Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: January 31, 2023

Citation: Silva I. F., Aguiar A., et al., 2023. Predictive Factors for Acute Liver Failure or Death in Patients with Acute Liver Injury: A Longitudinal Retrospective Study, Medical Research Archives, [online] 11(1). https://doi.org/10.18103/mra.v11i1.3566

DOI: https://doi.org/10.18103/mra.v11i1.3566

ISSN: 2375-1924

RESEARCH ARTICLE

Predictive Factors for Acute Liver Failure or Death in Patients with Acute Liver Injury: A Longitudinal Retrospective Study

Isabel Fonseca Silva[1], Ana Aguiar[2,3,4], Ana Oliveira[1], Arlindo Guimas[1], Pedro Vita[1], Rute Alves[1], Ana Luísa Rego[1], Álvaro Ferreira[1], Alexandre Pinto[1], Miguel Ricardo[1], Graziela Carvalheiras[1], Ana Novo[1], Ana Vigário[1], Ricardo Marinho[1], Ana Rita Costa[1], Diana Valadares[1], *Filipe Nery[1,2,3,4]

[1]Unidade de Cuidados Intermédios Médicos, Serviço de Cuidados Intensivos, Centro Hospitalar Universitário Do Porto, Porto, Portugal
[2]EPIUnit - Instituto De Saúde Pública, Universidade do Porto, Porto, Portugal
[3]Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Porto, Portugal
[4]ICBAS-UP, Instituto de Ciências Biomédicas de Abel Salazar - Universidade do Porto

*filipenery@clinicadofigado.com

ABSTRACT

Background & Aims: Severe acute liver injury (ALI) precedes acute liver failure (ALF). Risk factors related to ALI progression to ALF or death, are not well-known. We aimed to investigate which predictors of poor outcomes can be identified in patients with ALI.

Methods: We retrospectively analyzed 59 patients with ALI diagnosis, admitted from 2010 to 2021 in our Intermediate Medical Care Unit, and checked for clinical, biochemical and imagiological data, in order to explore their relationship with progression to ALF or death.

Results: From the 59 patients with ALI, 11 (18.6%) evolved to ALF and 9 (15.3%) died during the hospital staying or within the first month after discharge. Not having drug-induced liver injury or ischemic hepatitis as etiological factors was associated to increased progression to ALF (hazard ratio [HR] 0.17; 95% confidence interval [CI]: 0.03-0.96; P=0.041). In univariate analysis, ascites related to development to ALF (HR 0.25; 95% CI: 0.06-0.97; P=0.037) and death (HR 7.09; 95% CI: 1.55-33.04; P=0.006), while renal dysfunction, was only allied to death (HR 0.99; 95% CI: 0.97-1.00; P=0.035). Inflammatory markers at admission were not linked to progression to ALF. Yet, increased C reactive-protein levels were commonly found in patients who died (HR 1.01; 95% CI: 1.00-1.03; P=0.035). In multivariate analysis, only ascites remained significant (P=0.005) as predictor of death.

Conclusions: In patients with ALI, ascites at presentation is the only marker of poor prognosis (mainly to death). Still, those who are more inflamed or have renal dysfunction at baseline are more willing to die.

Keywords: Acute liver injury; acute liver failure; ascites; inflammation; renal dysfunction.

Abbreviations in order of appearance:

ALI, acute liver injury;
ALF, acute liver failure;
DILI, drug-induced liver injury;
HR, hazard ratio;
CI, confidence interval;
HE, hepatic encephalopathy;
LT, liver transplantation;
CHUPorto, Centro Hospitalar Universitário do Porto;
IntMCU, Intermediate Medical Care Unit;
EASL, European Association for the Study of the Liver;
INR, international normalized ratio;
HIV, human immunodeficiency virus;
HAV, hepatitis A virus;
HBV, hepatitis B virus;
HCV, hepatitis C virus;
HEV, hepatitis E virus;
PCR, polymerase chain reaction;
CMV, cytomegalovirus;
EBV, epstein-barr virus;
HSV-1, herpes simplex virus 1;
HSV-2, herpes simplex virus 2;
ANAs, antinuclear antibodies;
ASM, anti-smooth antibody;
LKM, anti-liver-kidney antibody;
SLA-LP, soluble liver/liver pancreas antibody;
AMAs, anti-mitochondrial antibody;
AST, aspartate aminotransferase;
ALT, alanine aminotransferase;
ALP, alkaline phosphatase;
GGT, gamma-glutamyltransferase;
TB, total bilirubin;
DB, direct bilirubin;
IB, indirect bilirubin;
CRP, C-reactive protein;
CBS, complete blood test;
SD, standard deviation;
AI, autoimmune hepatitis;
MELD, model for end-stage liver disease;
MDRD-6, modification of diet in renal disease 6;
ICU, intensive care unit;
NAC, N-acetyl cysteine;
SIRS, systemic inflammatory response;
IL-6, interleukin 6;

Highlights:

- Renal dysfunction and increased C-reactive protein increase the propensity to death in patients with acute liver injury;

- Ascites is related to death in patients presenting with acute liver injury;

- Progression from acute liver injury to acute liver failure is more common in patients with ascites.

