Hepatology Research

Hepatology Research 2016; **46**: 514–520 doi: 10.1111/hepr.12582

Original Article

Evaluation of neutrophil/leukocyte ratio and organ failure score as predictors of reversibility and survival following an acute-on-chronic liver failure event

Danai Agiasotelli, Alexandra Alexopoulou, Larisa Vasilieva, Georgia Kalpakou, Sotiria Papadaki and Spyros P Dourakis

2nd Department of Internal Medicine, Athens University Medical School, Athens, Greece

Aim: Acute-on-chronic liver failure (ACLF) is defined as an acute deterioration of liver disease with high mortality in patients with cirrhosis. The early mortality in ACLF is associated with organ failure and high leukocyte count. The time needed to reverse this condition and the factors affecting mortality after the early 30-day-period were evaluated.

Methods: One hundred and ninety-seven consecutive patients with cirrhosis were included. Patients were prospectively followed up for 180 days.

Results: ACLF was diagnosed in 54.8% of the patients. Infection was the most common precipitating event in patients with ACLF. On multivariate analysis, only the neutrophil/leukocyte ratio and Chronic Liver Failure Consortium Organ Failure (CLIF-C OF) score were associated with mortality. Hazard ratios for mortality of patients with ACLF compared with those without at different time end-points post-enrollment revealed that the relative risk of death in the ACLF group was 8.54 during the first 30-day period and declined to 1.94 during the second period of observation. The time varying effect of neutrophil/leukocyte ratio and CLIF-C score was negative (1% and 18% decline in the hazard ratio per month) while that of Model for End-Stage Liver Disease (MELD) was positive (3% increase in the hazard ratio per month).

Conclusion: The condition of ACLF was reversible in patients who survived. During the 30–180-day period following the acute event, the probability of death in ACLF became gradually similar to the non-ACLF group. The impact of inflammatory response and organ failure on survival is powerful during the first 30-day period and weakens thereafter while that of MELD increases.

Key words: acute-on-chronic liver failure, Chronic Liver Failure Consortium Organ Failure score, Model for End-Stage Liver Disease score, organ failure

INTRODUCTION

ACUTE-ON-CHRONIC LIVER FAILURE (ACLF) is an entity encompassing an acute deterioration of liver function in patients with cirrhosis either secondary to superimposed liver injury or due to extrahepatic precipitating factors.[1] A characteristic feature of the syndrome is a high short-term mortality due to one or more organ dysfunctions.[2,3] The prognostic factors determining the outcome of patients with compensated cirrhosis with or without a previous episode of decompensation who develop ACLF have recently been evaluated.[4] It seems that scoring systems evaluating the severity of liver disease such as the Child–Turcotte–Pugh (CTP) and Model for End-Stage Liver Disease (MELD),[5] performed less well in predicting short-term mortality than those addressing the degree of inflammatory reaction and/or organ failure in patients who developed ACLF.[1,4] It has therefore been demonstrated that leukocyte count and Sequential Organ Failure Assessment (SOFA) or modified-SOFA scores are the best predictors of ACLF-associated mortality.[6] More specifically, previous investigators showed that the Chronic Liver Failure Consortium (CLIF-C) ACLF, a score combining CLIF Organ Failure (OF), age and leukocyte count, showed a higher predictive accuracy than MELD, MELD-Na, and CTP, improving the prediction error rates at all main time points after ACLF diagnosis.[6] However, the performance of factors predicting survival in short, intermediate and long term and the time of reversibility of ACLF status needed further investigation.

In the current study, we investigated: (i) the effect on early, intermediate and late mortality of an episode of

Correspondence: Dr Alexandra Alexopoulou, 2nd Department of Medicine, Medical School, University of Athens, Hippokration General Hospital, 114 Vas Sofias Street, Athens 11528, Greece. Email: alexopou@ath.forthnet.gr
Received 8 July 2015; revision 15 August 2015; accepted 25 August 2015.

ACLF; (ii) the performance of prognostic markers in predicting early mortality at 30 days; and (iii) whether these factors still predict long-term mortality (after the 30-day period).

METHODS

Study population

THIS IS A prospective study conducted in a tertiary center from 2012 until 2014. Patients with acute decompensation of cirrhosis according to the criteria of the CANONIC study[4] were included. Patients with hepatocellular carcinoma, extrahepatic cholestasis, persistent hepatic encephalopathy and intrinsic renal disease were excluded.

