Dr. Rakhi Maiwall has been leading the research on diagnosis and prognosis of acute kidney injury (AKI) in patients with decompensated cirrhosis and acute on chronic liver failure (ACLF). She is currently the principal investigator of two projects funded by ICMR and DST-SERB aimed to use the “omics” approach for the discovery of biomarkers of AKI.

The key highlights of her research are as follows

1. **Establishing AKI in patients with ACLF is different from patients with decompensated cirrhosis**
   Maiwall R, Kumar S, Chandel SS, Kumar G, Rastogi A, Bihari C, Sharma MK,Thakur B, Jamwal K, Nayak S, Mathur RP, Sarin SK. AKI in patients with acute on chronic liver failure is different from acute decompensation of cirrhosis.
   Hepatol Int. 2015 Oct;9(4):627-39.

2. **Poor response of AKI to terlipressin in ACLF and superiority of continuous infusion over nor-epinephrine for management**

   Arora V, Maiwall R, Rajan V, Jindal A, Muralikrishna Shasthry S, Kumar G,Jain P, Sarin SK. Terlipressin Is Superior to Noradrenaline in the Management ofAcute Kidney Injury in Acute on Chronic Liver Failure. Hepatology. 2020
   Feb;71(2):600-610.

   Jindal A, Bhadoria AS, Maiwall R, Sarin SK. Evaluation of acute kidney injury and its response to terlipressin in patients with acute-on-chronic liver failure. Liver Int. 2016 Jan;36(1):59-67

   Maiwall R, Kumar G, Bharadwaj A, Jamwal K, Bhadoria AS, Jain P, Sarin SK. AKIpersistence at 48 h predicts mortality in patients with acute on chronic liver failure. Hepatol Int. 2017 Nov;11(6):529-539.

3. **Development and validation of predictive model for AKI in ACLF through a multinational database**

   Maiwall R, Sarin SK, Kumar S, Jain P, Kumar G, Bhadoria AS, Moreau R, Kedarisetty CK, Abbas Z, Amarapurkar D, Bhardwaj A, Bihari C, Butt AS, Chan A, Chawla YK, Chowdhury A, Dhiman R, Dokmeci AK, Ghazinyan H, Hamid SS, Kim DJ, Komolmit P, Lau GK, Lee GH, Lesmana LA, Jamwal K, Mamun-Al-Mahtab, Mathur RP, Nayak SL, Ning Q, Pamecha V, Alcantara-Payawal D, Rastogi A, Rahman S, Rela M, Saraswat VA, Shah S, Shiha G, Sharma BC, Sharma MK, Sharma K, Tan SS, Chandel SS, Vashishtha C, Wani ZA, Yuen MF, Yokosuka O, Duseja A, Jafri W, Devarbhavi H, Eapen CE, Goel A, Sood A, Ji J, Duan Z, Chen Y; of the APASL ACLF Research Consortium (AARC) working party. Development of predisposition, injury,

response, organ failure model for predicting acute kidney injury in acute on chronic liver failure. Liver Int. 2017 Oct;37(10):1497-1507

4. **Establishing the concept of AKI predisposes to repeated AKI in patients with decompensated cirrhosis**

   Maiwall R, Kumar A, Bhardwaj A, Kumar G, Bhadoria AS, Sarin SK. Cystatin C predicts acute kidney injury and mortality in cirrhotics: A prospective cohort study. Liver Int. 2018 Apr;38(4):654-664.

5. **Demonstrated acute kidney injury transitions to chronic kidney disease in patients with cirrhosis**

   Maiwall R, Pasupuleti SSR, Bihari C, Rastogi A, Singh PK, Naik V, Singh A, Jain P, Kumar A, Mukund A, Mathur RP, Kumar G, Sarin SK. Incidence, Risk Factors, and Outcomes of Transition of Acute Kidney Injury to Chronic Kidney Disease in Cirrhosis: A Prospective Cohort Study. Hepatology. 2020 Mar;71(3):1009-1022. doi: 10.1002/hep.30859. Epub 20

6. **Systemic inflammation as a driver of AKI in patients with ACLF with alcoholic hepatitis**

   Maiwall R, Chandel SS, Wani Z, Kumar S, Sarin SK. SIRS at Admission Is a Predictor of AKI Development and Mortality in Hospitalized Patients with Severe Alcoholic Hepatitis. Dig Dis Sci. 2016 Mar;61(3):920-9

7. **Impact of hemodynamic alterations, portal pressures and vasodilatory state in driving AKI and chronic kidney disease in cirrhosis**

   Maiwall R, Pasupuleti SSR, Jain P, Sarin SK. Degree of Portal and Systemic Hemodynamic Alterations Predict Recurrent AKI and Chronic Kidney Disease in Patients With Cirrhosis. Hepatol Commun. 2020 Nov 6;5(2):293-308.

