Biomarkers of macrophage activation and immune danger signals predict clinical outcomes in alcoholic hepatitis

Banishree Saha[1], banishree.saha@abcam.com*; David Tornai[1], David.Tornai@umassmed.edu*

Karen Kodys[1], Karen.Kodys@umassmed.edu; Adeyinka Adejumo[1], acadejumo@partners.org;

Patrick Lowe[1], Patrick.Lowe@umassmed.edu; Craig McClain[2], craig.mcclain@louisville.edu;

Mack Mitchell[3], Mack.Mitchell@UTSouthwestern.edu; Arthur McCullough[4],

MCCULLA@ccf.org; Dasarathi Srinivasan[6], DASARAS@ccf.org; Aimee Kroll-Desrosiers[5],

Aimee.Kroll-Desrosiers@umassmed.edu; Bruce Barton[5], Bruce.Barton@umassmed.edu;

Svetlana Radaeva[6], sradaeva@mail.nih.gov; Gyongyi Szabo[1], Gyongyi.Szabo@umassmed.edu

[1]Department of Medicine, University of Massachusetts Medical School, Worcester, MA

[2]Department of Medicine, University of Louisville, Louisville, KY

[3]Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX

[4]Gastroenterology and Hepatology, Cleveland Clinic, Cleveland, OH

[5]Department of Population and Quantitative Health Sciences, University of Massachusetts Medical School, Worcester, MA

[6]National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD

* These authors contributed equally to this work.

Corresponding Author: Gyongyi Szabo, MD, PhD

Department of Medicine

University of Massachusetts Medical School

364 Plantation Street, Worcester, MA 01605

Tel: 1-508-856-5275 Fax: 1-508-856-4770

Email: Gyongyi.Szabo@umassmed.edu

Supplementary material:

Inclusion and exclusion criteria for AH patients

Inclusion criteria were: ability to provide informed consent by the subject or appropriate family member, age 21-70, recent alcohol consumption >50 g/d for >6 months, continuing within two months before enrollment. The patients should show at least two of the following symptoms of AAH: anorexia, nausea, right upper quadrant pain, liver biopsy diagnostic of AAH or at least two of the following: jaundice, leukocytosis, or hepatomegaly. Inclusion criteria also included elevation of AST >80, but <500 U/L; AST>ALT and ALT<200 U/L; total bilirubin >3 mg/dL; ultrasound or CT scan suggesting fatty infiltration of the liver or liver biopsy showing AH. Exclusion criteria were: hypotension with BP <80/50 after volume repletion; pregnancy; incarceration; < 18 years old; chronic alcohol abuse; consumption of large quantities of alcohol 48 hours prior to blood donation; on any chronic medication; mentally handicapped; inability to provide consent or lack of consent from an appropriate family member; and/or signs of systemic infection and chronic diseases.

Diagnosis of cirrhosis

Information about the presence of cirrhosis was available in the previous clinical documentation of the patients in 81 cases (15 non-cirrhotic and 66 cirrhotic patients). The diagnoses were mainly based on imaging technologies (ultrasound, CT) and only 8 patients had biopsy confirmation.

Diagnostic criteria of adverse events

Bacteremia: positive blood culture. Sepsis: 2 points change in SOFA score. Spontaneous bacterial peritonitis: in ascitic fluid WBC count is more than 500 or absolute PMN is more than 250 or positive culture. Urinary tract infection: in culture of urine sample more than 100,000 colonies/cc or WBC > 20 while on antibiotics. Pneumonia: new infiltration detected by chest x-ray or CT scan. Acute kidney injury: more than 1.5 times increase in creatinine level within 7 days or 0.3 mg/dl absolute increase in creatinine level within 48 hours or less than 500 ml/24 hours urine output. Cardiovascular insufficiency: less than 90mmHg systolic blood pressure, acute myocardial infarct defined by EKG changes and elevation in troponin or acute onset acute congestive heart failure with pulmonary edema. Pulmonary insufficiency: PaO_2 less than 60mmHg or diffuse infiltrates by chest x-ray or CT scan.

Supplementary Figure Legends

Supplementary Figure 1. Patient selection and study design. (A) Flow chart of patient selection and study design for the study. (B) Diagram showing how chronic alcohol consumption leads to increased gut permeability allowing the entry of bacteria and bacterial components (LPS, 16S rDNA) to the portal and systemic circulation. Increased amount of LPS binds to LBP and sCD14 and can signal through the TLR4 pathway leading to activation of macrophages.