ACLF diagnosis

Acute-on-chronic liver failure grades were assessed according to the CANONIC study recommendations.[4] If a patient without ACLF developed ACLF during index hospitalization he/she was declassified from the group without ACLF and reclassified as having ACLF.

All patients were hospitalized and treated with standard of care for hepatic encephalopathy,[7] hepatorenal syndrome,[8] variceal bleeding[9] and bacterial infection.[8] The characteristics and time of survival of patients with ACLF were compared with those of patients with cirrhosis without ACLF hospitalized during the same period for other reasons.

Clinical and laboratory data collection

All patients were evaluated on admission and clinical and laboratory characteristics were recorded. They were followed up during hospitalization and, if discharged, at the outpatient clinic every 2 weeks for the first 2.5 months and every 2 months thereafter. All patients were intensively screened for infection using laboratory biomarkers (white blood cell count, neutrophil count, C-reactive protein) as well as diagnostic paracentesis and inoculation of blood samples and/or ascitic fluid into blood culture bottles for aerobic and anaerobic bacteria performed at bedside. All data required to compute CTP, MELD and CLIF-C OF scores[6] were recorded at the time of ACLF diagnosis for ACLF patients or on admission in patients without ACLF. Mortality at 30, 75 and 180 days post-enrollment was recorded. Three patients underwent liver transplantation. The time of observation was divided in three periods: (i) first 30 days; (ii) 31–75 days; and (iii) 76–180 days post-enrollment. Factors associated with mortality at different periods of observation and the time-dependent effect of factors related to mortality in patients with ACLF were evaluated.

Statistical analysis

Data are expressed as median (interquartile range [IQR]) for continuous and count with percentage for categorical variables. The Mann–Whitney *U*-test was used for continuous variables and χ^2-test for categorical variables. The Kaplan–Meier estimator evaluated the survival rates between groups. The Cox proportional hazards model was used to estimate the relative risk of death when comparing groups. The Cox proportional hazards model using time-dependent covariates was applied to assess the time-dependent effect of the mortality hazard of the ACLF group compared with the group without ACLF. The same methodology was used to estimate the time-varying effect of factors related to mortality. A two-tailed *P*-value of less than 5% was considered to be statistically significant. All statistical analyses were conducted by the Stata/SE 11.0 for Windows statistical package (StataCorp, College Station, TX, USA).

RESULTS

Patient characteristics

IN TOTAL, 197 consecutive patients with cirrhosis were included. Of the patients assessed, 108 (54.8%) had or developed ACLF and 89 did not have ACLF. There were 146 (74.1%) men with a median age of 61 years (IQR, 54–70.5). In most patients (48.7%), the etiology of cirrhosis was alcoholic, in 31.5% it was associated with chronic viral infection and in the remaining 19.8% cirrhosis was due to other causes (Table 1). All patients had ascites at enrollment and 58.2% used diuretics for the treatment of ascites (Table 1). Seventy-six (70.4%) patients were defined as ACLF grade 1, 28 (25.9%) as grade 2 and four (3.7%) as grade 3. The median follow up was 4.04 months (IQR, 1.5–6) for all patients, 2.1 months (IQR, 0.5–6) in the ACLF group and 6 months (IQR, 3.6–6) in the non-ACLF ($P < 0.001$).

Patients with ACLF compared with those without

At the time of enrollment, age, sex, diuretic use, presence of varices and/or portal gastropathy and history of variceal bleeding did not differ significantly between patients with and without ACLF (Table 1). Patients with ACLF had alcoholic cirrhosis more frequently (54%) and there was no statistically significant difference as far as etiology of cirrhosis was concerned between the two groups studied ($P = 0.051$). Patients with ACLF compared with those without had higher MELD ($P < 0.001$) and CTP scores ($P < 0.001$). CLIF-C OF values were higher in the group with ACLF compared with those without ($P < 0.001$),

Table 1 Characteristics and severity of liver disease of patients with and without ACLF