8. **The concept of organ cross-talk and influence of organ failures in drivibg AKI outcomes in critically ill cirrhotics**

   Maiwall R, Pasupuleti SSR, Chandel SS, Narayan A, Jain P, Mitra LG, Kumar G, Moreau R, Sarin SK. Co-orchestration of acute kidney injury and non-kidney organ failures in critically ill patients with cirrhosis. Liver Int. 2021 Jun;41(6):1358-1369.

9. **Established the role of plasma-exchange in management of organ dysfunction, amelioration of systemic inflammation in patients with acute and acute on chronic liver failure**

   Maiwall R, Sarin SK. Plasma Exchange in Acute and Acute on Chronic Liver

Failure. Semin Liver Dis. 2021 Nov;41(4):476-494. doi: 10.1055/s-0041-1730971.
Epub 2021 Jul 14. PMID: 34261138.

Maiwall R, Bajpai M, Singh A, Agarwal T, Kumar G, Bharadwaj A, Nautiyal N, Tevethia H, Jagdish RK, Vijayaraghavan R, Choudhury A, Mathur RP, Hidam A, Pati
NT, Sharma MK, Kumar A, Sarin SK. Standard-Volume Plasma Exchange Improves
Outcomes in Patients With Acute Liver Failure: A Randomized Controlled Trial.
Clin Gastroenterol Hepatol. 2021 Jan 29:S1542-3565(21)00086-0. doi: 10.1016/j.cgh.2021.01.036. Epub ahead of print. PMID: 33524593.

Maiwall R, Bajpai M, Choudhury AK, Kumar A, Sharma MK, Duan Z, Yu C, Hu J,
Ghazinian H, Ning Q, Ma K, Lee GH, Lim SG, Shah S, Kalal C, Dokmeci A, Kumar G,Jain P, Rao Pasupuleti SS, Paulson I, Kumar V, Sarin SK; AARC working Party.
Therapeutic plasma-exchange improves systemic inflammation and survival in acute-on-chronic liver failure: A propensity-score matched study from AARC.
Liver Int. 2021 May;41(5):1083-1096. doi: 10.1111/liv.14806. Epub 2021 Feb 24.
PMID: 33529450.

10. Developed International collaborations on AKI

Sujan R, Cruz-Lemini M, Altamirano J, Simonetto DA, Maiwall R, Axley P, Richardson T, Desai V, Cabezas J, Vargas V, Kamath PS, Shah VH, Sarin SK, Bataller R, Singal AK. A Validated Score Predicts Acute Kidney Injury and Survival in Patients With Alcoholic Hepatitis. Liver Transpl. 2018 Dec;24(12):1655-1664.

Sarmast N, Ogola GO, Kouznetsova M, Leise MD, Bahirwani R, Maiwall R, Tapper
E, Trotter J, Bajaj JS, Thacker LR, Tandon P, Wong F, Reddy KR, O'Leary JG,
Masica A, Modrykamien AM, Kamath PS, Asrani SK. Model for End-Stage Liver
Disease-Lactate and Prediction of Inpatient Mortality in Patients With Chronic Liver Disease. Hepatology. 2020 Nov;72(5):1747-1757. doi: 10.1002/hep.31199.
PMID: 32083761.

2: Singapura P, Ma TW, Sarmast N, Gonzalez SA, Durand F, Maiwall R, Nadim MK,

Fullinwider J, Saracino G, Francoz C, Sartin R, Trotter JF, Asrani SK. Estimating Glomerular Filtration Rate in Cirrhosis Using Creatinine-Based and Cystatin C-Based Equations: Systematic Review and Meta-Analysis. Liver Transpl.
2021 Nov;27(11):1538-1552. doi: 10.1002/lt.26216. Epub 2021 Aug 1. PMID: 34143570.

