Supporting Information

SYSTEMIC INFLAMMATION IN DECOMPENSATED CIRRHOSIS. CHARACTERIZATION AND ROLE IN ACUTE-ON-CHRONIC LIVER FAILURE.

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Diagnostic criteria of organ failure

Diagnostic criteria of organ failure were based on the CLIF-C organ failure score (1): Liver failure: serum bilirubin ≥12 mg/dl; Renal failure: serum creatinine ≥2 mg/dl; Cerebral failure: Grade III-IV hepatic encephalopathy (West-Haven classification) (2); Coagulation failure: INR ≥2.5; Circulatory failure: use of vasoconstrictors to treat severe arterial hypotension (use of vasoconstrictors for the treatment of type-1 hepatorenal syndrome not included); Respiratory failure: PaO2/FiO2 ≥214 or SpO2/FiO2 ≥200. Renal dysfunction was diagnosed in patients with serum creatinine values between 1.5 and 1.9 mg/dL. Cerebral dysfunction was diagnosed in patients with Grade I-II encephalopathy (3). Type 1 ACLF defines the presence of (a) renal failure alone; (b) liver, coagulation or respiratory failure alone when associated with renal and/or cerebral dysfunction; (c) cerebral failure alone when associated with renal dysfunction (3). Single non-renal organ failure without cerebral and/or renal dysfunction was, therefore, not considered as ACLF-1. Type 2 and Type 3 ACLF define the presence of 2 and 3 to 6 organ failures, respectively (3).

Supplementary methods

Measurement of plasma cytokines

Cytokine levels were determined in 25 μl of plasma using a multiplexed bead-based immunoassay (Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (Merck Millipore, Darmstadt, Germany)) on a Luminex 100 Bioanalyzer (Luminex Corp., Austin, TX). The readouts were analyzed with the standard version of the Milliplex Analyst software (Merck Millipore). A five-parameter logistic regression model was used to create standard curves (pg/mL) and to calculate the concentration of each sample. The intra-assay and inter-assay coefficients of variation are indicated in **Supplementary Table 1**.

The main biological effects of the cytokines included in the main analysis are described in **Supplementary Table 4** (4-19).

Criteria used to select the redox state of albumin as marker of systemic inflammation and methods of measurement of albumin oxidation.

The redox state of albumin was selected as marker of systemic inflammation because in our experience (Öttle and Stauber, unpublished observations) it does not require sample preparation and is stable for more than 2 years in samples obtained in EDTA tubes and kept at -70ºC. Other biomarkers of systemic oxidative stress (isoprostanes, myeloperoxidase, malondialdehyde, nitrotyroxine and total antioxidant capacity) require sample preparation prior to storage, are influenced by storage and/or time to analysis or have not been studied regarding long-term stability (20-22). The methodology used was as follows: albumin oxidation fractions were separated by high performance liquid chromatography (HPLC) and detected by fluorescence. Briefly, plasma was diluted 1:100 to a final volume of 1 ml in sample-buffer (0.1 M sodium phosphate, 0.3 M sodium chloride, pH 6.87) and filtered through a Whatman 0.45 µm nylon filter (Bartelt Labor-&Datentechnik, Graz, Austria). Twenty µL of the filtrate were injected into a high performance liquid chromatography (HPLC) system and separated on a Shodex Asahipak ES-502N 7C anion exchange column (7.5 x 100 mm, Bartelt Labor-&Datentechnik) with 50 mM sodium acetate, 400 mM sodium sulfate, pH 4.85 as the mobile phase. For elution, a gradient of 0 to 6% ethanol and a flow rate of 1 ml/min were used. The column was kept at 35°C. Detection was carried out by fluorescence at 280/340 nm. Quantification was based on the area of the individual peaks as determined by fitting Gaussian functions to the peaks using the Peak Fit software (SPSS Science, Chicago, IL).

Criteria used to select plasma renin concentration (PRC) and plasma copeptin concentration (PCC) as markers of systemic circulatory dysfunction (SCD) and methods of measurements.

PRC and PCC were selected to estimate systemic circulatory function because they are very sensitive to changes in effective arterial blood volume in cirrhosis (23,24). Other measurements estimating systemic circulatory function (ADH, renin activity and plasma neuropeptide Y) were not considered because they are affected by long-term storage (25,26). Plasma norepinephrine was not considered because it is frequently used in the management of patients with ACLF.

PRC was measured using the chemiluminescent immunoassay Liaison Direct Renin (DiaSorin; Saluggia, Italy). The test was performed on the LIAISON Analyzer (DiaSorin). The intra-assay and inter-assay coefficients of variation were <5% and <7%, respectively, for all the samples tested. PCC was measured using an immunoassay in a chemiluminescence-coated tube format (B.R.A.H.M.S., Kryptor, GmbH, Henningsdorf, Germany).

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Supplementary Figure Legends

Supplementary Figure 1: Pro-inflammatory/anti-inflammatory ratios as estimated by interleukin-6 (IL-6)/IL-10 and IL-8/IL-10 ratios in healthy subjects, patients with decompensated cirrhosis without acute-on-chronic liver failure (ACLF) and in patients with decompensated cirrhosis with ACLF. Data are mean ± SEM and P values are given as compared to healthy subjects.

Supplementary Figure 2: Upper Panel). Scatter graph of individual patients classified by the presence (red dots) or absence (green dots) of acute-on-chronic liver failure (ACLF) according to individual values of plasma renin concentration (PRC) and interleukin-6 (IL-6). The cut-off points for each marker divide the whole cohort into 3 tertiles (T1: lower values; T2: intermediate values; T3: higher values). Patients with hepatorenal syndrome (HRS) were predominantly in the right part of the panel. Lower Panel: Frequency of ACLF across the three tertiles of both PRC and IL-6.

Supplementary Figure 3: Upper Panels (A and B). Scatter plots of patients without active alcoholism and with (red dots) or without (green dots) acute-on-chronic liver failure (ACLF) according to individual values of plasma renin concentration (PRC) and interleukin-8 (IL-8) (left) or human nonmercaptalbumin-2 (HNA2) (right). Two cut-off points for each marker divide each cohort into 3 tertiles (T1: lower values; T2: intermediate values; T3: higher values). Patients with ACLF are predominantly in the right part of the panels (higher degree of SI). Lower Panels (C and D) show the presence of ACLF within each tertile of both PRC and IL-8 and PRC and HNA2.

Supplementary Figure 4: Upper Panels (A and B). Scatter plots of patients with (red dots) or without (green dots) acute-on-chronic liver failure (ACLF) with renal failure (ACLF-RF) according to individual values of plasma renin concentration (PRC), interleukin-8 (IL-8) (left) or human nonmercaptalbumin-2 (HNA2) (right). Two cut-off points for each marker divide each cohort into 3 tertiles (T1: lower values; T2: intermediate values; T3: higher values). Patients with ACLF-RF are predominantly in the right part of the panels (higher degree of SI). Lower Panels (C and D) show the presence of ACLF-RF within each tertile of both PRC and IL-8 and PRC and HNA2.

Supplementary Figure 5: Scatter plots of individual values of plasma renin concentration (PRC), interleukin-8 (IL-8), interleukin-6 (IL-6) and human nonmercaptalbumin-2 (HNA2) in patients with ACLF without active alcoholism (A) and with acute-on-chronic liver failure (ACLF) associated with renal failure without active alcoholism (B). Horizontal lines represent the upper normal limits (95% percentile of the distribution of values in healthy subjects).

Supplementary Figure 6: Upper Panels (A and B). Scatter plots of patients who died (red dots) or survived (green dots) at 90 days after enrolment according to individual values of plasma renin concentration (PRC) and interleukin-8 (IL-8) (left) or human nonmercaptalbumin-2 (HNA2) (right). Two cut-off points for each marker divide each cohort into 3 tertiles (T1: lower values; T2: intermediate values; T3: higher values). Patients who died are predominantly in the right part of the panels (higher degree of SI). Lower Panels (C and D) show the 90-day mortality within each tertile of both PRC and IL-8 and PRC and HNA2.

