	0.475 (0.1–2.22)	0.485 (0.28–0.68)
Lymphocytes (G/L)	0.79 (0.17–2.42)	0.95 (0.17–2.5)	1.7 (0.88–2.26)
Platelets (G/L)	95 (20–392)	84 (7–246)	102 (66–486)
APACHE II	19 (3–34)*	22 (9–35)	-
SAPS II	40 (9–69)	34.5 (13–54)	-
SOFA	13 (5–18)	13 (9–19)	-
MELD	40 (21–40)***	28.5 (12–40)	15.5 (7–20)###
Child–Pugh score	-	12 (10–15)	9 (6–11)###
CLIF-SOFA score	-	13 (7–19)	4.5 (1–7)###
NACSELD score	-	1 (0–4)	0 (0–1)##

Table 1. Continued

Variables (median, range)	ALF (n = 30)	ACLF (n = 34)	Stable cirrhosis (n = 8)
Baseline cortisol (CLIA) (nmol/L)	407 (52–1800)	427 (221–1800)	316.5 (200–913)
Time of baseline cortisol (CLIA)	10:00 (00:00–17:05)	10:30 (00:15–21:00)	11:28 (09:15–13:40)

Data indicated as median with minimum and maximum, $^{*}P = .05$, $^{**}P = .01$, $^{***}P = .001$ indicate ACLF vs ALF, $^{\#}P = .05$, $^{\#\#}P = .01$, $^{\#\#\#}P = .001$ indicate ACLF vs stable cirrhosis, comparisons by Mann-Whitney U tests.
ACLF, acute-on-chronic liver failure; AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALF, acute liver failure; AP, alkaline phosphatase; APAP, acetaminophen; AST, aspartate aminotransferase; CBG, corticosteroid-binding globulin; CLIA, chemiluminescence immunoassay; cvvHDF, continuous venovenous hemodiafiltration; DILI, drug-induced liver injury; DKA, diabetic ketoacidosis; GGT, gamma-glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HE, hepatic encephalopathy; HELLP, hemolysis elevated liver enzymes low platelet count; INR, international normalized ratio; MAP, mean arterial pressure; MELD, model of end-stage liver disease; NACSELD, North American Consortium for Study of End-stage Liver Disease; NAFLD, non-alcoholic liver disease; OLT, orthotopic liver transplantation; WBC, white blood cell.

did not follow a normal distribution, Mann–Whitney or Wilcoxon tests were applied. Proportions were compared using chi-square test. Correlations were analyzed using Spearman coefficients and area under the receiver operating characteristic curve. TFS was shown using Kaplan-Meier curves. *P*-value <.05 was considered statistically significant. Results are presented as median (interquartile range) unless otherwise specified.

Results

Patient Characteristics

N = 30 patients with ALF, n = 34 patients with ACLF, and n = 8 patients with compensated cirrhosis were included. Details on baseline characteristics are given in Tables 1 and A1. All ALF and ACLF patients were managed in intensive care. Sixteen (25.4%) died within 28 days and 19 (30.2%) within 3 months, respectively. Fifteen patients (23.8%) underwent liver transplantation within 28 days and 3 months, respectively.

Most frequent etiologies for ALF were acetaminophen overdose (n = 18, 60%) and other drug-induced liver injuries (DILI, n = 5, 16.7%). The main underlying diseases for ACLF were alcoholic liver disease (n = 15; 44.1%), and nonalcoholic liver disease (n = 4; 11.8%). The most frequent precipitating events were gastrointestinal bleeding (n = 14, 41.2%) and infection (n = 7, 20.6%). Alcoholic liver disease represented 75% (n = 6) among compensated cirrhosis patients.

Subsequent organ failure was frequent. Vasoactive drugs were needed in n = 16 (53.3%) ALF and n = 22 (64.7%) ACLF patients. Need for continuous venovenous hemofiltration was more frequent in ALF than ACLF (76.7% vs 50%, $P = .03$).