11. Biomarker discovery in AKI (Ongoing work in the lab under publication)

IDENTIFICATION OF SPECTRUM OF AKI

Change in urine supernatant metabolite profile in ACLF with and without AKI

MS-MS identified deferentially expressed metabolites (($p<0.05$) fold change >2 fold) with high FDR confidence PSM >2. Principal component analysis (PCA) documented a clear distinction between ACLF with and without AKI. (Figure 1A). Further pathway analysis of metabolites upregulated in ACLF-AKI showed enrichment of pathway associated with Tryptophan and nicotinamide metabolism, TCA- Cycle, amino acid and butanoate metabolism and down regulation of histidine and beta-alanine metabolism (Figure-1B). further random forest classification and AUROC analysis identified loss of Capryloylglycine acid, Pentadecanoic acid, Ethyl tetradecanoate, majoroside F6, biotin amide and gain of 3-(3-Methylthio) propylmalic acid, 1,2,3-Trimethyluric acid and Hyaluronic acid in ACLF AKI compare to no-AKI as potential urine metabolites-based marker to differentiate AKI and NO-AKI ACLF (Figure-1C).

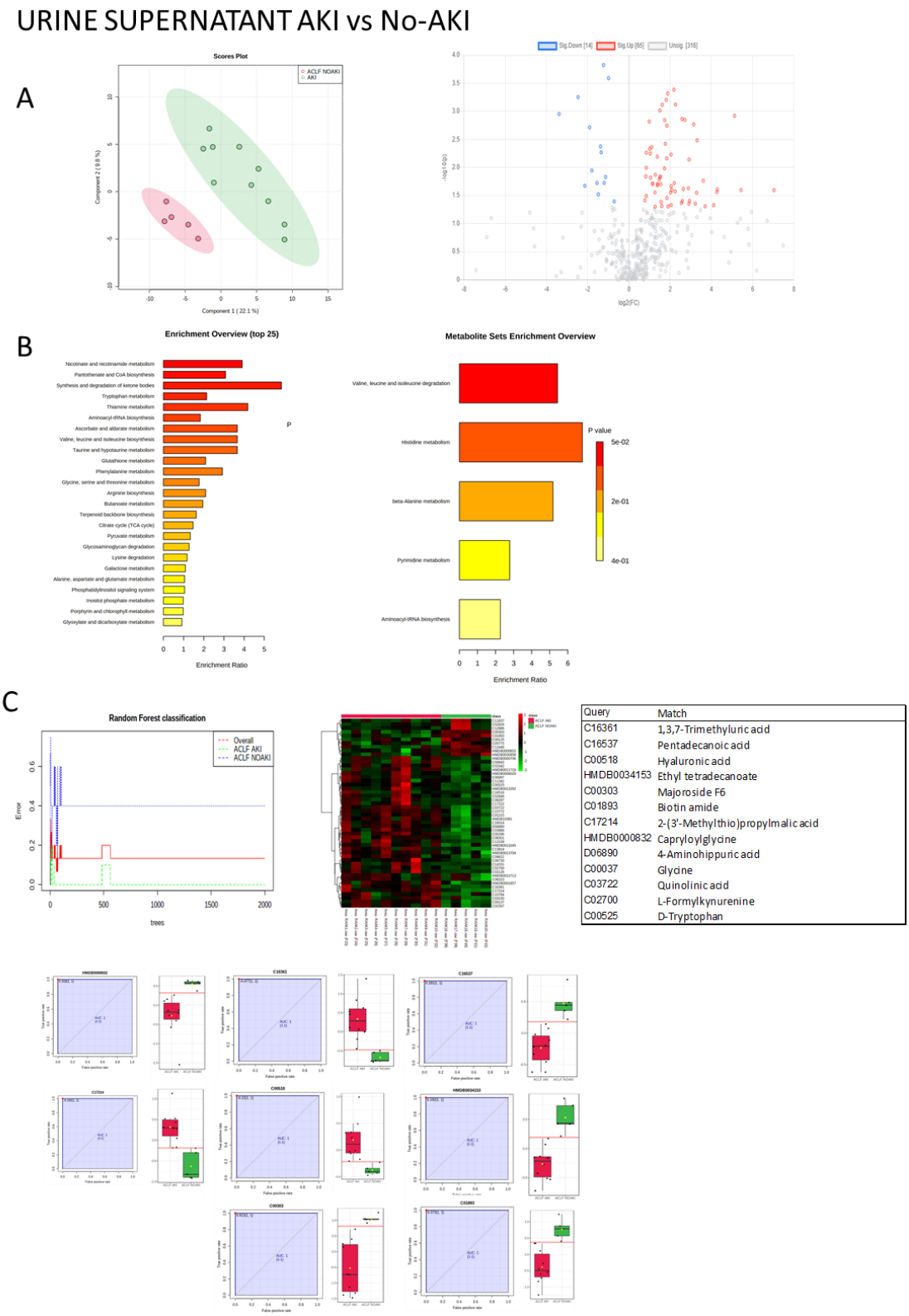


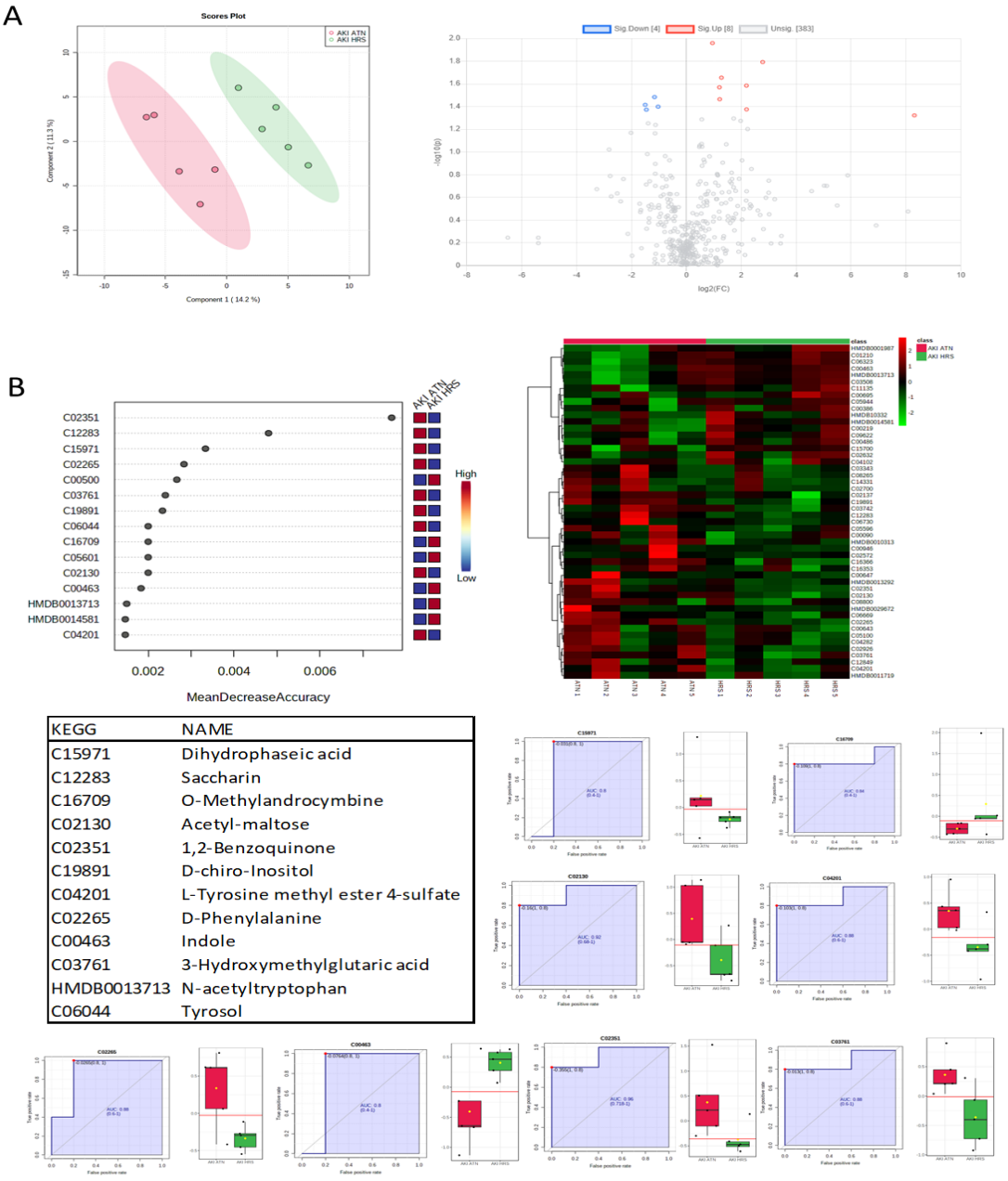
Fig1. (A). Score scatter plot of PCA model for AKI vs. No AKI in urine supernatant and volcano plot showing significant differential abundance (p value <0.001, absolute fold change >2). (B). KEGG enrichment pathways annotation based on significant different metabolites between AKI vs. NO AKI. The *x* axis indicates the proportion and