Supplementary Table 1: Intra-assay and inter-assay coefficients of variability (CV) of the 29-cytokine assay.

	Intra-assay %CV	Inter-assay %CV
IFNα2	2.4	13.3
IL-12P40	2.8	12.4
IL-12P70	2.2	16.7
IL-13	2.2	9.2
IL-15	2.7	8.1
IL-1α	3.3	12.8
IL-1β	2.3	6.7
IL-2	2.1	6.3
IL-3	3.4	6.1
IL-4	2.9	14.2
IL-5	2.6	10.8
MIP-1α	1.9	14.5
TNFβ	1.6	11.4
EGF	2.3	5.8
VEGF	3.7	10.4
TNFα	2.6	13.0
IL-6	2.0	18.3
IL-8	1.9	3.5
MCP-1	1.5	7.9
IP-10	2.6	15.3
MIP-1β	2.4	8.8
G-CSF	1.8	15.5
GM-CSF	3.1	10.1
IL-10	1.6	16.8
IL-1ra	2.1	10.7
IFNγ	1.6	12.0
Eotaxin	7.2	10.8
IL-17a	2.2	7.9
IL-7	1.7	16.1

Intra-assay precision was obtained from the mean of the % CV's from sixteen reportable results across two different concentrations of cytokines in a single assay. Inter-assay precision was obtained from the mean of the % CV's from four reportable results across two different concentrations of cytokines across six different experiments.

Supplementary Table 2. Limit of detection for each cytokine included in the study and percentage of samples with undetectable levels.

	Limit of detection (pg/ml) *	**Healthy subjects (N=40)**	**Cirrhotic patients without ACLF (n=285)**	**Cirrhotic patients with ACLF (n=237)**
Cytokines with undetectable levels in less than 30% of samples:				
TNFα	0.7	0	0	0
IL-6	0.3	87.5%	7.4%	0.8%
IL-8	0.4	10.0%	0	0
MCP-1	1.9	0	0	0
IP-10	8.6	0	0	0.4%
MIP-1β	3.0	0	0.7%	0
G-CSF	1.8	20.0%	2.5%	0.8%
GM-CSF	7.5	85.0%	4.2%	3.0%
IL-10	1.1	60.0%	5.6%	7.6%
IL-1ra	8.3	7.5%	0	0.8%
IFNγ	0.8	40.0%	0	1.3%
Eotaxin	4.0	17.5%	0.7%	0.4%
IL-17a	0.7	52.5%	1.1%	0.4%
IL-7	1.4	80.0%	9.1%	9.3%
EGF	2.8	17.5%	27.0%	31.6%
VEGF	26.3	65.0%	22.8%	16.9%
IFNα2	2.9	87.5%	19.3%	12.2%
Cytokines with undetectable levels in more than 30% of samples:				
IL-12P40	7.4	87.5%	71.2%	65.8%
IL-12P70	0.6	65.0%	66.3%	55.7%
IL-13	1.3	90.0%	83.5%	77.2%
IL-15	1.2	92.5%	70.2%	42.2%
IL-1α	9.4	72.5%	44.2%	30.4%
IL-1β	0.8	90.0%	83.2%	81.9%
IL-2	1.0	82.5%	74.4%	70.9%
IL-3	0.7	80.0%	96.8%	97.0%
IL-4	4.5	97.5%	77.9%	75.5%
IL-5	0.5	22.5%	81.1%	81.4%
MIP-1α	2.9	82.5%	34.7%	26.6%
TNFβ	1.5	85.0%	78.6%	73.8%

* The lower limit of detection was calculated…….

Supplementary Table 3. Patients' characteristics.

	No ACLF N=285	ACLF N=237	ACLF – I N=126	ACLF – II N=86	ACLF – III N=25
Age (y)	57±12	57±11	59±11	55±11	53±10*
Gender(Male)	191(67.0)	153(64.6)	85(67.5)	55(64.0)	13(52.0)
Etiology					
Alcohol	128(47.6)	128(57.7)*	67(58.3)	49(59.0)	12(50.0)
HCV	66(24.5)	41(18.5)	22(19.1)	16(19.3)	3(12.5)
Alcohol + HCV	26(9.7)	23(10.4)	11(9.6)	9(10.8)	3(12.5)
Time from previous decompensation					
No previous decompensation	83(31.7)	53(25.6)	22(20.2)	25(32.9)	6(27.3)*
Less than 3 months	21(8.0)	30(14.5)	16(14.7)	7(9.2)	7(31.8)
More than 3 months	158(60.3)	124(59.9)	71(65.1)	44(57.9)	9(40.9)
Clinical Features					
Ascites[1]	211(74.0)	226(95.4)^	119(94.4)	83(96.5)	24(96.0)
Gastrointestinal hemorrhage	54(19.0)	31(13.1)	13(10.3)	15(17.4)	3(12.0)
Mean Arterial Pressure (*mm Hg*)	84±12	81±13*	82±13	81±13	75±11*
SIRS	31 (10.9)	47 (19.9)*	17 (13.5)	20 (23.3)	10 (41.7)^
Potential precipitating events (PE)					
Bacterial Infection	61(21.4)	80(34.2)*	38(30.7)	29(34.1)	13(52.0)
Active alcoholism within the past 3 months	33(12.5)	39(17.8)	14(12.1)	19(24.4)	6(24.0)
Acute Alcoholic Hepatitis	18 (6.3)	29 (12.2)*	11 (8.7)	14 (16.3)	4 (16.0)
Other PE[2]	16(5.6)	28(11.8)*	12(9.5)	14(16.3)	2(8.0)
No PE	131(48.5)	94(41.8)	57(47.5)	29(36.3)	8(32.0)
More than one PE	27(10.0)	37(16.4)*	17(14.2)	16(20.0)	4(16.0)
Laboratory data					
WBC (x*10^9/L*)[3]	6.1(4.1-9.0)	7.9(5.3-11.8)^	7.1(5.0-10.2)	9.5(5.8-13.7)	11.2(5.7-19.0)^
Serum bilirubin (*mg/dL*)[3]	3.0(1.6-6.7)	6.0(2.1-14.5)^	2.9(1.4-8.4)	10.6(4.8-19.7)	24.4(5.8-30.0)^
INR[3]	1.5(1.3-1.7)	1.8(1.4-2.3)^	1.5(1.3-1.9)	2.0(1.6-2.6)	2.5(1.9-3.5)^
Serum creatinine (*mg/dL*)[3]	0.9(0.7-1.3)	1.7(0.9-2.7)^	1.9(1.1-2.9)	1.1(0.8-2.2)	1.9(1.1-3.4)*
Albumin (g/dL)	2.9±0.6	2.8±0.7	3.0±0.6	2.7±0.7	2.6±0.7*
C-reactive protein (*mg/L*)[3]	18 (6-35)	27 (12-20)*	33 (19-62)	18 (4-30)	51 (31-69) *
Platelet count (x*10^9/L*)[3]	87(57-139)	78(52-120)	84(55-141)	75(53-113)	64(30-104)
Sodium (*mmol/L*)	136±5	133±7^	134±7	133±6	134±10
IgG (g/L) [3]	16 (12-20)	15 (11-18)	14 (11-17)	16 (11-19)	17 (15-19)
IgA (g/L) [3]	4.7 (3.0-6.6)	5.1 (3.3-8.0)	4.8 (2.7-6.1)	6.8 (3.4-8.8)	5.1 (3.9-9.1)
IgM (g/L) [3]	1.4 (0.9-2.1)	1.2 (0.8-2.2)	1.1 (0.6-1.8)	1.4 (0.8-2.6)	2.3 (0.9-3.1)
Organ failures/dysfunction					
Liver	31(10.9)	79(33.3)^	22(17.5)	41(47.7)	16(64.0)^
Kidney	-	104(43.9)	61(48.4)	28(32.6)	15(60.0)*
Cerebral	6(2.1)	37(15.6)^	5(4.0)	21(24.4)	11(44.0)^
Coagulation	10(3.5)	46(19.4)^	8(6.4)	26(30.2)	12(48.0)^
Circulation	3(1.1)	31(13.1)^	2(1.6)	15(17.4)	14(56.0)^
Lungs	2(0.7)	15(6.3)^	4(3.2)	6(7.0)	5(20.0)*
Kidney dysfunction	51(17.9)	30(12.7)	17(13.5)	9(10.5)	4(16.0)
Mild to moderate hepatic encephalopathy	69(24.3)	86(36.3)*	55(43.7)	21(24.4)	10(40.0)*
Scores					
Child-Pugh	9.1±1.9	10.7±2.1^	9.7±1.9	11.5±1.8	12.4±1.8^
MELD	17±5	25±7^	23±6	27±7	32±8^
MELDna	19±6	27±7^	25±6	29±7	33±7^
CLIF-C OF	7.1±1.1	9.6±2.3^	8.4±1.3	10.0±1.8	12.5±3.3^
Mortality rates (%)					
28 days	6(2.1)	65(27.4)^	26(20.6)	22(25.6)	17(68.0)^