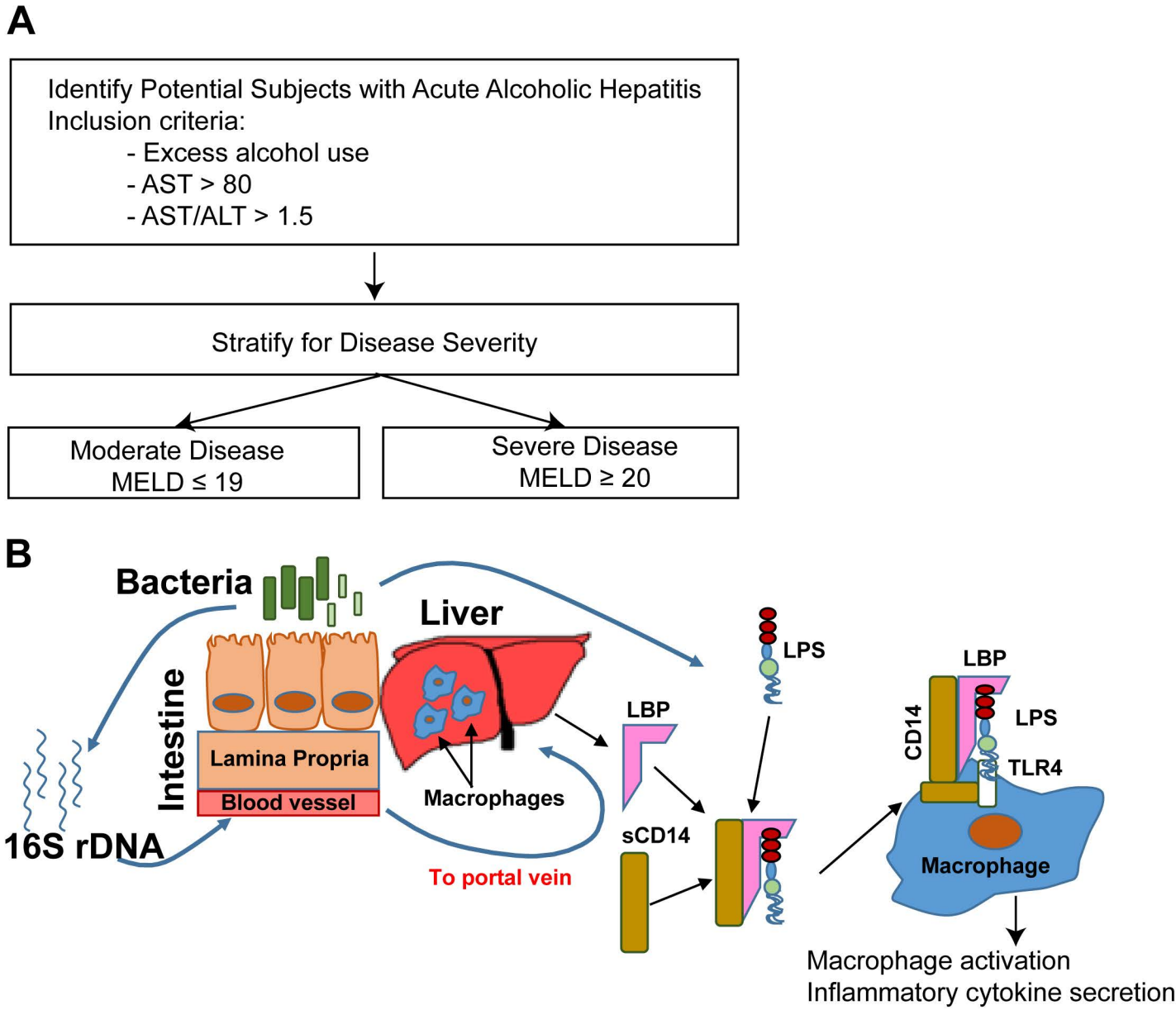
Supplementary Figure 2. Infection and organ failure development in a subpopulation of patients. From the 45 patients we had information about regarding infection and organ failure development 14 patient had infection, 1 organ failure and 5 death as a first event. 11 patients developed organ failure after infection and 9 of them died.