INTRODUCTION

Patients with an acute insult to an otherwise normal liver, may develop an increase in liver enzymes (acute hepatitis), eventually leading to abnormalities of coagulation or increase in bilirubin levels (acute liver injury) or, in the most extreme spectrum of disease severity, to acute liver failure (ALF), when hepatic encephalopathy (HE) arises.[1,2] While ALF is considered a rare condition, with an annual incidence of less than 10 cases per million in the developed world, the incidence of acute liver injury (ALI) is unknown.[3] Although ALF still carries a high mortality rate (up to 90% in historical series), this appears to be decreasing, based on i) earlier recognition of this condition with patients correctly allocated

to specific and dedicated medical facilities – intermediate or intensive liver care units, ii) identification of the underlying etiology with directed specific treatments, and iii) improvement of medical care, which comprises liver transplantation (LT) whenever indicated.[3-5]

It is known that ALI may precede ALF, but the factors that mediate this path are not yet well recognized. Some prognostic features related to ALF dismal prognosis have already been identified: disturbed analytic profile (coagulation tests, serum bilirubin) and clinical issues (etiology, timing of appearance of HE, liver volume).[6] Yet, studies on ALI's prognostic factors relating to the progression to ALF have been rarely reported. Factor VII or changes in factor V serum levels during hospitalization may serve as a reliable prognostic marker in patients with hepatitis and coagulopathy, without HE.[7] Furthermore, it has been demonstrated that infection and/or the resulting systemic inflammatory response may act as an important factor contributing to the development of HE, thus to ALF.[8]

Recognizing ALI and related factors that may be linked to ALF is important. Acting on them may eventually change the related clinical course of the disease, helping avoiding LT and possible deaths. Also, early referral to a LT tertiary center shall be considered when those prognostic factors are found at admission or soon in the course of the hospitalization due to ALI.[9] Therefore, our study aimed to identify ALI's predictors of poor outcomes that determine the evolution to ALF or death in a group of hospitalized patients with ALI in a specialized intermediate medical care unit (IntMCU) in a tertiary center with a LT facility in the North of Portugal.

METHODS

Patient selection and study design.

This retrospective longitudinal study was conducted in Centro Hospitalar Universitário do Porto (CHUPorto), particularly considering patients with ALI admitted in the IntMCU, which has expertise in the management of patients with acute and chronic liver diseases, and in close relationship with the liver transplantation and intensive care medical teams.

The IntMCU has, from the beginning of its existence, an own electronic database where all admitted patients with respective main and secondary diagnosis are recorded. All patients with ALI admitted in the IntMCU were first extracted from this database. Data was collected from the clinical institutional electronic medical records from May 2010 until May 2021. To define ALI, the definition proposed by the European Association for the Study of the Liver (EASL) guidelines in 2017 was used: transaminases elevation of at least two-times, associated with jaundice and coagulopathy, defined as International Normalized Ratio (INR) above 1.5 or a prolongation of prothrombin time, without encephalopathy, in the absence of pre-existing liver disease.[1]

Besides the diagnosis of ALI, all included patients for final analysis fulfilled the following criteria: patients older than 18 years; absence of cirrhosis (expressed by the absence of liver

dysmorphia or collaterals); absence of any previously known malignancy; absence of extrahepatic disease resulting in an estimated life expectancy of less than 1 year, or human immunodeficiency virus (HIV) infection. Concerning the exclusion criteria, patients with ALF (with HE) at admission were omitted. Although the exclusion was made from the formal analysis, the registration of its development (also defined at the EASL guidelines in 2017) as the development of encephalopathy within 8 weeks of appearance of the first symptoms of hepatic injury in a patient who met ALI's criteria, was recorded in order to stratify the risk of progression.[1]