90 days	29(10.3)	99(42.5)^	41(33.6)	40(46.5)	18(72.0)*
180 days	45(16.4)	122(52.8)^	57(47.1)	46(54.1)	19(76.0)*
365 days	74(35.9)	143(70.1)^	73(67.0)	50(69.4)	20(87.0)

[1]Diagnosed by the presence of clinically detectable ascites at enrolment or of surrogates of ascites (diuretic treatment, SBP, therapeutic paracentesis prior to or after enrolment).

[2]Other precipitating event (PE) was defined by the presence prior to or at enrolment of one of the following: transjugular intrahepatic portosystemic shunting, major surgery, therapeutic paracentesis without use of intravenous albumin or hepatitis.

[3]Data are median (IQR). Comparison between NO ACLF vs ACLF and among grades of ACLF: *p-value < 0.05; ^p-value < 0.001

Supplementary Table 4: Predominant biological properties of the 17 cytokines with detectable values in more than 70% of samples.

Gene symbol*	Protein name**	Protein short name**	Family	Target cells***	Function***	Reference
TNF (aliases TNFA, TNFSF2)	Tumor necrosis factor	TNFα	TNF superfamily	Neutrophils, macrophages, monocytes, endothelial cells	Innate immune response and inducer of local and systemic inflammation; promotes activation and production of acute-phase proteins	4
IL6	Interleukin-6	IL-6	Hematopoietin	Wide variety of cells: B cells, T cells, thymocytes, myeloid cells, osteoclasts	Inflammatory and co-stimulatory action; induces proliferation and differentiation; synergizes with TGF-β to drive Th17; promotes activation and production of acute-phase proteins	5
CXCL8	Interleukin-8	IL-8	Chemokine, CXC subfamily	Neutrophils, monocytes, mast cells	Proinflammatory mediator, chemotactic factor for neutrophils, induces respiratory burst and phagocytosis.	6
CCL2	C-C motif chemokine 2	MCP-1	Chemokine, CC subfamily	Monocytes, basophils, memory T cells, pDC	Pro-inflammatory; chemotactic activity for monocytes and basophils	7
CXCL10	C-X-C motif chemokine 10	IP-10	Chemokine, CXC subfamily	Monocytes, NK and T-cells	Inflammatory chemokine, associated with hepatitis C virus and autoimmunity; induced by interferons	8
CCL4	C-C motif chemokine 4	MIP-1-β	Chemokine, CC subfamily	Immature mDC, monocytes, Th1, Treg, NK cells, pDC	Chemokine with chemotactic properties for monocytes and natural killer cells, induces IL-1β, IL-6 and TNFα.	9
CSF3 (alias GCSF)	Granulocyte colony-stimulating factor	G-CSF	Hematopoietin	Committed progenitors	Differentiation and activation of granulocytes	10
CSF2	Granulocyte-macrophage colony-stimulating factor	GM-CSF	Hematopoietin	Committed progenitors	Differentiation and activation of granulocytes and monocytes	11
IL10	Interleukin-10	IL-10	IL-10 family	Macrophages, T cells, dendritic cells, B cells	Immune suppression; decreases antigen presentation and MHC class II expression of dendritic cells; down-regulates pathogenic Th1, Th2, and Th17 responses	12
IL1RN	Interleukin-1 receptor antagonist	IL-1ra	IL-1 family	Cells, monocytes and endothelial cells	IL-1ra and the soluble decoy receptor complex (composed of IL1R2 and IL1RAP) inhibit IL-1-mediated inflammatory responses	13
IFNG	Interferon gamma	IFNγ	Interferon family; type 2	Macrophages, NK cells, T cells, others	Promotes activation of APCs and cell-mediated immunity; increased MHC class II expression	14

IFNA2	Interferon alpha 2	IFNα2	Interferon family; type 1	NK cells, many others	Promotes resistance to viral pathogens; promotes increased expression of MHC class I; involved in auto-immunity	14
CCL11	Chemokine (C-C Motif) Ligand 11	Eotaxin	Chemokine, CC subfamily	Eosinophils	Chemotactic factor mainly involved in eosinophil recruitment and allergic responses.	15
IL17A	Interleukin-17A	IL-17a	IL-17 family	Mucosal tissues, epithelial and endothelial cells	Proinflammatory; protective immunity in lung; tight junction integrity; promotes mobilization of neutrophils and cytokine production by epithelial cells; promotes angiogenesis	16
IL7	Interleukin-7	IL-7	Hepatopoietin	B cells, T cells, thymocytes	Homeostasis, differentiation, and survival (in particular regulatory T cells (Tregs))	17
EGF	Epidermal Growth Factor	EGF	EGF family	Wide variety of cells	Epithelial cell growth. Stimulates cytokine release.	18
VEGFA	Vascular Endothelial Growth Factor A	VEGF-A	PDGF family, VEGF subfamily	Endothelial cells	Angiogenesis and vascular permeability. Leukocyte adhesion.	19

*From Hugo Gene Nomenclature Committee

**From UniProt (http://www.uniprot.org/)

***Abbreviations: TGFβ, transforming growth factor-β; Th17, IL-17-producing helper T cells; Th1, T helper 1 cells; Th2, T helper 2 cells; pDC, plasmacytoid dendritic cells (DC); mDC, myeloid DC (also called conventional or classical DC); Treg, regulatory T cells; NK, natural killer cells; APCs, antigen-presenting cells

Supplementary Table 5. Plasma concentrations (pg/mL) of the cytokines with detectable values in less than 70% of patients. Relationship with the presence of ACLF.

Cytokines (number of subjects with data)	Healthy controls	No ACLF	ACLF	p-value*
Pro-inflammatory Cytokines				
IL-1α (11/159/165)	9.1 (1.8-56.3)	31.9 (5.7-111.8)	20.5 (7.4-81.2)	0.5751
IL-1β (4/48/43)	3.3 (1.2-4.0)	8.8 (4.1-28.9)	6.7 (3.7-12.7)	0.2554
IL-12P40 (5/82/81)	12.7 (5.0-31.1)	29.4 (8.5-86.6)	29.6 (14.5-72.7)	0.8050
IL-12P70 (14/96/105)	1.9 (0.5-5.5)	9.8 (4.8-34.3)	9.6 (4.9-29.4)	0.5470
MIP-1α (7/186/174)	0.81 (0.18-2.52)	4.7 (1.8-9.8)	7.1 (3.4-15.5)	0.0006
TNFβ (6/61/62)	5.2 (0.1-22.5)	12.2 (2.0-9.5)	4.9 (2.7-22.6)	0.0718
Anti-inflammatory Cytokines				
IL-13 (4/47/54)	14.0 (2.2-26.2)	37.9 (7.3)	16.0 (5.2-65.8)	0.1068
IL-4 (1/63/58)	13.2	16.9 (6.4-82.0)	19.0 (6.9-46.0)	0.8643
Other Cytokines				
IL-2 (7/73/69)	0.5 (0.3-5.6)	5.5 (2.6-16.7)	4.6 (2.1-9.1)	0.5672
IL-3 (8/9/7)	0.06 (0.02-0.15)	4.3 (2.5-8.7)	4.7 (2.5-13.6)	0.9578
IL-5 (31/54/44)	0.34 (0.20-0.58)	4.3 (2.5-8.7)	4.9 (3.0-9.1)	0.3079
IL-15 (3/85/137)	1.6 (1.0-1.6)	5.8 (3.3-13.0)	6.1 (3.7-9.6)	0.8805

Data are median (Inter-Quartile Range, IQR)

*P value between ACLF and NO ACLF.