Main characteristics	Total ($n = 197$)	With ACLF ($n = 108$)	Without ACLF ($n = 89$)	P
Age (years)	61 (54–70.5)	60.5 (53–70)	62 (56–72)	0.105
Sex (male %)	146 (74.1)	80 (74.1)	66 (74.2)	0.989
Etiology of liver cirrhosis				
Viral (*n*, %)	62 (31.5)	30 (27.8)	32 (36)	
HBV	31 (15.7)	15 (13.9)	16 (18.0)	
HCV	26 (13.2)	13 (12)	13 (14.6)	
HBV + HDV	4 (2.0)	1 (0.9)	3 (3.4)	
HBV + HCV	1 (0.5)	1 (0.9)	0 (0.0)	
Alcoholic (*n*, %)	96 (48.7)	61 (56.5)	35 (39.3)	0.051
Other (*n*, %)	39 (19.8)	17 (15.7)	22 (24.7)	
Non-alcoholic steatohepatitis	19 (9.6)	6 (5.6)	13 (14.6)	
Cryptogenic cirrhosis	8 (4.1)	3 (2.8)	5 (5.6)	
Wilson's disease	5 (2.5)	3 (2.8)	2 (2.2)	
Primary biliary cirrhosis	5 (2.5)	4 (3.7)	1 (1.1)	
Autoimmune hepatitis	1 (0.5)	0 (0.0)	1 (1.1)	
Primary sclerosing cholangitis	1 (0.5)	1 (0.9)	0 (0.0)	
Active alcohol abuse‡	54 (27.4)	35 (32.4)	19 (21.3)	0.083
Use of diuretics (*n*, %)	113 (58.2)	59 (55.1)	54 (62.1)	0.330
History of variceal bleeding (*n*, %)	36 (18.6)	20 (18.9)	16 (18.2)	0.903
Findings on endoscopy† (*n*, %)				
No findings	21 (13.6)	12 (15.2)	9 (12)	0.935
Varices and/or portal gastropathy	133 (86.3)	67 (84.8)	66 (88)	
CTP score	11 (9–12)	11.5 (10–13)	9 (8–10)	<0.001
MELD score	18 (14–24)	23 (17–28)	15 (11–18.5)	<0.001
CLIF-C OF	7 (6–9)	8 (7–10)	6 (6–7)	<0.001
Laboratory characteristics				
Hemoglobin (g/dL)	10.3 (8.7–12.1)	10.3 (8.5–12.1)	10.4 (8.7–12.1)	0.871
Leukocyte count $\times 10^9$/L	7.3 (5.2–10.15)	8.5(6–12)	5.9 (4.4–7.7)	<0.001
Neutrophil count $\times 10^9$/L	5.0 (3.3–7.7)-	6.3 (4.3–9.2)	3.9 (2.7–5.2)	<0.001
Neutrophil/leukocyte ratio (%)	72 (64–80)	75 (65.8–82.7)	68 (60–75)	<0.001
Platelet count $\times 10^9$/L	116 (80–182)	110 (80–180)	118 (75–183)	0.905
C-reactive protein (mg/L)	20.7 (10–44)	26 (14–53)	16.7 (8–33)	0.002
AST (IU/L)	72 (43–124)	96 (65–160)	48 (31–77.5)	<0.001
ALT (IU/L)	36 (23–61)	46.5 (31–80)	25 (18–40)	<0.001
Albumin (g/dL)	2.7 (2.3–3.1)	2.5 (2.2–3)	2.8 (2.5–3.2)	0.002
Creatinine (mg/L)	1.0 (0.8–1.5)	0.95 (0.7–1.6)	1 (0.8–1.4)	0.917

Data are median (interquartile range) or numbers of patients (%).
†154 patients underwent endoscopy, 79 (51.3) of them with ACLF and 75 (48.7) without ACLF.
‡Patients who are active drinkers on admission and/or 3 months earlier and consume >30 g/day if male and >20 g/day if female.[21]
ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTP, Child–Turcotte–Pugh; CLIF-C OF, Chronic Liver Failure Consortium Organ Failure; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; INR international normalized ratio; MELD, Model for End-Stage Liver Disease.

respectively (Table 1). Aspartate aminotransferase and alanine aminotransferase values were higher and albumin was lower in patients with ACLF ($P < 0.001$, $P < 0.001$ and $P = 0.002$, respectively). Inflammation markers, as demonstrated by leukocyte, neutrophils, neutrophil/leukocyte ratio (%) and C-reactive protein, were also higher in the former group compared with the latter ($P < 0.001$ for each of the first three parameters and $P = 0.002$ for last one) (Table 1).