All patients admitted with ALI were submitted to the same standard protocol of etiological workup: i) complete medical history; ii) laboratory evaluation (viral serology's considering hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV) and HEV polymerase chain reaction (PCR), cytomegalovirus (CMV), epstein-barr virus (EBV), herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human immunodeficiency virus 1 and 2 (HIV-1 and HIV-2) and any other serology's whenever clinically relevant; autoimmune profile considering IgM, IgG, C3, C4, antinuclear antibodies (ANAs), anti-smooth antibody (ASM), anti-liver-kidney antibody (LKM), soluble liver/liver pancreas antibody (SLA-LP), anti-mitochondrial antibody (AMAs); ceruloplasmin and serum copper together with biochemical analysis comprising liver profile: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB); renal function, albumin, total proteins and protein electrophoresis, together with inflammatory markers [C-reactive protein (CRP) and procalcitonin whenever needed], complete blood count (CBC) and coagulation evaluation with prothrombin time/ INR, activated partial thromboplastin time and factor V, as well as systematic screening of infection (urine, blood, sputum, peritoneal fluid cultures, etc.), usually at admission and every 48 hours. Patients were daily monitored for CBC, liver profile and renal function, CRP, ionogram and coagulation evaluation as aforementioned. As patients were admitted in a 24h over 24h surveillance unit, all of them were invasively monitored and clinical status with HE evaluation checked several times per day. Data were recorded till evolution to ALF, and/or death, and/or hospital discharge.

All patients had an abdominal image performed at admission (abdominal Doppler-ultrasound and/or computed tomography) ruling out indirect signs of chronic liver disease, based on liver morphology and signs of portal hypertension (portal vein size diameter, presence of collaterals, increased spleen size), as well as systematic evaluation of splanchnic vessel bed (portal vein and supra-hepatic veins patency).

All patients were submitted to the best standard of care treatment. None experimental drug or intervention was performed in any patient. In order to guarantee strict confidentiality of each

patient, all data were duly anonymized and the study protocol was conducted according to the ethical principles of the Declaration of Helsinki.

Statistics

Continuous variables were summarized as mean and standard deviation (SD). Categorical variables were described as absolute and relative frequencies. Continuous variables were compared using Student's *t*-test while, for proportions, Chi-square test or Fishers exact test were used as appropriate to explore the relationship between ALF and death with the different categorical variables. Also, multivariate analysis – MANOVA - was computed by using Bonferroni adjustment at a level of alpha of 0.025 per test to reduce the instance of a false positive. In particular, Bonferroni designed an adjustment to prevent data from incorrectly appearing to be statistically significant, since our sample size is small. Afterward, a Crude logistic regression model was computed and hazard ratio (HR) and 95% confidence intervals (CI) were calculated. Statistical analysis was carried out using SPSS Statistics 26.0 (IBM Corp, Armonk, New York). A significance level of 5% was adopted.

RESULTS

During the 11-year study period, 59 patients fulfilling ALI criteria were selected. Clinical and laboratory characteristics are expressed in Table 1. Overall, there was a female preponderance (57.6%) and age at diagnosis ranged from 18 to 83 years (mean age of 48.1 years old). Drug-induced Liver Injury (DILI) was the most frequent cause of ALI (37.3%), followed by ischemic injury (16.9%) and autoimmune hepatitis (AI) (13.6%). Among DILI patients, 14 were paracetamol-induced, representing 63,6% of all DILIs. In 14 patients with dubious etiological cause, a liver biopsy was done in order to increase diagnostic accuracy. N-acetyl cysteine protocol was performed in 45 patients irrespectively of the etiology, 14 of which had paracetamol induced ALI.

Table 1 – Baseline clinical and laboratory characteristics of patients admitted with acute liver injury.

	Number of patients (*n*=59)	%
Gender		
Male	25	42.4%
Age (mean ± SD)	48.1 ± 15.4	
Evolution to ALF	11	18.6%
Mortality	9	15.3%
Etiology of ALI		
Autoimmune	8	13.6%
Amanita intoxication	6	10.2%
Drug-induced Liver Injury	22	37.3%
Ischemic	10	16.9%
Viral	7	11.9%
Budd Chiari Syndrome	3	5.1%
Acute fatty liver of pregnancy	1	1.7%
Unknown	2	3.4%
Biological markers (mean ± SD)		
Hemoglobin (g/dL)	13.1 ± 2.5	
Leukocytes (10^9/L)	9.3 ± 4.6	
Neutrophils (10^9/L)	6.0 ± 3.9	
Lymphocytes (10^9/L)	1580.0 ± 1464.3	
Platelets (10^9/L)	192.5 ± 90.0	
NLR	6.9 ± 6.6	
MDRD 6 (mL/min)	94.0 ± 61.2	
MELD	21.3 ± 6.0	
MELD Na	22.6 ± 6.1	
CRP (mg/L)	32.3 ± 47.0	

SD - standard deviation; ALF – Acute Liver Failure; ALI – Acute Liver Injury; NLR – Neutrophil-lymphocyte ratio; MDRD 6 – Modification of Diet in Renal Disease 6; MELD - Model for End-Stage Liver Disease; CRP - C reactive protein.