Supplementary Table 6. Plasma levels of the cytokines (pg/mL) with detectable values in less than 70% of patients. Relationship with the ACLF grades.

Cytokines (number of patients with data)	ACLF – I	ACLF – II	ACLF – III	p-value*
Pro-inflammatory Cytokines				
IL-1α (87/59/19)	21.7 (8.2-96.3)	21.9 (6.6-85.6)	11.3 (8.8-27.3)	0.4129
IL-1β (23/17/3)	7.6 (3.5-12.7)	6.9 (3.7-19.3)	3.7 (3.6-5.5)	0.4088
IL-12P40 (43/30/8)	28.9 (14.3-51.4)	37.2 (16.1-159.7)	36.3 (5.3-104.9)	0.6347
IL-12P70 (52/41/12)	8.1 (4.7-21.2)	14.2 (6.5-38.5)	6.6 (3.7-20.6)	0.1629
MIP-1α (93/62/19)	7.6 (3.4-12.0)	6.3 (3.1-15.5)	8.6 (4.0-22.0)	0.4819
TNFβ (33/25/4)	3.6 (2.7-18.0)	8.0 (2.9-26.5)	7.5 (2.8-86.2)	0.4988
Anti-inflammatory Cytokines				
IL-4 (29/23/6)	16.5 (6.6-28.4)	26.9 (6.0-96.3)	18.8 (10.0-45.1)	0.4586
IL-13 (26/24/4)	10.8 (5.2-40.4)	27.4 (7.1-85.2)	15.0 (7.8-200.5)	0.2651
Other Cytokines				
IL-2 (36/26/7)	4.2 (1.9-8.1)	5.8 (3.4-14.5)	3.2 (1.8-8.4)	0.2187
IL-3 (3/4/0)	4.0 (2.5-4.7)	13.4 (7.8-22.6)	---	0.4108
IL-5 (23/19/2)	4.3 (2.8-8.0)	5.8 (3.2-11.1)	51.4 (3.4-99.5)	0.5309

Data are median (Inter-Quartile Range, IQR)

* Comparison between ACLF grades

Supplementary Table 7. Markers of systemic circulatory dysfunction (SCD), plasma cytokine levels and redox state of albumin in all patients with alcoholic cirrhosis according to the presence of active alcoholism and Acute Alcoholic Hepatitis (AAH).

	Active alcoholism without AAH (n=18)	***Active alcoholism with AAH (n=42)***	***No active alcoholism (n=175)***	***p-value***
Markers of SCD				
PRC (micro IU/ml)	108 (19-268)	111 (32-274)	84 (25-277)	0.8833
PCC (pmol/L)	12 (1-32)	15 (5-36)	16 (5-40)	0.6336
Pro-inflammatory Cytokines				
TNFα (pg/ml)	19 (14-24)	21 (14-31)	23 (15-35)	0.1613
IL-6 (pg/ml)	24 (12-75)	23 (12-44)	29 (16-69)	0.3822
IL-8 (pg/ml)	98 (60-181)	199 (113-344)	56 (30-106)	<0.0001
MCP-1 (pg/ml)	413 (302-635)	500 (354-690)	373 (279-526)	0.0366
IP-10 (pg/ml)	1086 (653-1362)	893 (580-1689)	987 (606-1957)	0.7524
MIP-1β (pg/ml)	27 (16-50)	35 (24-54)	25 (15-44)	0.0531
G-CSF (pg/ml)	32 (24-91)	30 (16-47)	29 (13-70)	0.4161
GM-CSF (pg/ml)	6.8 (4.0-13.9)	6.5 (4.0-12.5)	5.9 (2.4-15.5)	0.5596
Anti-inflammatory Cytokines				
IL-10 (pg/ml)	2.6 (1.2-5.5)	5.9 (2.2-12.5)	4 (1-19)	0.3676
IL-1ra (pg/ml)	18 (9-62)	18 (10-44)	16 (6-58)	0.6236
Other Cytokines				
IFNγ (pg/ml)	16 (4-50)	11 (3-30)	7 (3-29)	0.4725
IFNα2 (pg/ml)	35 (14-74)	16 (5-33)	116 (78-166)	0.1161
Eotaxin (pg/ml)	130 (93-180)	118 (89-197)	6 (2-14)	0.4940
IL-17a (pg/ml)	12.3 (3.1-26.0)	6.0 (1.7-23.4)	3 (1-11)	0.2286
IL-7 (pg/ml)	2.8 (1.3-12.2)	3.8 (1.8-11.4)	27 (8-70)	0.8507
EGF (pg/ml)	19 (5-33)	15 (8-47)	26 (8-71)	0.2188
VEGF (pg/ml)	62 (40-365)	112 (54-276)	99 (35-282)	0.7537
Albumin Oxidation Fractions*				
HMA (%)	52 (42-70)	49 (34-61)	51 (36-61)	0.5060
HNA1 (%)	37.2 (29.5-41.3)	46.9 (34.3-57.3)	41.7 (32.9-54.0)	0.1597
HNA2 (%)	9.2 (4.2-11.4)	5.7 (4.2-9.8)	6.5 (3.3-11.0)	0.6939

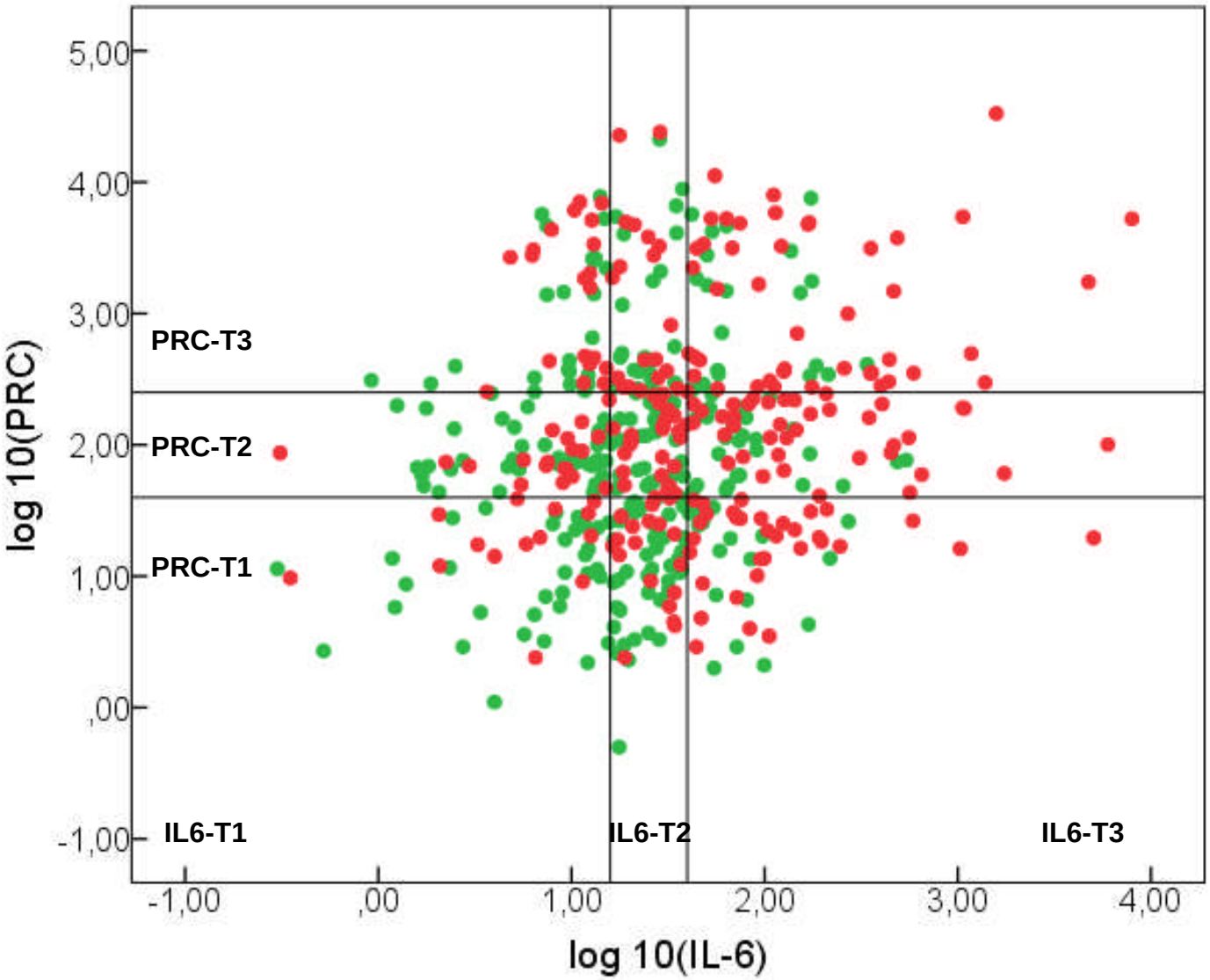
Data are median (Inter-Quartile Range, IQR)