Supplementary Table 1. Baseline characteristics of patients with acute alcoholic hepatitis. Clinical and laboratory parameters of alcoholic hepatitis patients together and grouped by diseases severity as evaluated by MELD score. Data are displayed as n (%) or mean ± SE.

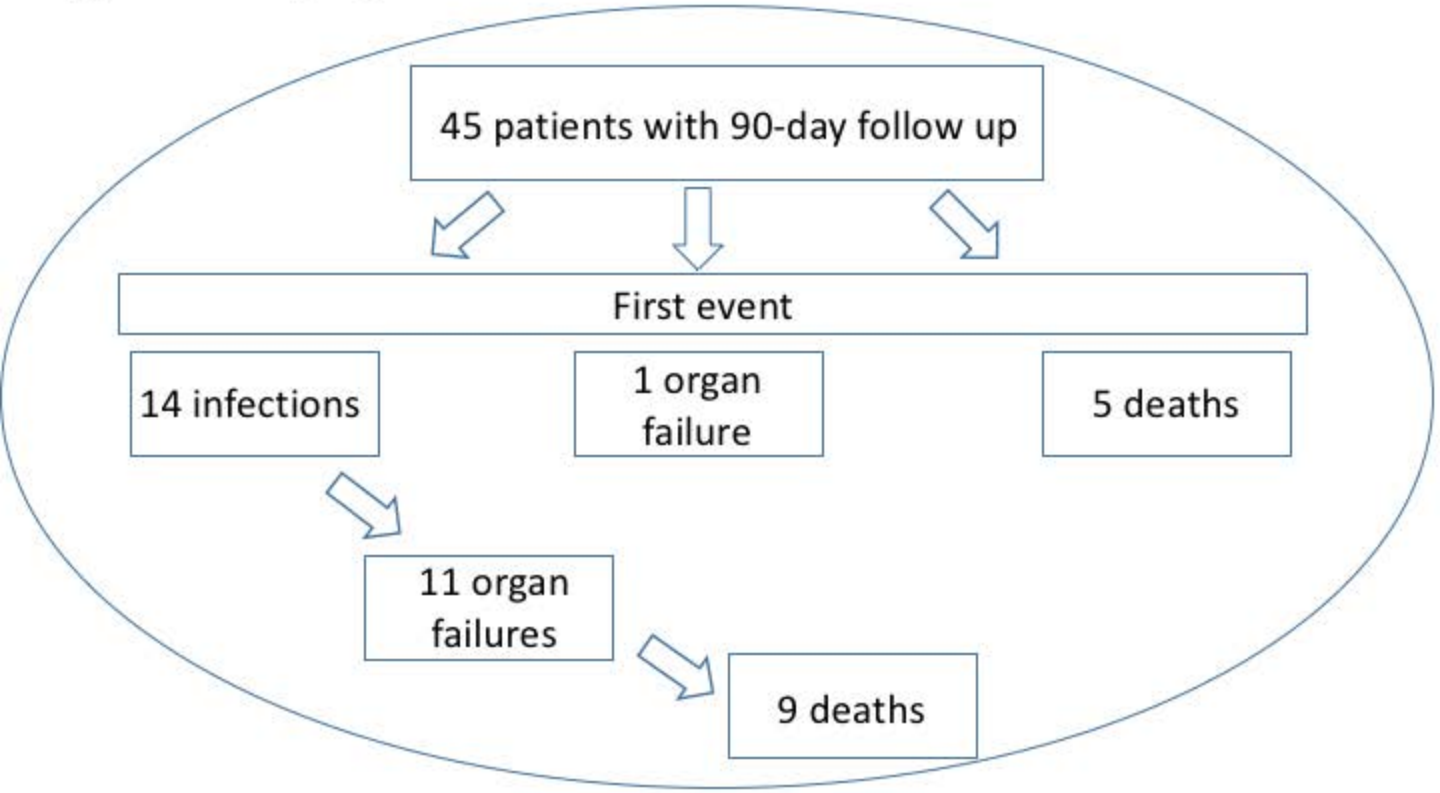
Supplementary Table 2. Ability of the measured markers to predict 90-day mortality. ROC (A) analyses were preformed to test efficiency of the markers in 90days mortality prediction and identify best cut-off values. Area under the curve (AUROC) and p values were calculated. Cut-off values and the corresponding sensitivity and specificity are given. Kaplan-Meier estimator with Log-Rank test (B) were preformed to test the survival function. The percentages of deaths per group are given with Log-Rank p value.