Eleven patients (18.6%) progressed to ALF, with a time interval between admission in IntMCU and the onset of ALF ranging from 0 to 8 days (median of 3 days). Women and older patients tended to evolve more frequently to ALF. In univariate analyses, patients developing ALF were the ones presenting with ascites (HR 0.25; 95% CI: 0.06-0.97), and with DILI as related etiology (HR 0.17; 95% CI: 0.03-0.96) (Table 2). Baseline severity scores [either Model for End-Stage Liver Disease (MELD) and MELD-Na], as well as markers of systemic inflammation [leukocytes, lymphocytes, neutrophil/ lymphocyte ratio and CRP] were not predictive of progression from ALI to ALF.

Table 2 – Baseline comparable variables considering patients with acute liver injury evolving to acute liver failure or not.

n (%)	non-acute liver failure (n=48)	acute liver failure (n=11)	*p*-value	Crude HR (95%CI)
Gender			0.655	
Women	27 (56.3)	7 (63.6)		1.36 (0.35-5.27)
Men	21 (43.8)	4 (36.4)		1 [Reference]
Age (years) [mean (SD)]	47.2 (16.4)	52.2 (9.3)	0.334	1.02 (0.98-1.07)
Outcome			**0.002**	
Death	4 (8.3)	5 (45.5)		**0.11 (0.02-0.52)**
Alive	44 (91.7)	6 (54.5)		1
MDRD 6 [mean (SD)]	92.3 (66.8)	101.6 (26.6)	0.651	1.00 (0.99-1.01)
Upgrade ICU			**<0.001**	
Yes	1 (2.1)	6 (54.5)		**0.02 (0.00-0.18)**
No	47 (97.9)	5 (45.5)		1
Ascites			**0.037**	
Yes	11 (22.9)	6 (54.5)		**0.25 (0.06-0.97)**
No	37 (77.1)	5 (45.5)		1
NAC protocol			0.206	
Yes	35 (72.9)	10 (90.9)		0.27 (0.03-2.32)
No	13 (27.1)	1 (9.1)		1
Etiology			**0.041**	
DILI	20 (41.7)	2 (18.2)		**0.17 (0.03-0.96)**
Ischemic	10 (20.8)	0 (0)		(-)†
AI	6 (12.5)	2 (18.2)		0.57 (0.09-3.64)
Others	12 (25.0)	7 (63.6)		1
MELD [mean (SD)]	20.83 (6.20)	23.4 (4.8)	0.199	1.08 (0.96-1.20)
MELD-Na [mean (SD)]	22.10 (6.13)	25.10 (5.30)	0.157	1.09 (0.97-1.23)
Leukocytes (10^9/L) [mean (SD)]	9.2 (4.1)	9.6 (6.4)	0.763	1.00 (1.00-1.00)
Lymphocytes (10^9/L) [mean (SD)]	1.6 (1.6)	1.7 (0.9)	0.847	1.00 (1.00-1.00)
NLR [mean (SD)]	7.2 (6.5)	6.2 (7.7)	0.682	0.98 (0.87-1.10)
CRP (mg/L) [mean (SD)]	34.5 (51.5)	23.0 (17.9)	0.493	0.99 (0.97-1.01)

Bold values correspond to p<0.05

SD - standard deviation; HR – Hazard ratio; MDRD 6 – Modification of Diet in Renal Disease 6; ICU - Intensive Care Unit; NAC – N-acetyl cysteine; DILI- Drug-induced Liver Injury; AI - Autoimmune hepatitis; MELD - Model for End-Stage Liver Disease; NLR – Neutrophil-lymphocyte ratio; CRP - C reactive protein.

Early death, occurring during the hospital stay or in the first 28 days after hospital discharge, was found in 9 (15.3%) patients. Acute liver failure was the cause of death in 5 patients, representing 55.6% of all causes, septic shock in 3, and dysrhythmia in 1 patient. A decreased renal function [expressed by Modification of Diet in Renal Disease method (MDRD-6)] (HR 0.99; 95% CI: 0.97-1.00), need of intensive care unit (ICU) (HR 12.53; 95% CI: 2.16-72.71), presence of ascites (HR 7.09; 95% CI: 1.55-33.04), increased lymphocytes count (HR 1.00; 95% CI: 1.00-1.00) and those with higher mean value of CRP (HR 1.01; 95% CI: 1.00-1.03), all predicted death at baseline, as found in univariate analysis (Table 3). Afterwards, we conducted a multivariate analysis for death/alive and ALF. For the first, there was a significant difference between alive and dead patients when considering jointly on the variables CRP, ascites and MDRD-6, Wilk's Ą = 0.791, F (3,48) = 4.227, p = 0.010, partial ŋ2 = 0.20. On the other hand, no significant difference between evolving to ALF or not was found when considering jointly on the variables CRP, ascites and MDRD-6, Wilk´s Ą = 0.854, F (3,48) = 2.727, p = 0.054, partial ŋ2 = 0.14.