Precipitant factors in ACLF

Bacterial infections were the most common precipitating events in patients with ACLF, accounting for 58 (53.7%) cases, followed by active alcohol abuse (32.4%) and active

variceal bleeding (12%). Systemic inflammatory response syndrome or sepsis[10] were recorded in 17 (15.7%) patients. Spontaneous bacterial peritonitis was the most common infection (13.9%), followed by urinary tract infection (12%) and respiratory infection (8.3%). Twenty percent of patients had more than one precipitating factor. It is noteworthy that in 25 (23.1%) patients the precipitant factor was unknown.

Mortality and factors associated with mortality

Patients with ACLF had a worse survival compared with those without, as shown by Kaplan–Meier survival curve (log rank, $P < 0.001$) (Fig. 1). It is noteworthy that survival of patients with and without ACLF at 30, 75 and 180 days of follow up was 64.5%, 33.3% and 25.9% for the former and 94.4%, 65.2% and 52.8% for the latter, respectively.

Univariate Cox regression analysis showed that the variables exhibiting a significant association with mortality at 30 days in patients with ACLF were sex ($P = 0.012$), neutrophil/leukocyte ratio ($P = 0.008$), MELD ($P < 0.001$), CTP score ($P < 0.001$) and CLIF-C OF ($P < 0.001$) (Table 2). However, on multivariate Cox regression analysis when adjusted for potential cofounders including age, sex, MELD, CLIF-C OF and neutrophil/leukocyte ratio, only the neutrophil/leukocyte ratio ($P = 0.017$) and CLIF-C OF score ($P = 0.010$) were predictive of death at 30 days (Table 2).

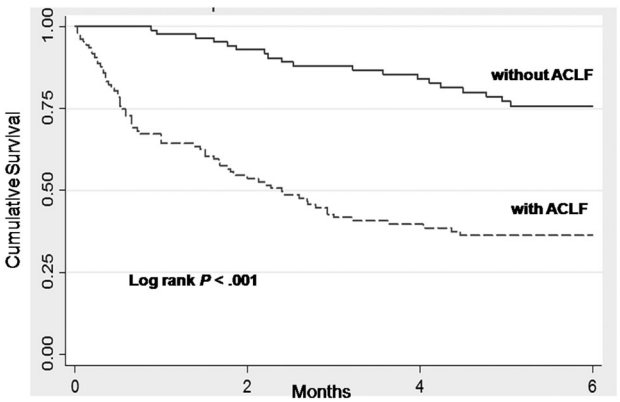

Figure 1 Comparison of survival in patients with and without acute-on-chronic liver failure (ACLF).

Ability of CLIF-C OF to predict outcome

Figure 2 shows the predictive ability of CLIF-C OF score for the 30-day mortality in patients with ACLF. In receiver–operator curve (ROC) analyses, CLIF-C OF score offered the best accuracy in predicting 30-day mortality (c-statistic, 0.744). The optimal cut-off of 9 offered sensitivity of 74%, specificity of 64% and a negative predictive value of 81%. A CLIF-C OF value of 7 or lower had a 93% negative predictive value and 97% sensitivity, while a score of 12 or higher showed an 86% positive predictive value and 99% specificity.

Neutrophil/leukocyte ratio (%) offered less accuracy to predict outcome in patients with ACLF during the first 30-day period (c-statistic, 0.679). Based on the ROC, the optimal cut-off for neutrophil/leukocyte ratio was 72.6%,

Table 2 Univariate and multivariate Cox regression analysis of factors predicting mortality at 30 days in 108 patients with ACLF

	Univariate analysis		Multivariate analysis†	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (per 1 year)	1.01 (0.98–1.03)	0.555	1.01(0.99–1.04)	0.371
Sex (female vs male)	2.30 (1.20–4.41)	0.012	1.10 (0.51–2.38)	0.810
Leukocyte count	1.02 (0.98–1.07)	0.350		
Neutrophil count	1.03 (0.98–1.08)	0.233		
Neutrophil/leukocyte ratio (%)	1.04 (1.01–1.08)	0.008	1.04(1.01–1.08)	0.017
C-reactive protein	1.00 (0.99–1.01)	0.448		
Child–Turcotte–Pugh score	1.51 (1.27–1.80)	<0.001		
Bacterial infection	0.79 (0.42–1.51)	0.489		
ALT	1.00 (1.00–1.00)	0.100		
MELD score	1.09 (1.05–1.13)	<0.001	0.99 (0.91–1.07)	0.715
CLIF-C OF	1.49(1.26–1.75)	<0.001	1.55 (1.11–2.17)	0.010
Variceal bleeding	1.25 (0.44–3.51)	0.67		

†Mutually adjusted for: age, sex, neutrophil/leukocyte ratio (%), MELD score, CLIF-C OF.
ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; CI, confidence interval; CLIF-C OF, Chronic Liver Failure Consortium Organ Failure; HR, hazard ratio; MELD, Model for End-Stage Liver Disease.