Supplementary Table 3. Ability of the measured markers to predict 90-day infection and organ failure development. ROC (A) analyses were preformed to test efficiency of the markers in 90days infection development prediction and identify best cut-off values. Area under the curve (AUROC) and p values were calculated. Cut-off values and the corresponding sensitivity and specificity are given. When tested for organ failure development the analyses displayed the same cut-off levels. Aalen-Johansen estimator was used to test the cumulative incidence function of infection (B) and organ failure development (C) taking mortality under account as a competing risk. The percentages of the first adverse events per group are given with Log-Rank p value for the event of interest.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Table 1

	Total	MELD ≤19	MELD ≥20
Frequency (count), n	89	43 (48%)	46 (52%)
Age, y	47.87 ± 1.04	48.12 ± 1.57	47.63 ± 1.39
Sex(male), n(%)	53 (62%)	23 (55%)	30 (70%)
MELD	19.76 ± 0.83	13.09 ± 0.62	26.02 ± 0.70
Creatinine, mg/dL	0.81 ± 0.07	0.52 ± 0.08	0.88 ± 0.08
INR	1.81 ± 0.08	1.39 ± 0.15	1.93 ± 0.08
Albumin, g/L	2.50 ± 0.10	2.54 ± 0.23	2.50 ± 0.11
Bilirubin, mg/dL	11.66 ± 1.05	4.32 ± 0.74	18.67 ± 1.18
AST, IU/L	129.97 ± 13.26	130.14 ± 39.24	129.92 ± 13.65
ALP, IU/L	174.94 ± 11.78	235.43 ± 28.87	158 ± 10.79
WBC, $10^3/mm^3$	10.14 ± 0.77	8.63 ± 1.16	11.26 ± 1.00
90 days Mortality, n(%)	15 (17%)	1 (2%)	14 (30%)

Supplementary Table 2A

	AUROC	P value	Cut-off (ng/ml)	Sensitivity (%)	Specificity (%)
sCD14	0.7151	0.0090	>4250.99	53.33	84.93
sCD163	0.6749	0.0336	>2217.23	93.33	43.84
sCD206	0.6941	0.0184	>1805.88	86.67	60.27
OPN	0.7394	0.0048	>518.41	92.86	54.93
HMGB1	0.5513	0.5492	-	-	-
LBP	0.623	0.1469	-	-	-
Endotoxin	0.5029	0.9724	-	-	-
16S rDNA	0.513	0.8820	-	-	-

Supplementary Table 2B

	Low marker level	High marker level	Log Rank p
sCD14	10.61%	42.11%	0.003
sCD163	3.23%	25.93%	0.035
sCD206	4.55%	31.71%	0.006
OPN	2.63%	31.11%	0.012

Supplementary Table 3 A

	AUROC	P value	Cut-off (ng/ml)	Sensitivity (%)	Specificity (%)
sCD14	0.6161	0.2112	-	-	-
sCD163	0.8575	0.0001	>2277.62	93.33	75.86
sCD206	0.8644	<0.0001	>1436.99	86.67	82.76
OPN	0.9039	<0.0001	>432.74	92.86	72.41
HMGB1	0.6084	0.2568	-	-	-
LBP	0.6995	0.0358	>21.05	50	89.66
Endotoxin	0.5057	0.9506	-	-	-
16Sr DNA	0.5431	0.6501	-	-	-

Supplementary Table 3 B

	Low marker level		High marker level		Infection Log Rank p
	Infection	Death before inf.	Infection	Death before inf.	
sCD163	4.55%	4.55%	56.52%	17.39%	0.0001
sCD206	8.33%	0%	57.14%	23.81%	0.0003
OPN	9.09%	0%	52.17%	21.74%	0.0014
LBP	18.18%	6.06%	66.67%	25.00%	0.0023

Supplementary Table 3 C

	Low marker level		High marker level		Organ failure Log Rank p
	Organ failure	Death before OF.	Organ failure	Death before OF.	
sCD163	4.55%	4.55%	47.83%	17.39%	0.0007
sCD206	4.17%	0%	52.38%	23.81%	0.0002
OPN	4.55%	0%	47.83%	21.74%	0.0008
LBP	21.21%	6.06%	41.67%	25.00%	0.1325