Table 3 – Baseline comparable variables considering survival outcome (alive vs death).

n (%)	Alive (n=50)	Death (n=9)	*p*-value	Crude HR (95%CI)
Gender			0.385	
Women	30 (60.0)	4 (44.4)		0.53 (0.13-2.23)
Men	20 (40.0)	5 (55.6)		1 [Reference]
Age (years) [mean (SD)]	46.92 (15.92)	54.67 (10.50)	0.167	1.04 (0.99-1.09)
DILI (paracetamol)			0.069	
Yes	14 (28.0)	0 (0)		(-)†
No	36 (72.0)	9 (100)		1
MDRD 6 [mean (SD)]	100.17 (64.68)	59.67 (35.35)	**0.035**	0.99 (0.97-1.00)
Upgrade ICU			**0.001**	
Yes	3 (6.0)	4 (44.4)		**12.53 (2.16-72.71)**
No	47 (94.0)	5 (55.6)		1
Ascites			**0.006**	
Yes	11 (22.0)	6 (66.7)		**7.09 (1.55-33.04)**
No	39 (78.0)	3 (33.3)		1
NAC protocol			0.113	
Yes	40 (80.0)	5 (55.6)		0.31 (0.07-1.38)
No	10 (20.0)	4 (44.4)		1
MELD [mean (SD)]	21.14 (5.97)	23.73 (7.07)	0.187	1.01 (0.90-1.14)
MELD-Na [mean (SD)]	22.30 (6.02)	25.38 (6.74)	0.116	1.05 (0.93-1.18)

(Continued on next page)

n (%)	Alive (n=50)	Death (n=9)	*p*-value	Crude HR (95%CI)
Leukocytes (10^9/L) [mean (SD)]	8.9 (4.1)	11.5 (6.7)	0.111	1.00 (1.00-1.00)
Lymphocytes (10^9/L) [mean (SD)]	1.4 (8.5)	2.5 (3.1)	**0.048**	1.00 (1.00-1.00)
NLR [mean (SD)]	7.3 (7.1)	5.5 (3.6)	0.465	0.95 (0.83-1.09)
Etiology			**0.027**	
DILI	22 (44.0)	0 (0)		(-)†
Ischemic	7 (14.0)	3 (33.3)		1.61 (0.28-9.20)
AI	6 (12.0)	2 (22.2)		1.25 (0.18-8.73)
Others	15 (30.0)	4 (44.4)		1
CRP (mg/L) [mean (SD)]	26.0 (39.1)	62.0 (69.5)	**0.035**	1.01 (1.00-1.03)

† OR<0.001

Bold values correspond to p<0.05

SD - standard deviation; HR – Hazard ratio; DILI- Drug-induced Liver Injury; MDRD 6 – Modification of Diet in Renal Disease 6; ICU - Intensive Care Unit; NAC – N-acetyl cysteine; MELD - Model for End-Stage Liver Disease; NLR – Neutrophil-lymphocyte ratio; AI - Autoimmune hepatitis; CRP - C reactive protein.

A separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alfa level of 0.025. For the first outcome, there was a significant difference between alive and dead patients concerning only the presence of ascites, F (1,50) = 8.539, p = 0.005, partial ŋ2 = 0.146, with deceased patients (M = 0.667) scoring higher than alive patients (M = 0.209). There was not a significant difference between dead and alive patients concerning CRP and MDRD-6 levels (p = 0.035 and p = 0.222, respectively). Then, a significant result between evolving and not evolving to ALF was only found concerning ascites, F (1,50) = 6.344, p = 0.015, partial ŋ2 = 0.113. For CRP and MDRD-6 levels, no significant differences on evolving to ALF were found (p=0.493 and p=0.745, respectively).

N-acetyl cysteine (NAC) protocol was performed in 45 patients, irrespective of the underlying etiology. Its administration did not prevent the progression to ALF or death and was more commonly done in those with DILI (p<0.001) and AI hepatitis (p<0.001) (Table 4).