Cortisol Concentrations in Relation to 28-Day TFS Measured by CLIA

Median baseline cortisol concentrations measured by CLIA were similar among ALF and ACLF patients (407 vs 427 nmol/L) and not significantly higher in ACLF compared to compensated cirrhosis patients (427 vs 316.5 nmol/L; Table 1 and Figure 1A). In ALF, results of baseline cortisol and SST acquired by CLIA were available in 29 and 28 patients, respectively, baseline cortisol concentrations by LC-MS were assessed in 19 patients. In ACLF, baseline cortisol and SST results were obtained in 33 patients (CLIA) and baseline cortisol concentrations in 29 patients (LC-MS). Technical issues prohibited assessment of baseline cortisol by CLIA in 1 patient in each group. Cortisol increment following SST was significantly higher in ALF compared to ACLF ($P = .0014$, Figure 1A). Albumin and CBG concentrations were similar among ALF and ACLF patients (Figure 1A).

ALF patients with 28-day TFS had significantly lower baseline cortisol concentrations measured by CLIA ($P = .0083$) and cortisol concentrations < 387 nmol/L predicted TFS (sensitivity 91%, specificity 53%, $P = .0474$). Accordingly, 28-day TFS was significantly higher in ALF patients with baseline cortisol <387 nmol/L ($P = .039$, Figure 2A). Also in ACLF, baseline cortisol concentrations measured by CLIA were relatively lower in patients with 28-day TFS ($P = .0885$). Baseline cortisol concentrations <392 nmol/L were associated with 28-day TFS (sensitivity 80%, specificity 62%, $P = .0154$, Figures 1C and 2B). If we consolidate all patients with LF, a cortisol concentration <399 nmol/L predicted for 28-day TFS and TFS-survival rates were significantly higher in patients with baseline cortisol <399 nmol/L ($P = .017$, Figures 2C and A1). Hundred percent 28-day TFS was observed in compensated cirrhosis.

The different previously proposed definitions of AI were variably met in 7%–50% of ALF and in 9%–66% of ACLF patients reflecting the difficulty of diagnostic accuracy and the controversy of CIRCI (Table A2). Similarly, there was a heterogeneous association with 28-day TFS. In ALF, patients with AI defined as baseline cortisol <275 nmol/L had better 28-day TFS compared to patients without AI ($P = .06$, Figure A2A) which further supports our finding that low baseline cortisol is predictive for TFS. In ACLF, there was no clear association of 28-day TFS and any AI definition (Figure A2B and D). Interestingly, AI criteria were also met in 13%–75% of compensated cirrhosis patients (Table A2), however, there was no association with 28-day TFS. Overall, there was no difference in 28-day TFS when AI was defined by SST results (Figure A2C and D).