number of metabolites annotated to the pathway, and the *y* axis indicates name of the KEGG metabolic pathway (p value <0.001). (C). Random forest classifier, Heat map showing differential metabolite between AKI vs. NO AKI and AUROC box plot showing up regulated and down regulated metabolites in urine supernatant.

Change in urine supernatant metabolite profile in ACLF HRS and ATN-AKI

MS-MS identified deferentially expressed metabolites ($p<0.05$) fold change >2 fold) with high FDR confidence PSM >2. Principal component analysis (PCA) documented a clear distinction between HRS and ATN AKI in ACLF. (Figure 2A). Further random forest classification and AUROC analysis identified increase in dihydrophaseic acid, Acetyl-maltose, L-tyrosine methyl,ester 4-sulphate, D-phenylanine, 1,2-benzoqunone, 3-hydroxymethylglutaric acid and decrease in O-methylandrocymbine and indole ATN compare to HRS as potential urine metabolites-based marker to differentiate ATN and HRS (Figure-2B).

URINE SUPERNATANT ATN vs HRS



KEGG	NAME
C15971	Dihydrophaseic acid
C12283	Saccharin
C16709	O-Methylandrocymbine
C02130	Acetyl-maltose
C02351	1,2-Benzoquinone
C19891	D-chiro-Inositol
C04201	L-Tyrosine methyl ester 4-sulfate
C02265	D-Phenylalanine
C00463	Indole
C03761	3-Hydroxymethylglutaric acid
HMDB0013713	N-acetyltryptophan
C06044	Tyrosol

Fig 2.(A)Score scatter plot of PCA model for HRS vs. ATN in urine supernatant and volcano plot showing significant differential abundance(p value<0.001, absolute fold change>2). (B) MeanDecreaseAccuracy plot (random forests) and heat map showing differential metabolite between ATN vs. HRS and AUROC box plot showing up regulated and down regulated metabolites in urine supernatant.

Change in exfoliate urine cells metabolite profile in ACLF with and without AKI

MS-MS identified deferentially expressed metabolites ((p<0.05) fold change >2 fold) with high FDR confidence PSM >2. Principal component analysis (PCA) documented

a clear distinction between ACLF with and without AKI (Figure 3A) . Further pathway analysis of metabolites upregulated in exfoliate urine cells of ACLF-AKI showed enrichment of pathway associated with pantothenate and CoA biosynthesis, propanoate , taurine, hypotaurine and selenocompound metabolism and dounregulation of phenylamine and pentose phosphate metabolism (Figure-3b). further random forest classification and AUROC analysis identified loss of 2-hydroxybutyric acid, 3-dehydrocarnitine, Majoorsode F6, erythr0-isoleucine and gain of Homoferreirin,4-deoxythreonic acid, Oxolinic acid, biotine amide in ACLF AKI compare to no-AKI as potential exfoliate urine cells metabolites-based marker to differentiate AKI and NO-AKI ACLF (Figure-3c).