Table 4 –N-acetyl cysteine protocol administration considering different etiologies of Acute Liver Injury.

n (%)	No NAC protocol	NAC protocol	*p*-value	Crude HR (95%CI)
Etiology			<0.001	
DILI	3 (21.4)	19 (42.2)		1.187 (0.210-6.718)
Ischemic	8 (57.1)	2 (4.4)		**0.047 (0.006-0.340)**
AI	0 (0)	8 (17.8)		(-)†
Others	3 (21.4)	16 (35.6)		1 [Reference]

† OR<0.001

Bold values correspond to p<0.05

NAC – N-acetyl cysteine; HR – Hazard ratio; DILI- Drug-induced Liver Injury; AI - Autoimmune hepatitis.

Twelve patients with ALI had infection at admission. Three of them evolved to ALF, and 4 died. Infection at admission was not related with progression to ALF or death. Of notice, HE preceded the diagnosis of declared infection (24-48 hours) in 4 patients (57,1%).

DISCUSSION

This longitudinal retrospective study lightens the relationship between biological and clinical parameters that, in patients admitted with ALI, relate to progression to ALF or death, gathering patients always managed by the same medical team with standardized protocols regarding laboratory and medical evaluation. Importantly, this report excludes patients with ALF at inclusion, unlike the vast majority of the previously conducted studies.

Autoimmune hepatitis is more frequent in women.[10] Drug-induced liver injury has also been more frequently recognized within this gender.[11,12] Being female has been found to be a risk factor for ALF development after a DILI episode.[13] Being DILI and AI hepatitis important etiologies in our study, the predominance of female gender is not a surprise. Yet, in our study, gender was not a determinant risk factor for progression to ALF. Gender may rather be related to specific causes of ALI. Particular etiologies may lead more frequently to ALI. As ALI is a syndrome and not a specific disease itself, with quite heterogeneous and different inherent etiologies, gender may not be a risk factor itself for ALI but for the inherent etiological cause.

Drug-induced liver injury was the most frequently found etiology in our cohort, with paracetamol as the predominantly related drug. These findings are consistent with previous studies.[2,14] The most frequent etiology of ALF in Europe is DILI, paracetamol or non-paracetamol, depending on the region.[1] The two previous Portuguese's studies evaluating ALF's etiologies, concluded that DILI is more often caused by non-paracetamol DILI.[2,14] This shift from non-paracetamol DILI to paracetamol induced ALI

seen in our study may be justified by i) the less severe enrolled patients (ALI versus ALF studies), as it is known that paracetamol induced liver disease has a better outcome than other DILI etiologies;[15] ii) different period of time of patient inclusion with modifications concerning drug policy (in Portugal, we assisted to the liberalization of drug market in 2005 with a new created category of over-the-counter medicines in 2013, in which paracetamol falls), with paracetamol being easily available for general population. Ischemic hepatitis is a well-known cause of ALF, in which treatment directed towards cardiocirculatory resuscitation leads to clinical improvement.[16] Patients with ischemic-induced ALI did not evolve to ALF yet, 1/3 died. This reflects a different pathophysiology leading to liver insult, with a different phenotype of patients (older, increased comorbidities), and eventually prognosis. In future studies, this subgroup of patients with ALI or developing ALF may be considered for different analysis. The number of patients with AI hepatitis (13.6%) in this study was superior to other previous studies about ALF,[2,10,17-20], probably allied to the inclusion of less severe patients (ALI versus ALF) as well as an increase of the incidence and recognition/awareness of AI hepatitis over the years.[21,22]

We found exactly the same proportion of patients (19%) evolving from ALI to ALF than Koch et al in his multicentric prospectively conducted study.[9] Yet, etiologies differ substantially as half of the patients enrolled in this large study had paracetamol related ALI. When considering mortality, 9 of our patients died, and 50 patients survived corresponding to an overall survival of 84,7%, superior to the previous Portuguese studies about ALF, reflecting early referral, more accurate and early diagnosis and better supporting measures along time, as well as less severe patients included (ALI vs ALF).[2,14] When comparing the same reality, ALI's overall survival is superior to ALF's overall survival, demonstrating that these entities are part of a continuum liver insult.

Ascites can be present in ALF. This finding has been related with severe portal hypertension.[23] Its presence in ALI patients, though, less severe ones, has not been systematically described, also demonstrating the spectrum of progression of the ALI from less severe (ALI/ eventually no ascites) to more severe (ALF/ eventually ascites) along time. In all of our patients, cirrhosis has been consistently ruled out, though, excluding portal hypertension in this context. Ascites has been found in a total of 17 patients, almost half of them in the setting of ischemic etiology, probably reflecting other mechanisms for the development of ascites. Ascites may also mirror the severity of liver damage, which has also been documented to relate to portal hypertension.[24] Ascites was definitely a marker of severity in our cohort, being its presence at admission related to an increased risk of death.