Supplementary Table 8. Plasma concentrations of renin (PRC), copeptin (PCC) and cytokines and albumin oxidation fractions in patients with/without SIRS.

	Patients without SIRS (n=443)	**Patients with SIRS (n=78)**	**p-value**
Markers of SCD			
PRC (micro IU/ml)	80 (24-277)	101 (32-447)	0.1365
PCC (pmol/L)	14 (5-36)	26 (9-45)	0.0126
Pro-inflammatory Cytokines			
TNFα (pg/ml)	22 (15-33)	27 (17-38)	0.1118
IL-6 (pg/ml)	26 (13-53)	61 (20-168)	<0.0001
IL-8 (pg/ml)	50 (25-104)	96 (38-182)	<0.0001
MCP-1 (pg/ml)	352 (247-509)	412 (279-756)	0.0193
IP-10 (pg/ml)	1036 (606-1917)	1005 (624-2005)	0.7744
MIP-1β (pg/ml)	24 (14-40)	30 (16-47)	0.0376
G-CSF (pg/ml)	25 (13-54)	29 (16-93)	0.0520
GM-CSF (pg/ml)	5.5 (2.6-12.5)	6.8 (2.7-16.0)	0.2764
Anti-inflammatory Cytokines			
IL-10 (pg/ml)	4.2 (1.4-13.9)	9.2 (2.9-19.8)	0.0077
IL-1ra (pg/ml)	14 (6-33)	26 (10-71)	0.0003
Other Cytokines			
IFNγ (pg/ml)	6 (2-21)	6 (3-15)	0.8457
IFNα2 (pg/ml)	23 (10-61)	28 (10-59)	0.9013
Eotaxin (pg/ml)	120 (86-165)	101 (72-150)	0.0594
IL-17a (pg/ml)	4.0 (1.6-13.0)	3.0 (1.5-15.2)	0.8516
IL-7 (pg/ml)	2.9 (1.2-8.9)	3.5 (1.5-10.1)	0.4890
EGF (pg/ml)	23 (9-60)	17 (8-45)	0.1950
VEGF (pg/ml)	87 (28-263)	91 (28-202)	0.7148
Albumin Oxidation Fractions*			
HMA (%)	49 (37-60)	51 (40-62)	0.4032
HNA1 (%)	41.2 (33.3-52.2)	39.5 (29.7-48.9)	0.1953
HNA2 (%)	7.0 (3.4-11.6)	7.7 (3.3-12.4)	0.6424

Data are median (Inter-Quartile Range, IQR)