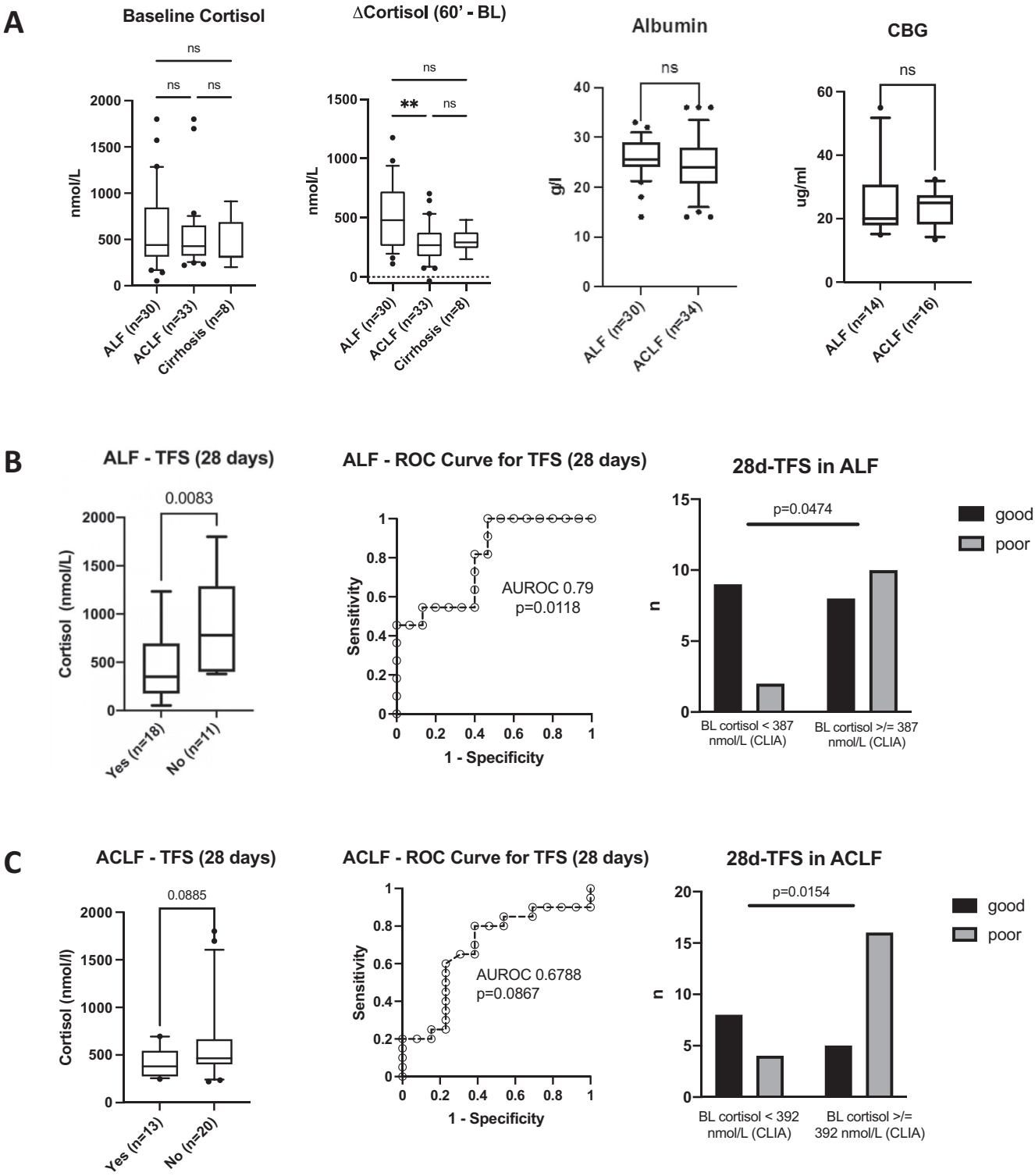

Figure 1. Cortisol concentrations and definitions of prognostic cortisol values. (A) Baseline and delta cortisol levels (nmol per liter) assessed by the short synacthen test in acute liver failure(ALF), acute-on-chronic liver failure(ACLF), and stable cirrhosis groups. Albumin and corticosteroid binding globulin levels (grams per liter and micrograms per liter, respectively) were similar in ALF and ACLF. (B, C) 28-day transplant-free survival (TFS) of patients with either ALF or ACLF in relation to baseline cortisol levels. Receiver operating characteristic (ROC) curve analysis for 28-day TFS with baseline cortisol value of 387 nmol per liter for ALF patients and 392 nmol per liter for ACLF patients, respectively. Bar plots displaying contingency analysis of good (28-day TFS) and poor (no 28-day TFS) outcomes for patients with baseline cortisol values below or above 387 nmol per liter in ALF and below or above 392 nmol per liter in ACLF, respectively. Results displayed as box plots showing median with 10–90 percentile and all points min-max unless stated otherwise. Comparisons by Mann-Whitney U tests, $^{*}P = .05$, $^{**}P = .01$, $^{***}P = .001$.