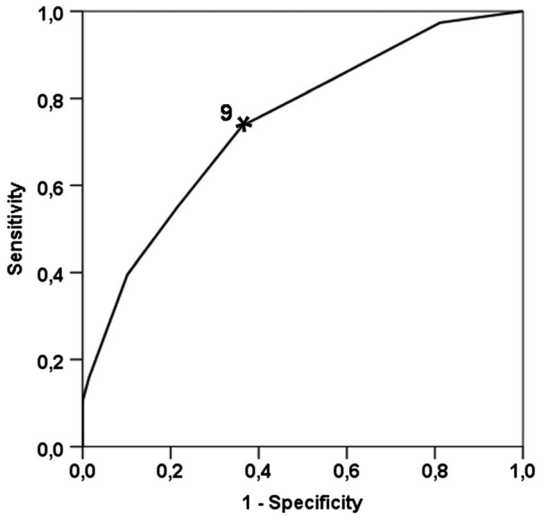

Figure 2 Accuracy of Chronic Liver Failure Consortium Organ Failure (CLIF-C OF) in patients with acute-on-chronic liver failure (c-statistic, 0.744). The cut-off point of 9 for CLIF-C OF offers sensitivity of 74% and specificity of 64% in predicting 30-day mortality.

offering sensitivity of 74% and specificity of 57% for predicting mortality.

Reversibility of risk of death during follow up in patients with ACLF

Hazard ratios (and 95% confidence interval [CI]) for mortality of patients with ACLF compared with those without (reference group) at different time intervals following enrollment, using the Cox proportional hazards model, adjusting for age, sex, neutrophil/leukocyte ratio, MELD and CLIF-C OF, revealed that the relative risk of death in the ACLF group was 8.54 (95% CI, 1.99–36.70; $P = 0.004$) at 30 days and declined to 1.94 (95% CI, 0.78–4.87; $P = 0.154$) during the second period of observation (>30 days and ≤75 days) and to 1.26 during the third period of observation (>75 and <180 days) (95% CI, 0.53–3.00; $P = 0.609$).

Time-varying effect of factors CLIF-C OF, neutrophil/leukocyte ratio and MELD on mortality in the ACLF group

In Table 3, the interaction with time of CLIF-C OF, neutrophil/leukocyte ratio and MELD score during the 180-day period of observation was studied. It was shown that the interaction with time of the CLIF-C OF and neutrophil/leukocyte ratio was negative (18% and 1% decline in the hazard ratio per month of CLIF-C OF and neutrophil/leukocyte ratio, respectively), while that of MELD score was positive (3% increase in the hazard ratio per month). This is an indication that, as time goes by, CLIF-C OF and neutrophil/leukocyte ratio become weaker risk factors for mortality, while MELD score becomes stronger.

Table 3 Multivariate Cox regression analysis† studying interaction with time of factors predicting mortality during 180-day observation period in patients with ACLF

	HR	95% CI for HR	*P*
Main model			
Age	1.02	(1.00–1.04)	0.063
Sex	1.00	(0.53–1.89)	0.990
CLIF-C OF score	1.54	(1.08–2.22)	0.019
Neutrophil/leukocyte ratio	1.05	(1.01–1.09)	0.017
MELD score	1.00	(0.91–1.09)	0.928
Interaction with time			
CLIF-C OF score†	0.82	(0.67–1.00)	0.050
Neutrophil/leukocyte ratio†	0.99	(0.97–1.01)	0.151
MELD score†	1.03	(0.98–1.07)	0.281

†Mutually adjusted for age, sex, neutrophil/leukocyte ratio (%), MELD score and CLIF-C OF.
ACLF, acute-on-chronic liver failure; CI, confidence interval; CLIF-C OF, Chronic Liver Failure Consortium Organ Failure; HR, hazard ratio; MELD, Model for End-Stage Liver Disease.