The role of NAC treatment protocol, as antidote for paracetamol induced liver failure has been well described. In 2017, EASL´s clinical practice guidelines for the management of ALF also recommended its use irrespectively of inherent etiology, as it

improves the outcome of patients with mild grades of HE.[1,25] To our knowledge there are no systematic conducted studies outside paracetamol-induced liver injury considering the role of NAC protocol on the outcome of the patient with ALI. In our study, ¾ of the patients with ALI underwent NAC treatment together with specific treatment directed towards respective etiologies and support. N-acetyl cysteine did not prevent progression to ALF or death, when considering etiologies all together. The benefit of NAC treatment in this setting must be analyzed in studies comparing groups considering similar etiologies of ALI, being submitted to NAC perfusion or not, together with standard of care treatment.

No association was documented between the severity of liver failure, as expressed by the MELD or MELD-Na score and progression to ALF. The MELD score was initially proposed to quantify end-stage liver disease for transplant planning and its use for ALF is not well accepted because of the lack of value of bilirubin as an early marker for ALF's severity. This may explain our results because during early stages of ALI, bilirubin may be normal or near normal, making this score less accurate to evaluate an acute liver dysfunction. This may be even more evident in this early stage of severity (ALI versus ALF).[26]

Patients more inflamed at admission were not more prone to develop ALF or die, even though those with increased CRP levels had a tendency to die more. The reason why inflammatory markers were not related to ALF development seems intriguing, as an association between systemic inflammatory response (SIRS) and worsening of HE in patients with ALF, independent of infection, has been well documented.[1,8] Yet, CRP is derived from interleukin 6 (IL-6) that is mainly produced in the liver. Also, in the context of liver failure, the increase of CRP may not be as expected, due to peripheral blood mononuclear cells, which also have a decrease potential to produce IL-6.[27] Yet, we have included, once again, patients in a less severe phase of the spectrum of disease than the ones usually included in the studies with ongoing ALF, meaning that the burden of SIRS on prognosis may not be the same when comparing ALI to ALF, particularly when considering this item as a risk factor at hospital admission and ALI diagnosis.

The retrospective nature and relative small number of included patients are the most important limitations to this study. In order to mitigate the selection bias related to its retrospective nature, we selected consecutive patients with ALI based on specific criteria accepted worldwide. Also, even if retrospective in character, all data were prospectively collected as a thorough protocol is followed since the constitution of our intermediate care unit. Portugal is a small sized country with an estimated population of around 10 million. Being ALF a rare medical condition and with an unknown incidence of ALI, the number of patients included in this 11-year period seems adjusted and representative of our Portuguese reality.

CONCLUSION

This retrospective longitudinal study shows ascites at presentation as a determinant of poor prognosis, and that more inflamed patients or those with decreased renal function are more willing to die. Patients in these conditions shall be promptly transferred and be managed in dedicated liver care Units. Also, NAC protocol, which is indicated in ALF patients irrespective of etiology, did not show any advantage (nor disadvantage) in avoiding progression to ALF or death.

Corresponding Author:
Filipe Nery, M.D. PhD.,
Centro Hospitalar Universitário do Porto,
Largo Prof. Abel Salazar S/N 4099-001 Porto.
Email: filipenery@clinicadofigado.com

Author contributions:
FN, AO, IFS and DV were involved in the study concept and design; IFS, AO, FN and DV were involved in data acquisition; FN, AA and IFS were involved in interpretation and data analysis; IFS, AA and FN were involved in manuscript draft; IFS, AA, AO, AG, PV, RA, ALR, AF, AP, MR, GC, AN, AV, RM, ARC, DV and FN gave critical revision of the manuscript for important intellectual content; AA, IFS and FN were involved in statistical analysis; All authors revised the manuscript, data analysis and data interpretation.

Grant support:
No specific grants were used. Ana Aguiar holds a PhD Grant (2020. 09390.BD), co-funded by the Fundação para a Ciência e a Tecnologia (FCT) and the Fundo Social Europeu (FSE) Program.

Conflict of interest:
The authors declare no conflicts of interest that pertain to this work.

Data availability statement:
Data are available from the corresponding author (Prof. Filipe Nery) upon reasonable request.

Acknowledgments:
None

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why

	Item No	Recommendation
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

References:

1. European Association for the Study of the Liver. Clinical practice guidelines panel, Wendon J, et al. EASL Clinical Practical Guidelines on the management of acute (fulminant) liver failure. *J Hepatol.* 2017;66(5):1047-1081.