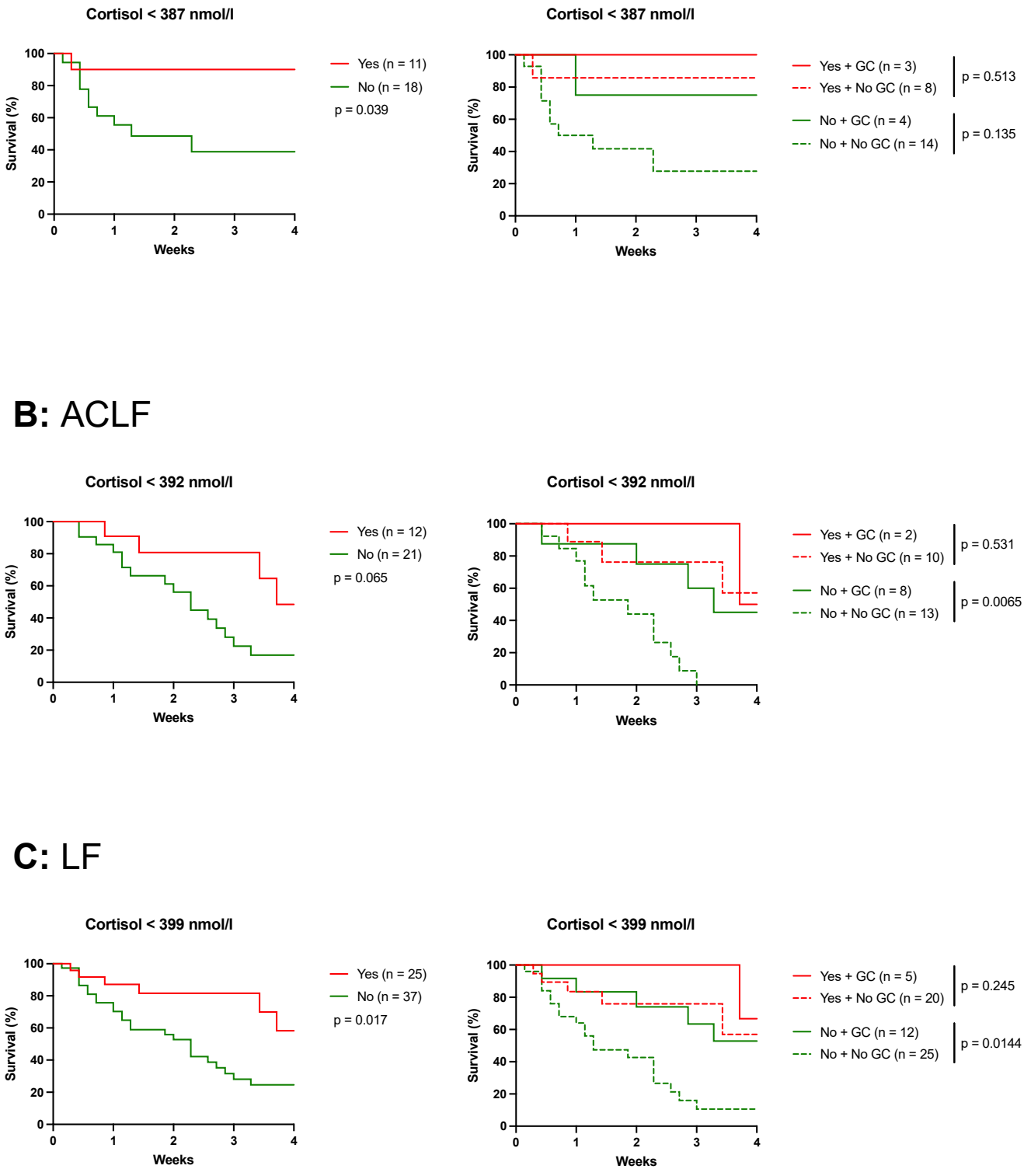

Figure 2. 28-day TFS in relation to prognostic cortisol values and effect of glucocorticoid treatment, Kaplan-Meier analysis of 28-day TFS for (A) ALF patients with baseline cortisol below 387 nmol per liter, (B) ACLF patients with baseline cortisol below 392 nmol per liter, and (C) Liver failure (LF) patients with baseline cortisol below 399 nmol per liter. Analogous Kaplan-Meier analysis with patients further stratified based on glucocorticoid treatment. Comparisons of TFS curves by log-rank tests, $P < .05$ indicates statistical significance.