DISCUSSION

PATIENTS WHO DEVELOP an acute deterioration of cirrhosis and organ dysfunction due to a precipitating event have a worse outcome than those who do not develop organ dysfunction.[11] ACLF was therefore defined as "an acute deterioration of pre-existing liver disease usually related to a precipitating event and associated with increased mortality at 3 months due to multisystem organ failure".[12] However, consequent studies have been focused on short term mortality of 28 days because this was the most crucial period of high mortality.[4,6] MELD score has been shown to correlate with intermediate mortality risk at 3 months and has been used to determine priorities for liver transplantation.[13] It seemed that the conventional scoring systems addressing the severity of liver disease such as CTP or MELD performed less well than the scoring systems addressing organ dysfunction or severity of inflammation in the setting of ACLF.[14]

Our study demonstrates a high 30-day-mortality, a reversible component (in those who survived) and the presence of an obvious precipitating event in the majority of cases. Patients with ACLF were more frequently alcoholic, and had higher leukocyte and neutrophil counts and

C-reactive protein and aminotransferase values. It is remarkable that despite higher MELD and CTP scores of the ACLF compared with non-ACLF group, these parameters were not related to the 30-day mortality.

In the recent study by Moreau *et al.*, patients with ACLF were reported to have a 28-day mortality ranging 29.7–34% as compared with only 1.9% among patients who did not develop ACLF.[4] Similarly, the mortality rate during the first month in our ACLF group was 34.5% compared with 5.6% in the group without ACLF. However, in patients with ACLF who overcame the acute episode, the difference in survival tended to attenuate over time to the same level as in patients without ACLF. The deviation in mortality between the two groups weakened soon after the first month from the acute event and the ACLF group became similar to the group without ACLF. The survival of the ACLF group appears to be still dismal even after the first month but it is rather influenced by the high rate of the first 30-day mortality. When the effect of the ACLF group on mortality is estimated exclusively on the second period of time (31–75 days post-enrollment), no significant difference was apparent between the two groups.

Furthermore, our study shows that a high CLIF-C OF was an independent predictor of 30-day mortality in the ACLF group. However, instead of leukocyte count, neutrophil/leukocyte ratio was found to be an independent predictor of mortality. Excessive inflammatory reaction is considered to be severe in patients with ACLF causing acute systemic inflammation and oxidative stress to organs.[15] Both organ failure score and neutrophil/leukocyte ratio are influenced by the degree of systemic inflammation. Plasma-induced neutrophil phagocytic dysfunction was shown to be greater in patients with more severe liver cirrhosis and was associated with increased expression of inflammatory mediators.[16] Neutrophil/leukocyte ratio compared with leukocyte count had a higher impact as a predictor of mortality in the present study probably due to the degree of portal hypertension and the subsequent pancytopenia in the population studied.[17] As it was shown previously in ACLF studies,[14,18–20] infection was found to be a major precipitating event in patients with ACLF. Many of the patients with infection developed systemic inflammatory response syndrome or sepsis. It seems, therefore, that severity of inflammation, lack of its resolution and severity of organ failure were associated with significantly higher risk of death.

More specifically, CLIF-C OF is a simple organ failure score which was used to diagnose and grade organ failures and ACLF in cirrhotics.[4,6] It included parameters commonly used in clinical practice in ACLF patients. Our data showed that CLIF-C OF could be a useful predictor of outcome. The cut-off points of the parameter were found to provide good prognostic information in predicting 30-day mortality. The clinical application of this predictor is easy and can be used in patients with ACLF at risk, who may require intensive care and/or evaluation for liver transplantation.

As mentioned earlier, our results showed that in patients who recovered from an episode of acute deterioration of liver disease, the long-term outcome became similar to that of patients without acute deterioration and that was our aim; namely, to study the after "28-day mortality" and the factors that influence it. Our model of survival provided further evidence that predictors of survival change over time. The impact of neutrophil/leukocyte ratio and CLIF-C OF on predicting survival tended to reduce, while that of MELD tended to grow over time. In fact, the effect on survival of neutrophil/leukocyte ratio and CLIF-C OF score is powerful only during the first 30 days after the episode of deterioration of liver disease. This is another indication that the outcome of patients who overcame an acute deterioration episode, becomes similar to that of patients without acute event.

It is noteworthy that in our study, coming from a single center in Greece, renal dysfunction/failure was less common in ACLF patients, unlike the CANONIC study. Furthermore, ACLF was diagnosed in half of the patients. It seems, therefore, that the epidemiological and clinical pattern of ACLF may differ slightly among geographical areas and even among hospitals.