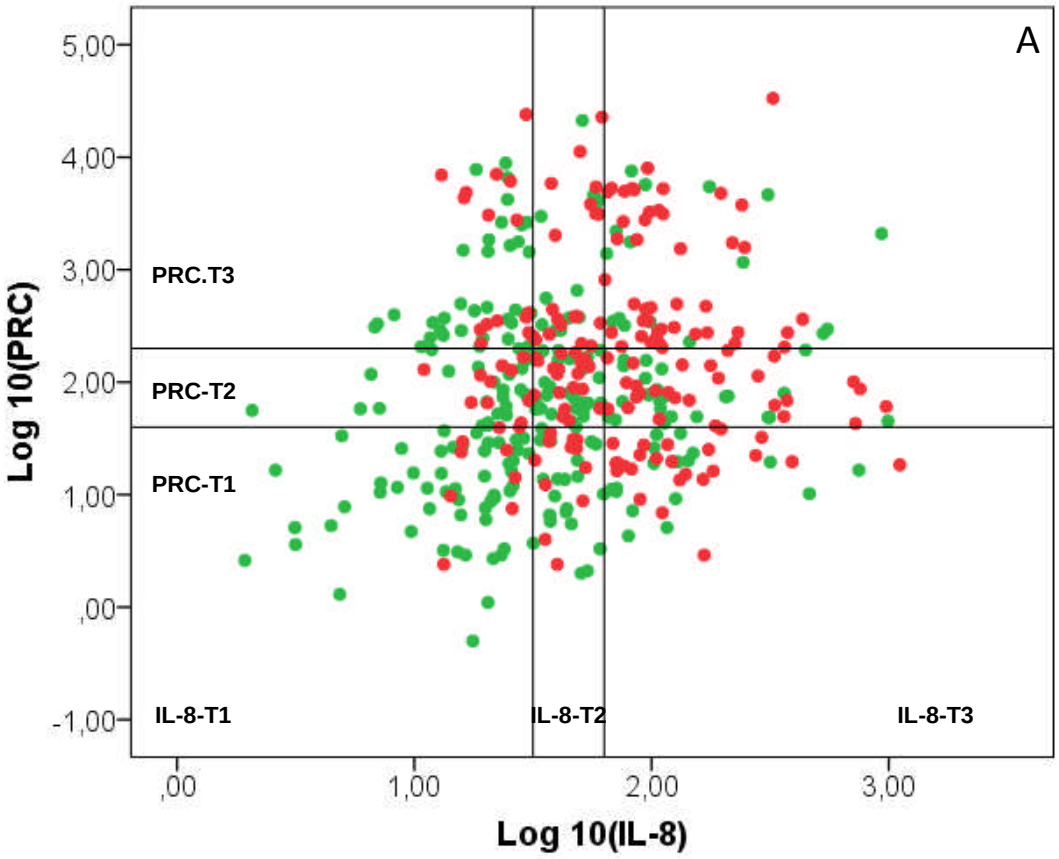
Supplementary Figure 2

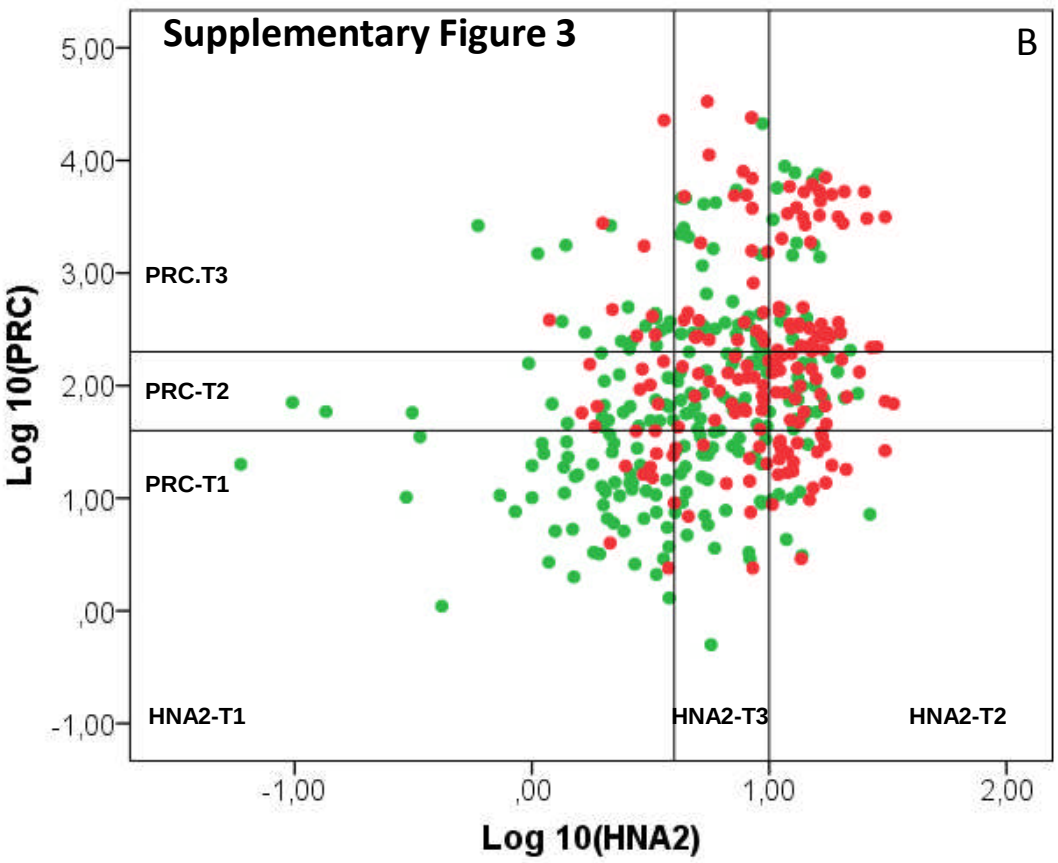


	IL6-T1	IL6-T2	IL6-T3	Overall PRC
PRC-T3	22/48 (46%)	28/54 (52%)	48/68 (71%)	98/170 (58%)*
PRC-T2	20/59 (34%)	25/51 (49%)	29/52 (56%)	74/162 (46%)
PRC-T1	14/51 (27%)	18/59 (30%)	29/46 (63%)	61/156 (39%)
Overall IL-6	56/158 (35%)	71/164 (43%)	106/166 (64%)#	N=488

***p<0.05 vs previous tertile; #p<0.01 vs previous tertile**

Supplementary Figure 3





C

	IL8-T1	IL8-T2	IL8-T3	Overall PRC
PRC-T3	15/50 (30%)	23/36 (64%)	41/55 (75%)	79/141 (56%)*
PRC-T2	10/34 (29%)	21/50 (42%)	25/46 (54%)	56/130 (43%)
PRC-T1	9/61 (15%)	13/35 (37%)	23/39 (59%)	45/135 (33%)
Overall IL-8	34/145 (23%)	57/121 (47%)#	89/140 (64%)#	n=406

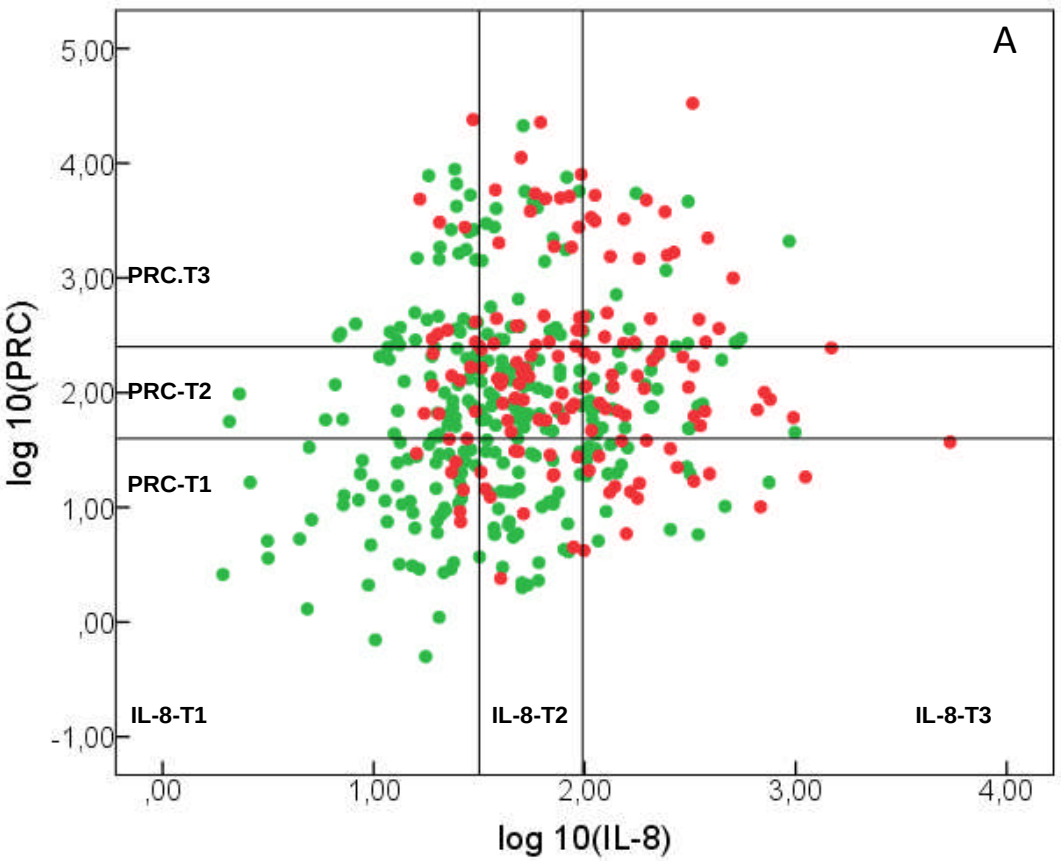
***p<0.05 vs previous tertile; #p<0.01 vs previous tertile**

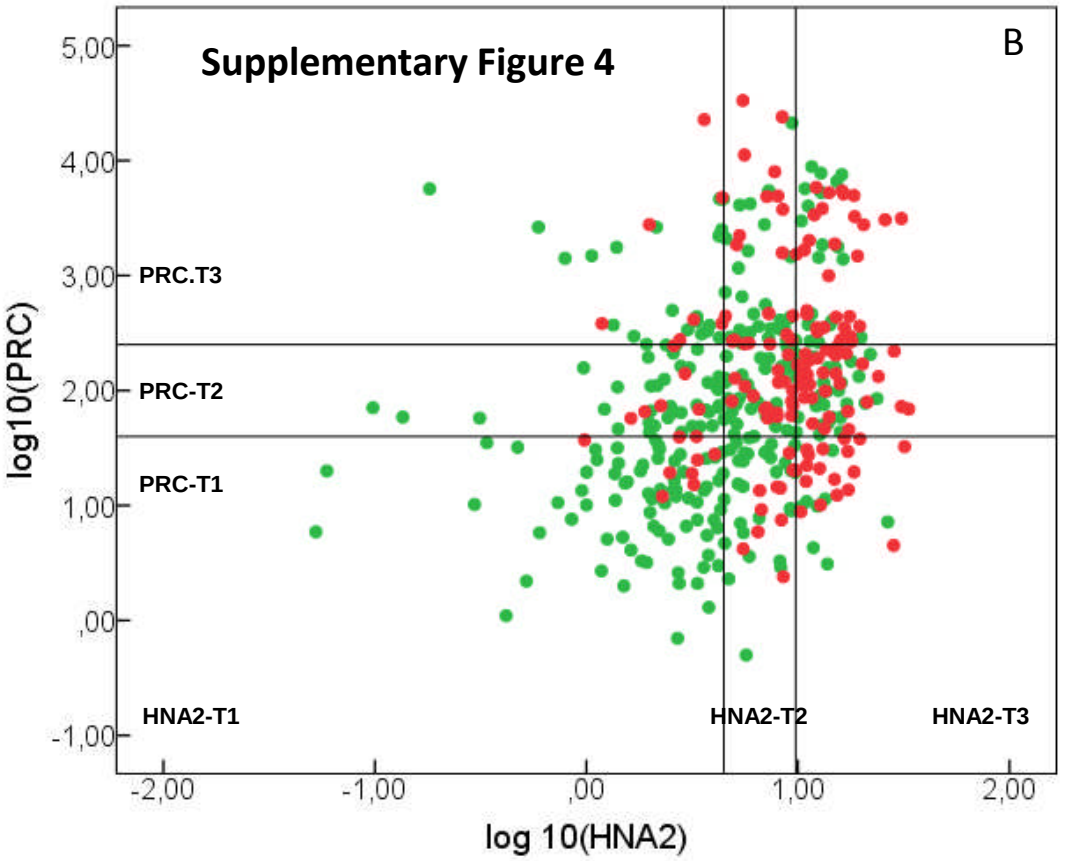
D

	HNA2-T1	HNA2-T2	HNA2-T3	Overall PRC
PRC-T3	8/25 (32%)	25/53 (47%)	46/63 (73%)	79/141 (56%)*
PRC-T2	9/33 (27%)	23/52 (44%)	24/44 (55%)	56/129 (43%)
PRC-T1	10/61 (16%)	11/42 (26%)	22/30 (73%)	43/133 (32%)
Overall HNA2	27/119 (23%)	59/147 (40%)#	92/137 (67%)#	n=406