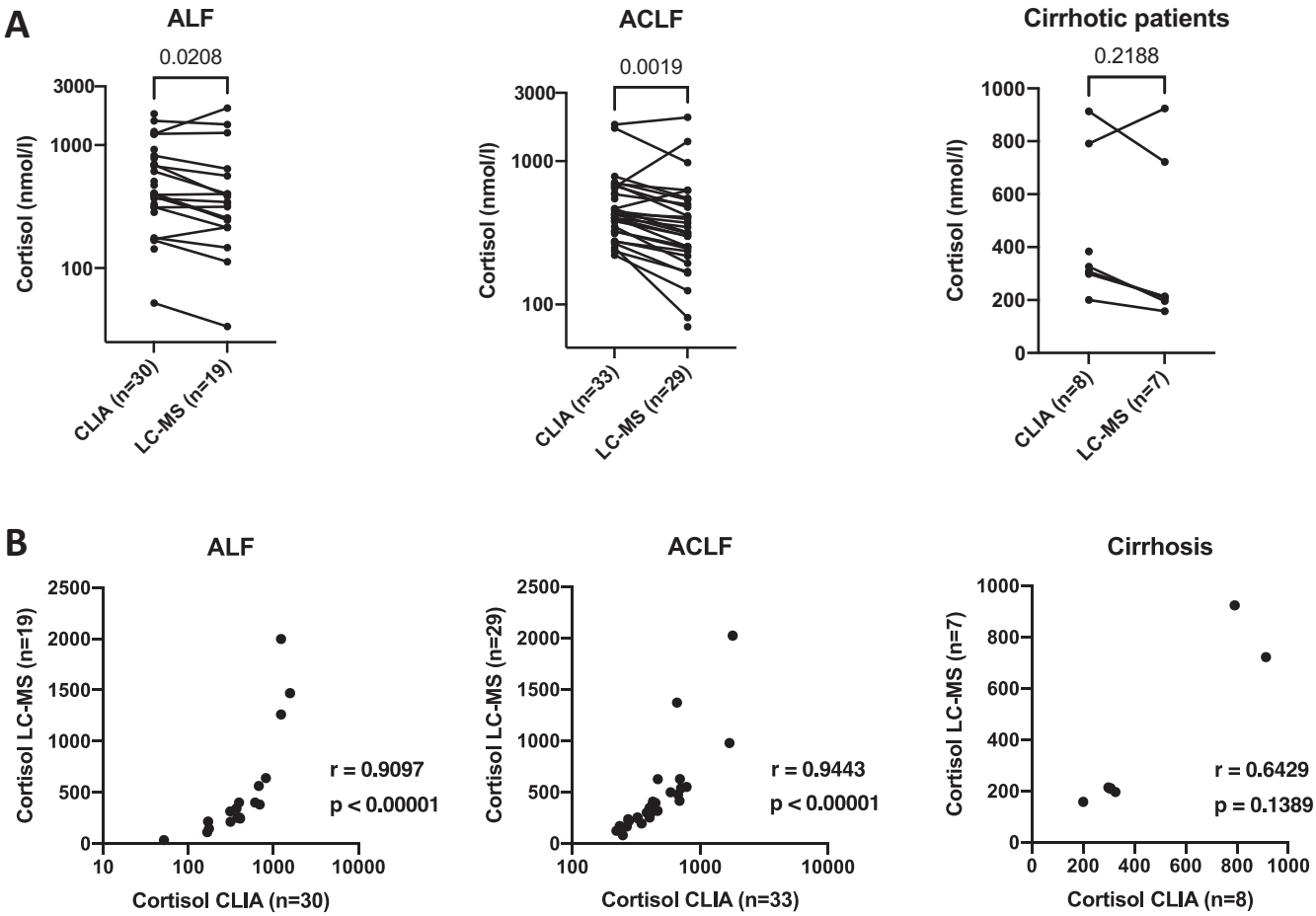

Figure 3. Comparison of cortisol quantification techniques. (A) Quantification of cortisol levels by both chemiluminescence immunoassay (CLIA) and liquid chromatography-mass spectrometry (LC-MS) within each subject of ALF, ACLF, and cirrhotic cohorts. Statistical significance assessed by Wilcoxon signed-rank test. (B) Scatterplots displaying cortisol values obtained from CLIA and LC-MS measurements, comparisons by Spearman r coefficient, $P < .05$ indicates statistical significance.

Steroid Hormone Assessment in Relation to 28-Day TFS Using LC-MS

In both, ALF and ACLF, baseline cortisol concentrations assessed by LC-MS were lower when compared with those assessed by CLIA (Figure 3).

Cortisol concentrations <230 nmol/L in ALF (sensitivity 100%, specificity 36%, $P = .09$) and <250 nmol/L in ACLF (sensitivity 81%, specificity 46%, $P = .11$) correlated with 28-day TFS, respectively. Baseline cortisol <227 nmol/L was predictive for survival in the consolidated LF cohort (Figure A3). Previous definitions of AI based on LC-MS were not distinctly associated with TFS in ALF and ACLF (Figure A4).

Similarly, low concentrations of steroid derivatives were associated with 28-day and 3-month TFS (Figure A5).

28-Day TFS in Relation to GC Treatment

In our cohort, GC treatment decision-making was not related to baseline cortisol values or the diagnosis of AI by any definition. GC treatment was initiated more frequently in patients with low delta cortisol (Table A2). Duration of GC treatment depended on clinician's decision (median time of hydrocortisone treatment 7 days, interquartile range 3–9 days). Seventy percent of patients treated with GCs had concomitant vascular hypotension.