The results provide evidence that, despite high early mortality, acute deterioration in patients with liver cirrhosis is a potentially reversible condition and these patients may benefit from appropriate care. This information is important in countries where liver transplants are scarce because of a limited organ supply and most of liver transplant candidates die on the waiting list.

REFERENCES

1 Jalan R, Gines P, Olson JC, *et al.* Acute-on chronic liver failure. *J Hepatol* 2012; **57**: 1336–48.
2 Olson JC, Wendon JA, Kramer DJ, *et al.* Intensive care of the patient with cirrhosis. *Hepatology* 2011; **54**: 1864–72.
3 Wlodzimirow KA, Eslami S, Abu-Hanna A, Nieuwoudt M, Chamuleau RA. A systematic review on prognostic indicators of acute on chronic liver failure and their predictive value for mortality. *Liver Int* 2013; **33**: 40–52.
4 Moreau R, Jalan R, Gines P, *et al.* Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426–37.

5 Brown RS Jr, Kumar KS, Russo MW, *et al.* Model for end-stage liver disease and Child-Turcotte-Pugh score as predictors of pretransplantation disease severity, posttransplantation outcome, and resource utilization in United Network for Organ Sharing status 2A patients. *Liver Transpl* 2002; **8**: 278–84.
6 Jalan R, Saliba F, Pavesi M, *et al.* Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol* 2014; **61**: 1038–47.
7 Vilstrup H, Amodio P, Bajaj J, *et al.* Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases. *J Hepatol* 2014; **61**: 642–59.
8 European Association for the Study of the Liver. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397–417.
9 Sarin SK, Kumar A, Angus PW, *et al.* Diagnosis and management of acute variceal bleeding: Asian Pacific Association for Study of the Liver recommendations. *Hepatol Int* 2011; **5**: 607–24.
10 Fernández J, Gustot T. Management of bacterial infections in cirrhosis. *J Hepatol* 2012; **56**: S1–12.
11 Jalan R, Stadlbauer V, Sen S, Cheshire L, Chang YM, Mookerjee RP. Role of predisposition, injury, response and organ failure in the prognosis of patients with acute-on-chronic liver failure: a prospective cohort study. *Crit Care* 2012; **16**: R227.
12 Sarin SK, Kumar A, Almeida JA, *et al.* Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int* 2009; **3**: 269–82.
13 Said A, Williams J, Holden J, *et al.* Model for end stage liver disease score predicts mortality across a broad spectrum of liver disease. *J Hepatol* 2004; **40**: 897–903.
14 Moreau R, Arroyo V. Acute on Chronic Liver Failure: A New Clinical Entity. *Clin Gastroenterol Hepatol* 2015; **13**: 836–41.
15 Arroyo V, García-Martinez R, Salvatella X. Human serum albumin, systemic inflammation, and cirrhosis. *J Hepatol* 2014; **61**: 396–407.
16 Tritto G, Bechlis Z, Stadlbauer V, *et al.* Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. *J Hepatol* 2011; **55**: 574–81.
17 Minemura M, Tajiri K, Shimizu Y. Systemic abnormalities in liver disease. *World J Gastroenterol* 2009; **15**: 2960–74.
18 Katoonizadeh A, Laleman W, Verslype C, *et al.* Early features of acute-on-chronic alcoholic liver failure: a prospective cohort study. *Gut* 2010; **59**: 1561–9.
19 Cordoba J, Ventura-Cots M, Simón-Talero M, *et al.* Characteristics, risk factors, and mortality of cirrhotic patients hospitalized for hepatic encephalopathy with and without acute-on-chronic liver failure (ACLF). *J Hepatol* 2014; **60**: 275–81.
20 Agrawal S, Duseja A, Gupta T, Dhiman RK, Chawla Y. Simple Organ Failure Count Versus Canonic Grading System for Predicting Mortality in Acute on Chronic Liver Failure. *J Gastroenterol Hepatol* 2015; **30**: 575–81.
21 O'Shea RS, Dasarathy S, McCullough AJ, and the Practice Guideline Committee of the American Association for the Study of Liver Diseases and the Practice Parameters Committee of the American College of Gastroenterology. AASLD practice guidelines. Alcoholic liver disease. *Hepatology* 2010; **51**: 307–27.