***p<0.05 vs previous tertile; #p<0.01 vs previous tertile**

Supplementary Figure 4





C

	IL8-T1	IL8-T2	IL8-T3	Overall PRC
PRC-T3	12/48 (25%)	20/41 (49%)	34/51 (67%)	66/140 (47%)#
PRC-T2	9/42 (21%)	19/53 (36%)	23/49 (47%)	51/144 (35%)
PRC-T1	7/63 (11%)	9/42 (21%)	18/38 (47%)	34/143 (24%)
Overall IL-8	28/153 (18%)	48/136 (35%)#	75/138 (54%)#	n=427

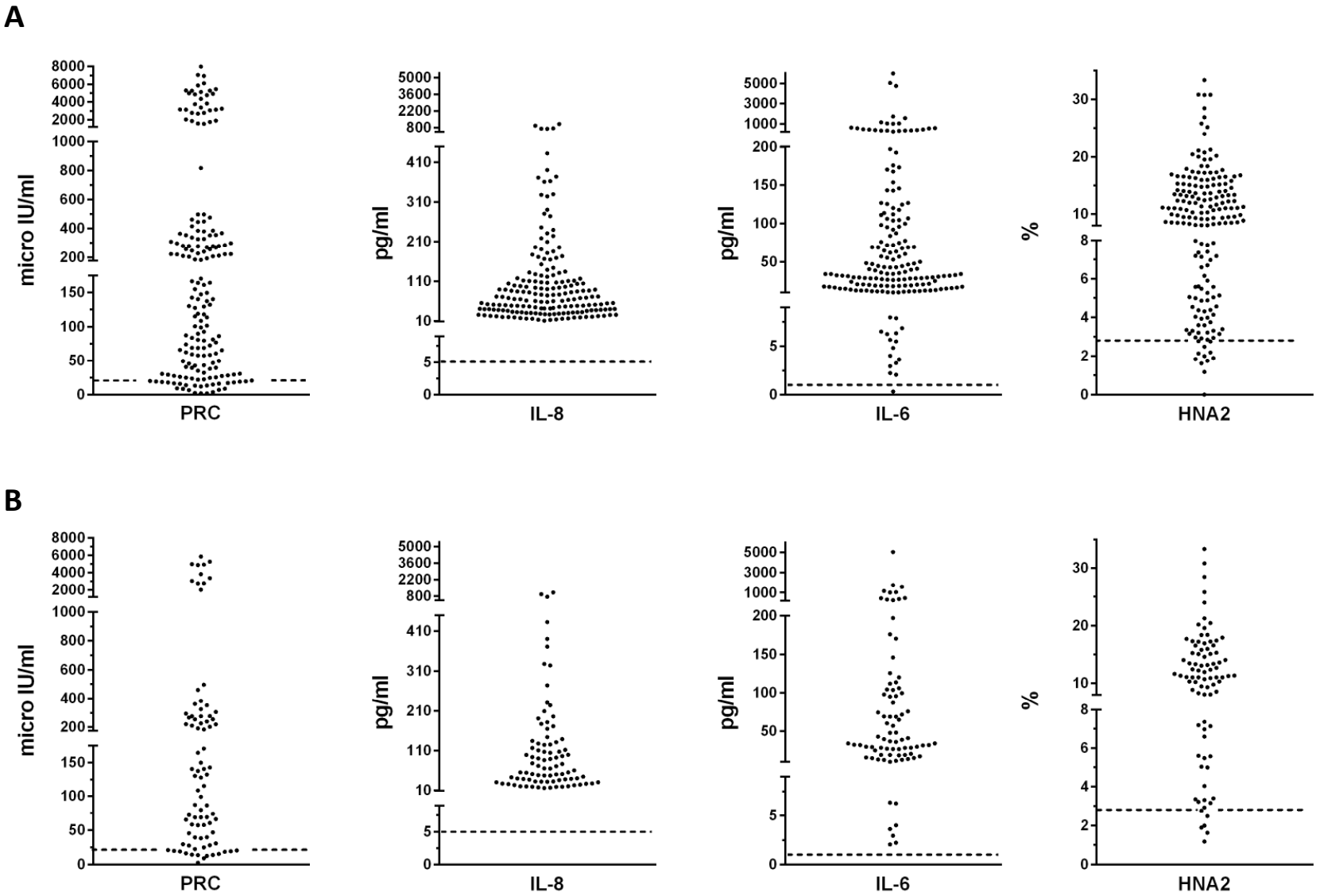
***p<0.05 vs previous tertile; #p<0.01 vs previous tertile**

D

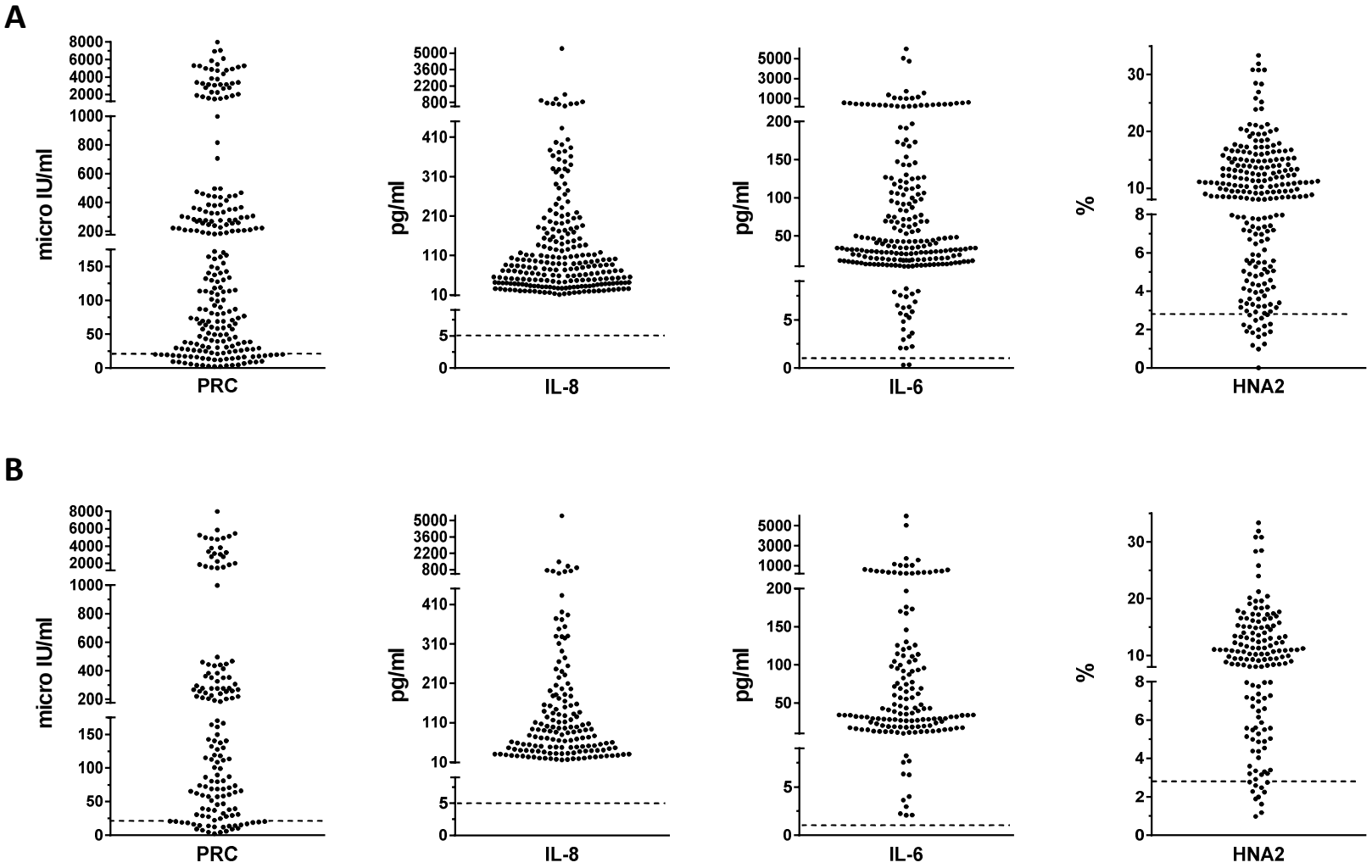
	HNA2-T1	HNA2-T2	HNA2-T3	
PRC-T3	6/29 (21%)	21/50 (42%)	37/57 (65%)	64/136 (47%)
PRC-T2	7/42 (17%)	17/50 (34%)	28/52 (54%)	52/144 (36%)*
PRC-T1	7/77 (9%)	10/39 (26%)	16/24 (67%)	33/140 (24%)
Overall HNA2	20/148 (14%)	48/139 (35%)#	81/133 (61%)#	N=420