2. Areia M, Romaozinho JM, Ferreira M, Amaro P, Leitao MC. Fulminant hepatic failure: a Portuguese experience. *Eur J Gastroenterol Hepatol.* 2007;19(8):665-669.

3. Cardoso FS, Marcelino P, Bagulho L, Karvellas CJ. Acute liver failure: An up-to-date approach. *J Crit Care.* 2017;39:25-30.

4. Germani G, Theocharidou E, Adam R, et al. Liver transplantation for acute liver failure in Europe: outcomes over 20 years from the ELTR database. *J Hepatol.* 2012;57(2):288-296.

5. Rovegno M, Vera M, Ruiz A, Benitez C. Current concepts in acute liver failure. *Ann Hepatol.* 2019;18(4):543-552.

6. O'Grady J. Timing and benefit of liver transplantation in acute liver failure. *J Hepatol.* 2014;60(3):663-670.

7. Elinav E, Ben-Dov I, Hai-Am E, Ackerman Z, Ofran Y. The predictive value of admission and follow up factor V and VII levels in patients with acute hepatitis and coagulopathy. *J Hepatol.* 2005;42(1):82-86.

8. Vaquero J, Polson J, Chung C, et al. Infection and the progression of hepatic encephalopathy in acute liver failure. *Gastroenterology.* 2003;125(3):755-764.

9. Koch DG, Speiser JL, Durkalski V, et al. The Natural History of Severe Acute Liver Injury. *Am J Gastroenterol.* 2017;112(9):1389-1396.

10. Francque S, Vonghia L, Ramon A, Michielsen P. Epidemiology and treatment of autoimmune hepatitis. *Hepat Med.* 2012;4:1-10.

11. Sahebjam F, Vierling JM. Autoimmune hepatitis. *Front Med.* 2015;9(2):187-219.

12. Amacher DE. Female gender as a susceptibility factor for drug-induced liver injury. *Hum Exp Toxicol.* 2014;33(9):928-939.

13. Castiella A, Zapata E, Lucena MI, Andrade RJ. Drug-induced autoimmune liver disease: A diagnostic dilemma of an increasingly reported disease. *World J Hepatol.* 2014;6(4):160-168.

14. Simoes C, Santos S, Vicente M, Sousa Cardoso F. Epidemiology of Acute Liver Failure from a Regional Liver Transplant Center in Portugal. *GE Port J Gastroenterol.* 2018;26(1):33-39.

15. Marrone G, Vaccaro FG, Biolato M, et al. Drug-induced liver injury 2017: the diagnosis is not easy but always to keep in mind. *Eur Rev Med Pharmacol Sci.* 2017;21(1 Suppl):122-134.

16. Tyagi A, Pruthi HS. Ischemic Hepatitis. *Med J Armed Forces India.* 1999;55(4):359-360.

17. Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med.* 2002;137(12):947-954.

18. Escorsell A, Mas A, de la Mata M, Spanish Group for the Study of Acute Liver Failure. Acute liver failure in Spain: analysis of 267 cases. *Liver Transpl.* 2007;13(10):1389-1395.

19. Hadem J, Tacke F, Bruns T, et al. Etiologies and outcomes of acute liver failure in Germany. *Clin Gastroenterol Hepatol.* 2012;10(6):664-669 e662.

20. Kayaalp C, Ersan V, Yilmaz S. Acute liver failure in Turkey: a systematic review. *Turk J Gastroenterol.* 2014;25(1):35-40.

21. Takahashi A, Ohira H, Abe K, et al. Increasing incidence of acute autoimmune hepatitis: a nationwide survey in Japan. *Sci Rep.* 2020;10(1):14250.

22. Schramm C, Lohse AW. Autoimmune hepatitis on the rise. *J Hepatol.* 2014; 60(3):478-479.

23. Navasa M, Garcia-Pagan JC, Bosch J, et al. Portal hypertension in acute liver failure. *Gut.* 1992;33(7):965-968.

24. Valla D, Flejou JF, Lebrec D, et al. Portal hypertension and ascites in acute hepatitis: clinical, hemodynamic and histological correlations. *Hepatology.* 1989;10(4):482-487.

25. Lee WM, Hynan LS, Rossaro L, et al. Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. *Gastroenterology.* 2009;137(3):856-864, 864 e851.

26. McPhail MJ. Improving MELD for use in acute liver failure. *J Hepatol.* 2011;54(6):1320; author reply 1320-1321.

27. Wigmore SJ, Walsh TS, Lee A, Ross JA. Pro-inflammatory cytokine release and mediation of the acute phase protein response in fulminant hepatic failure. *Intensive Care Med.* 1998;24(3):224-229.