In both, ALF and ACLF, GC treatment improved 28-day TFS in those patients with high baseline cortisol values (CLIA and LC-MS, Figures 2 and A3). Interestingly, GC treatment did not affect 28-day TFS in patients with low baseline cortisol concentrations. In regard to previous AI definitions, patients with AI defined by baseline cortisol treated with GCs had better survival rates, whereas no clear impact was found when AI definition was based on SST (Figures A2 and A4).

Disease Severity and Secondary Infection in Relation to AI

At admission, organ failure (vascular hypotension, renal failure, encephalopathy) and ascites were not associated with baseline cortisol cutoffs mentioned before or AI according to previous definitions in all cohorts (Table A3).

Occurrence of secondary infection was not associated with baseline cortisol below the newly identified cutoffs predictive for 28-day TFS or any previous AI definition

(Table A3B and D). However, ALF patients with secondary infections displayed numerically higher cortisol concentrations compared to patients without (CLIA, $P = .166$; LC-MS, $P = .1061$, Figure A6). Interestingly, ALF patients with baseline cortisol concentration >387 nmol/L and without AI according to most previous definitions who received GC treatment developed significantly more secondary infections compared to those without GC treatment (Table A3B).

In both, ALF and ACLF, APACHE II and SAPS II scores at admission were higher in patients with baseline cortisol concentrations >387 and >392 nmol/L, respectively, reflecting disease severity (Table A4). In ALF, APACHE II score appeared to correlate with baseline cortisol concentrations (CLIA and LC-MS) (Figure A7A). In ACLF, baseline cortisol (LC-MS) correlated positively with CLIF-SOFA, North American Consortium for Study of End-stage Liver Disease, and SAPS II (CLIA and LC-MS, Figure A7B). No correlation between baseline cortisol and SOFA, model of end-stage liver disease and Child–Pugh score was found among patients with ALF and ACLF, respectively.

Differentiation of Circulating Monocytes

Occurrence of HLA-DR_{low} M-MDSC has previously been linked to poor prognosis in liver failure syndromes.[23,24] In our study, baseline cortisol (CLIA and LC-MS) correlated negatively with HLA-DR expression on monocytes in ALF (CLIA, $P = .02$; LC-MS, $P = .018$) and ACLF (CLIA, $P = .011$; LC-MS, $P = .0056$; Figure 4). Vice versa, monocytes from patients with baseline cortisol <387 nmol/L (ALF), <392 nmol/L (ACLF) and AI as previously defined, respectively, had higher HLA-DR expression (Figure 4 and A8). In ACLF, we observed a negative correlation between baseline cortisol and monocytic IL-6 production in response to lipopolysaccharide (Figure A8B). We did not identify an association between cortisol concentrations and TNF-α production (Figure A8), phenotypic markers (MERTK, CD163, CX3CR1), phagocytosis or oxidative burst (data not shown).

Discussion

The present study evaluating the relationship between cortisol concentrations and short-term TFS in acute liver failure syndromes suggests that low cortisol concentrations predict 28-day TFS. Moreover, GC treatment was associated with improved prognosis independent of baseline cortisol values, while secondary infections under GC occurred more frequently in ALF patients with high baseline cortisol.

In the context of critical illness, adrenal response and cortisol production are incompletely understood and the definition of CIRCI and its therapeutic implications remain indeterminate. Previous studies applied variable AI definitions obtained in healthy individuals[7] and revealed controversial results in liver disease.

Consistent with our data, Marik et al[10] reported significantly higher cortisol concentrations in nonsurviving patients with fulminant liver failure, chronic liver disease, and post liver transplantation compared to survivors. Additionally, patients with AI had significantly lower mortality rates compared with patients without AI (38.8% vs 55.9%, $P = .005$).[10] Further, high cortisol levels were identified as an independent predictor of disease severity and mortality in pneumonia.[25] In septic shock, Annane et al[21] reported highest mortality rates in patients with baseline cortisol concentrations >938 nmol/L and delta cortisol <250 nmol/L. This finding is in line with our data, where all 3 patients fulfilling these criteria died within 23 days. In contrast, Harry et al[11] described higher incidence of AI (baseline cortisol <250 nmol/L, delta cortisol <250 nmol/L or peak cortisol <500 nmol/L) among nonsurviving patients with acute hepatic dysfunction. Tsai et al[26] reported increased mortality in cirrhotic patients with sepsis and AI (baseline cortisol <414 nmol/L or delta cortisol <250 nmol/L with baseline cortisol between 414 and 938 nmol/L). Cirrhotic patients with variceal bleeding and pathologic SST (delta cortisol <250 nmol/L with baseline cortisol <970 nmol/L) had lower 45-day survival than patients with normal SST results.[27] In acutely decompensated cirrhotic patients, an independent association of AI (delta cortisol <250 nmol/L with baseline cortisol <965 nmol/L) and death within 90 days was reported.[28]

These controversial findings mainly relate to the enormous variability of distinct AI definitions and tests used and the heterogeneity of the study populations. Interestingly, all studies reporting an association of AI with poor prognosis used low delta cortisol to define AI.