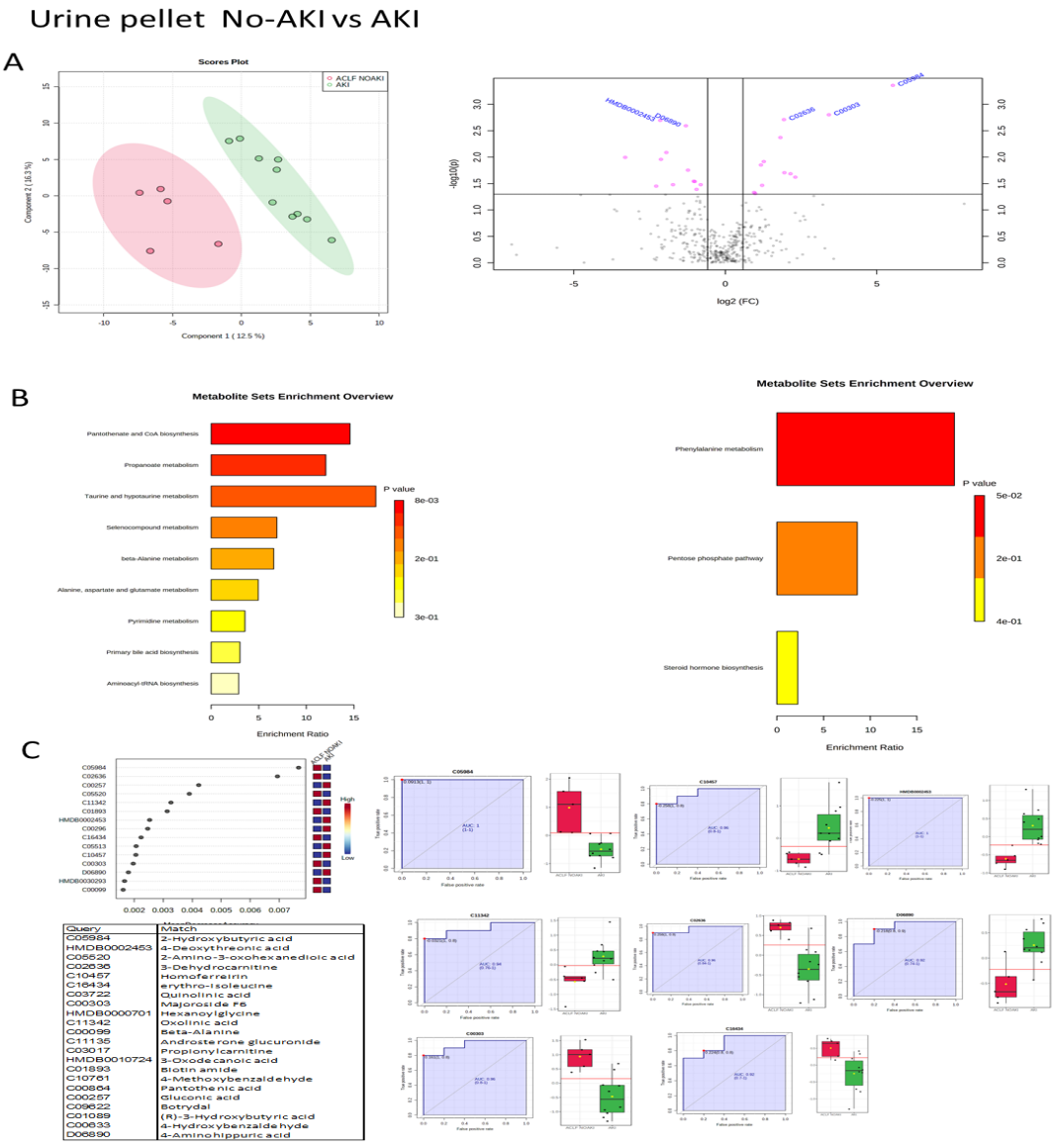


Fig 3. (A). Score scatter plot of PCA model for AKI vs. No AKI in urine pellet and volcano plot showing significant differential abundance (p value <0.001, absolute fold change >2). (B). KEGG enrichment pathways annotation based on significant different metabolites between AKI vs. NO AKI. The *x* axis indicates the proportion and number of metabolites annotated to the pathway, and the *y* axis indicates name of the KEGG