***p<0.05 vs previous tertile; #p<0.01 vs previous tertile**

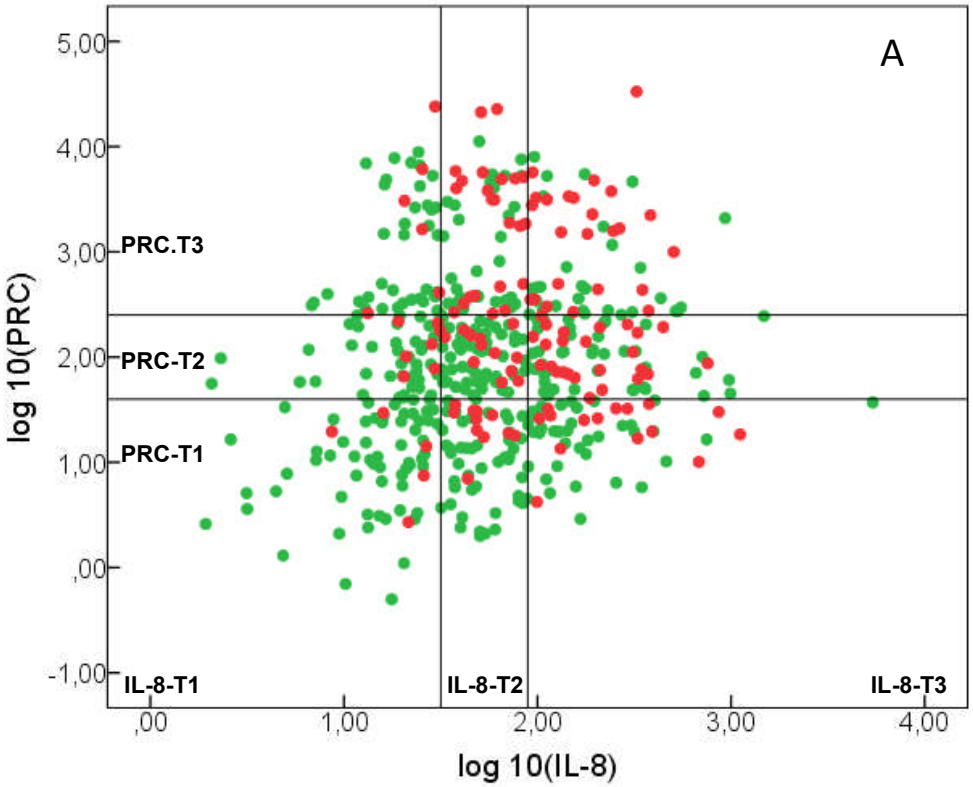
Supplementary Figure 5

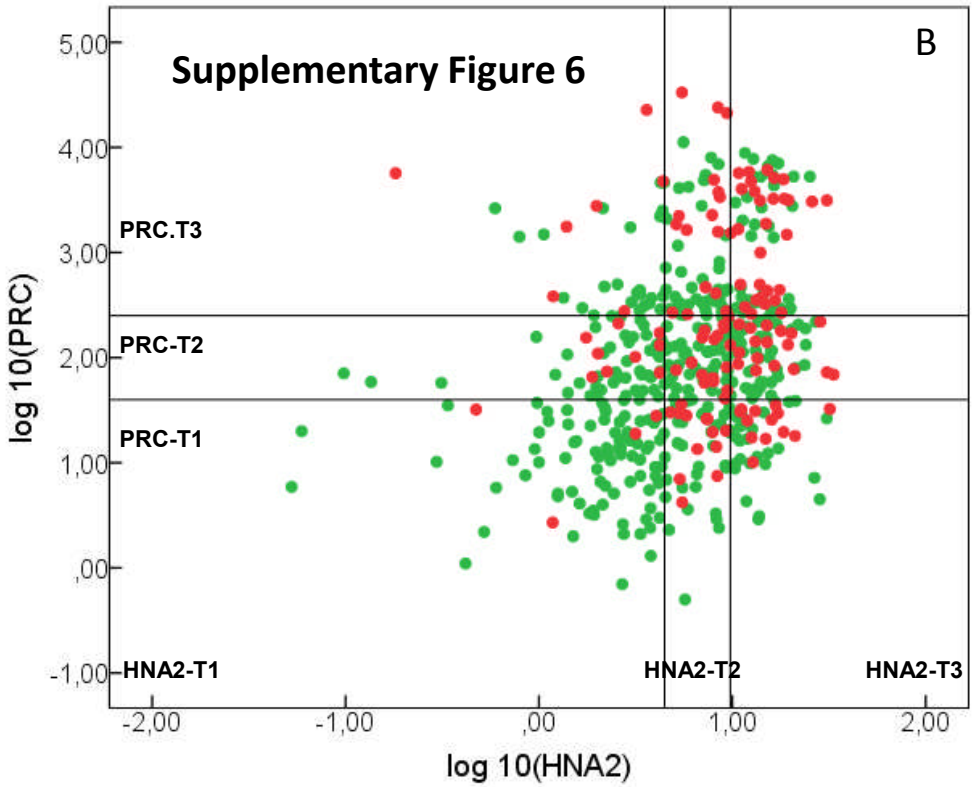


Supplementary Figure 4



Supplementary Figure 6





C

	IL8-T1	IL8-T2	IL8-T3	Overall PRC
PRC-T3	8/52 (15%)	24/56 (43%)	27/64 (42%)	59/172 (34%) *
PRC-T2	5/46 (11%)	11/58 (19%)	22/60 (37%)	38/164 (23%)
PRC-T1	5/65 (8%)	11/53 (21%)	15/50 (30%)	31/168 (19%)
Overall IL-8	18/163 (11%)	46/167 (28%)#	64/174 (37%)	N=504

***p<0.05 vs previous tertile; #p<0.01 vs previous tertile**

D

	HNA2-T1	HNA2-T2	HNA2-T3	Overall PRC
PRC-T3	7/33 (21%)	18/60 (30%)	33/75 (44%)	58/168 (35%)
PRC-T2	8/51 (16%)	15/59 (25%)	14/54 (26%)	37/164 (23%)*
PRC-T1	4/82 (5%)	13/46 (28%)	13/36 (36%)	30/164 (18%)
Overall HNA2	19/166 (5%)	46/165 (14%)#	60/165 (22%)#	N=496

***p<0.05 vs previous tertile; #p<0.01 vs previous tertile**