Our findings reported here suggest higher 28-day TFS rates in ALF and ACLF patients with low baseline cortisol concentrations (<387 nmol/L in ALF and <392 nmol/L in ACLF, respectively) and consolidate the potential of high baseline cortisol as a poor prognostic marker. The negative correlation between cortisol concentrations and HLA-DR_{low} expression, previously identified as poor prognostic marker,[23,24] further supports the notion that exceeding cortisol concentrations predict poor prognosis in liver failure syndromes. On the other hand, previously proposed AI definitions based on baseline cortisol or SST results did not correlate well with short-term TFS in patients with liver failure.

The term "relative AI" defined by blunted cortisol increment following SST[20] was introduced to describe a maximally activated adrenal cortex producing large yet insufficient amounts of cortisol to deal with the severe stress of illness.[8] Given hypoproteinaemia is common in critical illness, blunted cortisol increment following SST could reflect reduced cortisol binding capacity, while free cortisol increases appropriately.[7] In our analysis, AI assessed by SST did not relate to 28-day TFS. Additionally, we did not observe a correlation between CBG or albumin concentrations and TFS. We thus suggest that SST is dispensable in patients with acute liver failure syndromes. We assume that in maximally stressed organisms, stress levels are sufficiently reflected by baseline cortisol

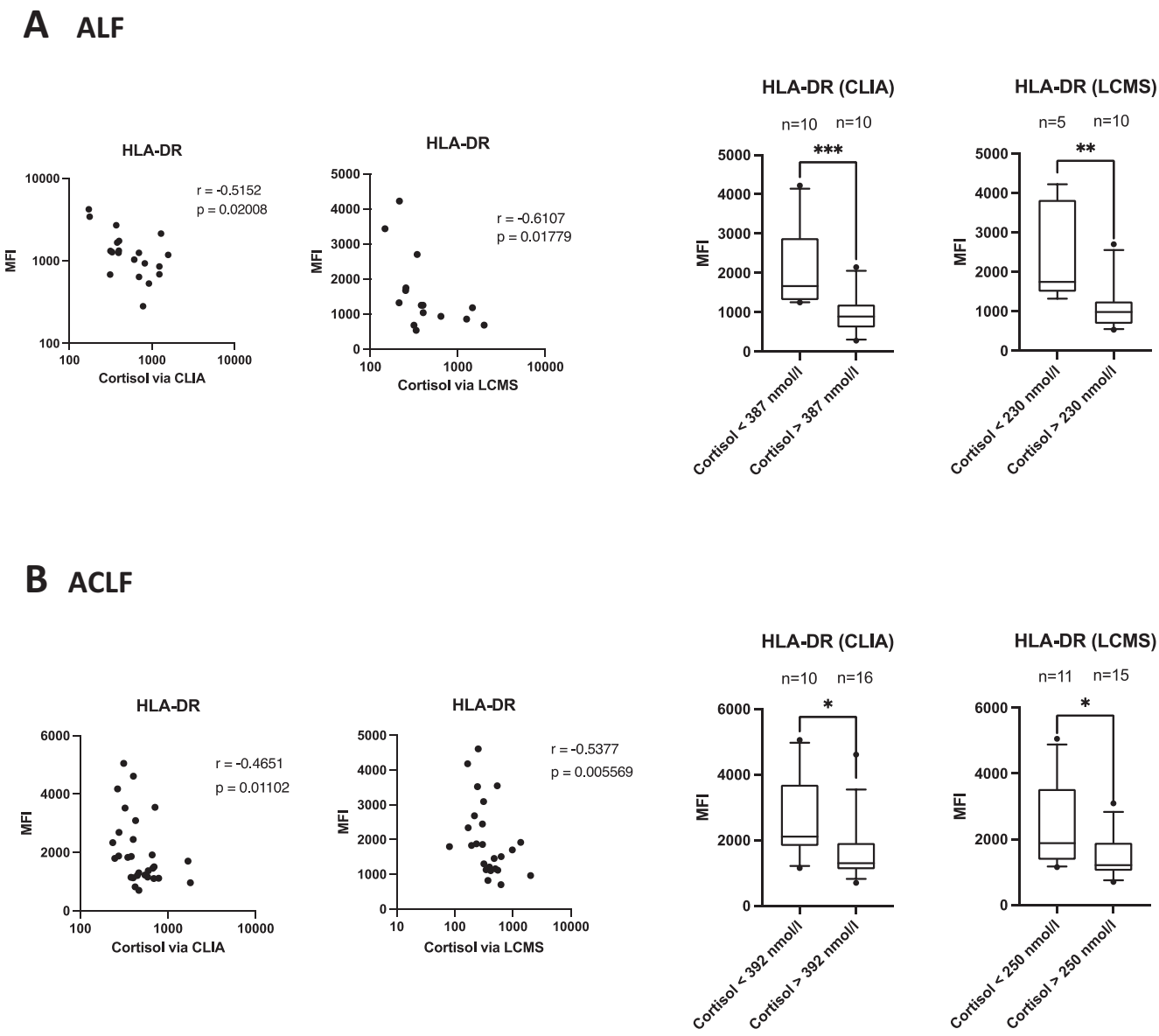

Figure 4. HLA-DR expression on circulating monocytes in relation to cortisol concentrations and prognostic cortisol values. (A) ALF. Scatterplots showing correlation between HLA-DR expression on circulating monocytes and cortisol concentrations quantified either by CLIA or LC-MS. Comparisons by Spearman r coefficient. HLA-DR expression in relation to prognostic cortisol values determined for either CLIA or LC-MS measurements. Box plots show median with 10–90 percentile and all points min-max. Comparisons by Mann-Whitney U tests, $^{*}P = .05$, $^{**}P = .01$, $^{***}P = .001$. (B) ACLF. See panel A.

concentrations while SST is an excessive, unnatural demand, and does not add diagnostic accuracy in LF.

Due to changes in binding protein concentrations in patients with liver dysfunction, assessment of free cortisol instead of total cortisol has been postulated.[12] In our study, we measured free cortisol levels by LC-MS exemplarily in 9 ACLF patients, and observed a trend associating lower free cortisol values either measured by LC-MS or calculated (free cortisol index) with better TFS (Figure A9). Therefore, we endorse its prospective differential evaluation.