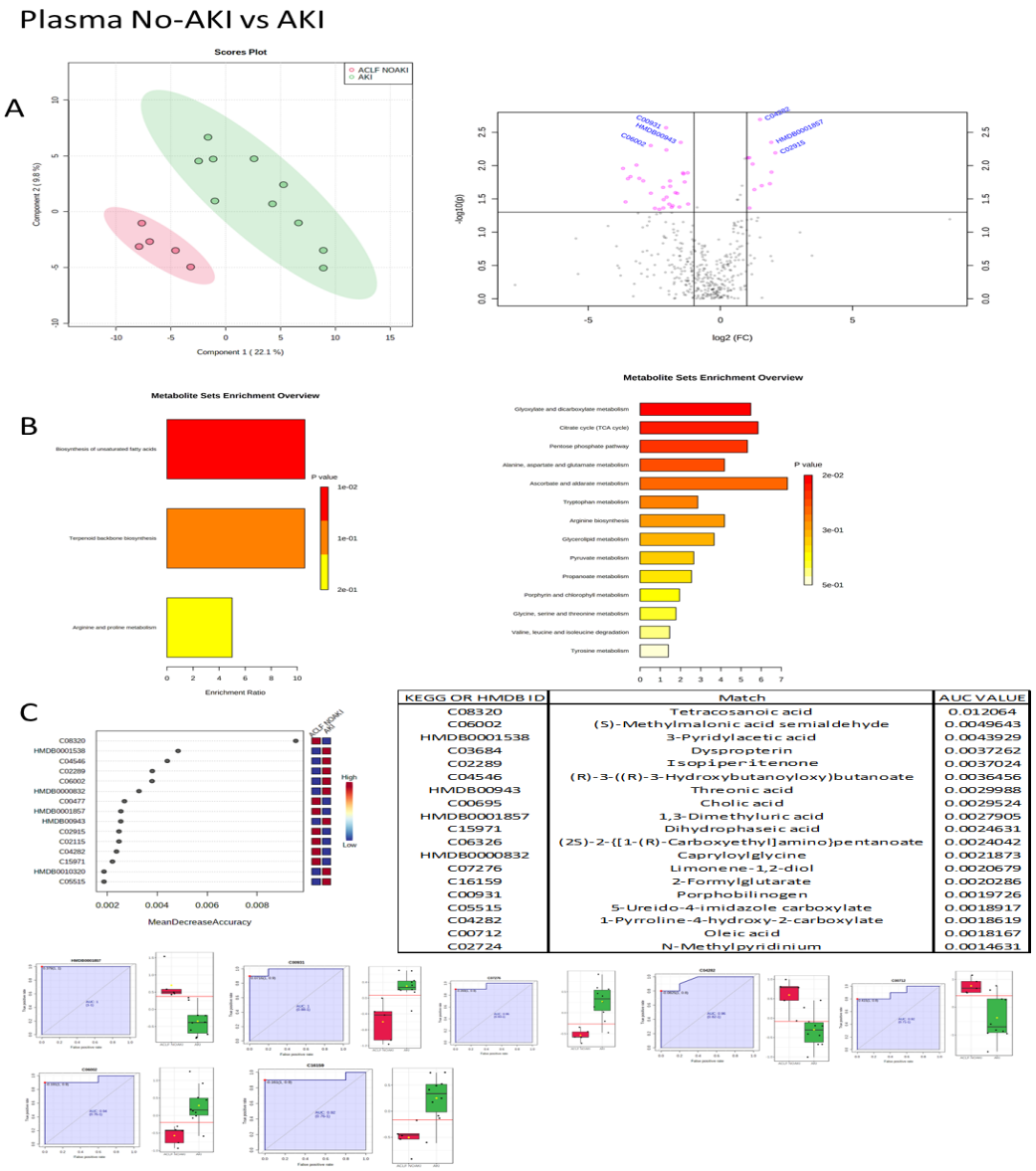
metabolic pathway (p value <0.001). (C). MeanDecreaseAccuracy plot (random forests), Heat map showing differential metabolite between AKI vs. NO AKI and AUROC box plot showing up regulated and down regulated metabolites in urine pellet.

Change in exfoliate urine cells metabolite profile in ACLF HRS and ATN-AKI

MS-MS identified deferentially expressed metabolites (($p<0.05$) fold change >2 fold) with high FDR confidence PSM >2. Principal component analysis (PCA) documented a clear distinction between HRS and ATN (Figure 4A) . Further pathway analysis of metabolites upregulated in exfoliate urine cells of ATN showed enrichment of pathway associated with Ketone bodies metabolism, butanoate metabolism and amino acid metabolism in ATN compare to HRS. (Figure-4b). further random forest classification and AUROC analysis identified loss 2-formylglutrate, Carnosine and gain of 3-methoxy-4-hydroxyphenylglycol glucuronide, 2-methylpropanoyl phosphate, 2-methylbenzoic acid in ATN compare to HRS as potential exfoliate urine cells metabolites-based marker to differentiate HRS and ATN ACLF (Figure-4c).