CLIA remains the most widely used method for steroid assessment. In our cohort, CLIA and LC-MS results correlated well but the actual values were lower using the LC-MS method given its higher specificity. However, when using LC-MS baseline cortisol for survival prediction we did not find a significantly distinctive cutoff. This may implicate that CLIA has higher prognostic value despite or even because of its lower specificity.

Stress-dose GC treatment is proposed in patients with ALF/ACLF and septic shock.[29] Otherwise, recommendations are lacking due to contradictory data. Harry et al[30] did not find better survival in liver failure patients with AI treated with corticosteroids, while Marik et al[10] reported reduced mortality in patients with AI and fulminant liver failure, chronic liver disease, or liver transplantation under steroid

treatment. In our cohort, AI patients under GC treatment had better 28-day TFS when AI was defined by baseline cortisol <414 nmol/L in ALF and <275 nmol/L in ACLF, respectively. Hence, we suggest assessing baseline cortisol concentrations at the indicated cutoff levels for treatment guidance.

Overall, our observational and single-center design limited our results to hypothesis generating only. GC treatment did not underlie defined criteria or randomization, which limits reproducibility and validity. Furthermore, patient numbers were small given the rare nature of the conditions limiting statistical significance.

In conclusion, we suggest that baseline cortisol concentrations serve as a prognostic marker in patients with liver failure given concentrations <387 nmol/L in ALF and <392 nmol/L in ACLF (<399 nmol/L in all patients with LF) indicated better short-term survival. Interestingly, patients with baseline cortisol concentrations exceeding these cutoffs had better TFS when treated with GC. We therefore propose a systematic assessment of baseline cortisol upon hepatic failure while conduction of SST can be omitted. The definition for CIRCI in liver failure remains indeterminate. Multicenter prospective studies with high sample size using the above identified definitions as treatment guidance are required for better understanding.

Supplementary Materials

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.gastha.2022.08.006.

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
Data, analytical methods, and study materials are available upon reasonable request to the corresponding author.