Change in plasma metabolite profile in ACLF with and without AKI

MS-MS identified deferentially expressed metabolites ($p<0.05$) fold change >2 fold) with high FDR confidence PSM >2. Principal component analysis (PCA) documented a clear distinction between ACLF with and without AKI (Figure 5A). Further pathway analysis of metabolites upregulated in plasma of ACLF-AKI showed enrichment of pathway associated with biosynthesis of unsaturated fatty acid, terpenoiide backbone biosynthesis and downregulation of TCA-cycle, pentose phosphate, pyruvate metabolism and tryptophan metabolism (Figure-5b). further random forest classification and AUROC analysis identified loss of 1,3-dimethyluric acid, 1-pyrroline-4-hydroxy-2-carboxylate, olic acid, and gain of porphobilinogen, Limonene,1,2-diol, methylmalonic acid semialdehyde and 2-formylglutrate in ACLF AKI compare to no-AKI as potential plasma metabolites-based marker to differentiate AKI and NO-AKI ACLF (Figure-5c).



KEGG OR HMDB ID	Match	AUC VALUE
C08320	Tetracosanoic acid	0.012064
C06002	(S)-Methylmalonic acid semialdehyde	0.0049643
HMDB0001538	3-Pyridylacetic acid	0.0043929
C03684	Dyspropterin	0.0037262
C02289	Isoperitenone	0.0037024
C04546	(R)-3-((R)-3-Hydroxybutanoyloxy)butanoate	0.0036456
HMDB00943	Threonic acid	0.0029988
C00695	Cholic acid	0.0029524
HMDB0001857	1,3-Dimethyluric acid	0.0027905
C15971	Dihydrophaseic acid	0.0024631
C06326	(2S)-2-{[1-(R)-Carboxyethyl]amino}pentanoate	0.0024042
HMDB0000832	Capryloylglycine	0.0021873
C07276	Limonene-1,2-diol	0.0020679
C16159	2-Formylglutarate	0.0020286
C00931	Porphobilinogen	0.0019726
C05515	5-Ureido-4-imidazole carboxylate	0.0018917
C04282	1-Pyrroline-4-hydroxy-2-carboxylate	0.0018619
C00712	Oleic acid	0.0018167
C02724	N-Methylpyridinium	0.0014631

Fig 5. (A). Score scatter plot of PCA model for AKI vs. No AKI in plasma and volcano plot showing significant differential abundance (p value <0.001, absolute fold change >2). (B). KEGG enrichment pathways annotation based on significant different metabolites between AKI vs. NO AKI. The *x* axis indicates the proportion and number of metabolites annotated to the pathway, and the *y* axis indicates name of the KEGG metabolic pathway (p value <0.001). (C). MeanDecreaseAccuracy plot (random forests), Heat map showing differential metabolite between AKI vs. NO AKI and AUROC box plot showing up regulated and down regulated metabolites in plasma.

Change in plasma metabolite profile in ACLF HRS and ATN-AKI

MS-MS identified deferentially expressed metabolites (($p<0.05$) fold change >2 fold) with high FDR confidence PSM >2. Principal component analysis (PCA) documented a clear distinction between HRS and ATN (Figure 6A). Further pathway analysis of metabolites upregulated in plasma of ATN showed enrichment of pathway associated

with amino acid metabolism, taurine and hypotaurie metabolism and down regulation of Nicotinate and nicotinamide metabolism, TCA cycle, pyruvate metabolism and tryptophan metabolism in ATN compare to HRS. (Figure-6b). further random forest classification and AUROC analysis identified loss alpha-L-arabinose, protein disusphide 2-chloro-4-methylphenol and gain of D-saccharic acid, botrydial, N5-(L-1carboxyethyl)-L-ornithine, 2-amino-3-oxohexanedioic acid, vitamin C and taurolithocholiclate sulphate in ATN compare to HRS as potential plasma metabolites-based marker to differentiate HRS and ATN ACLF (Figure-6c).

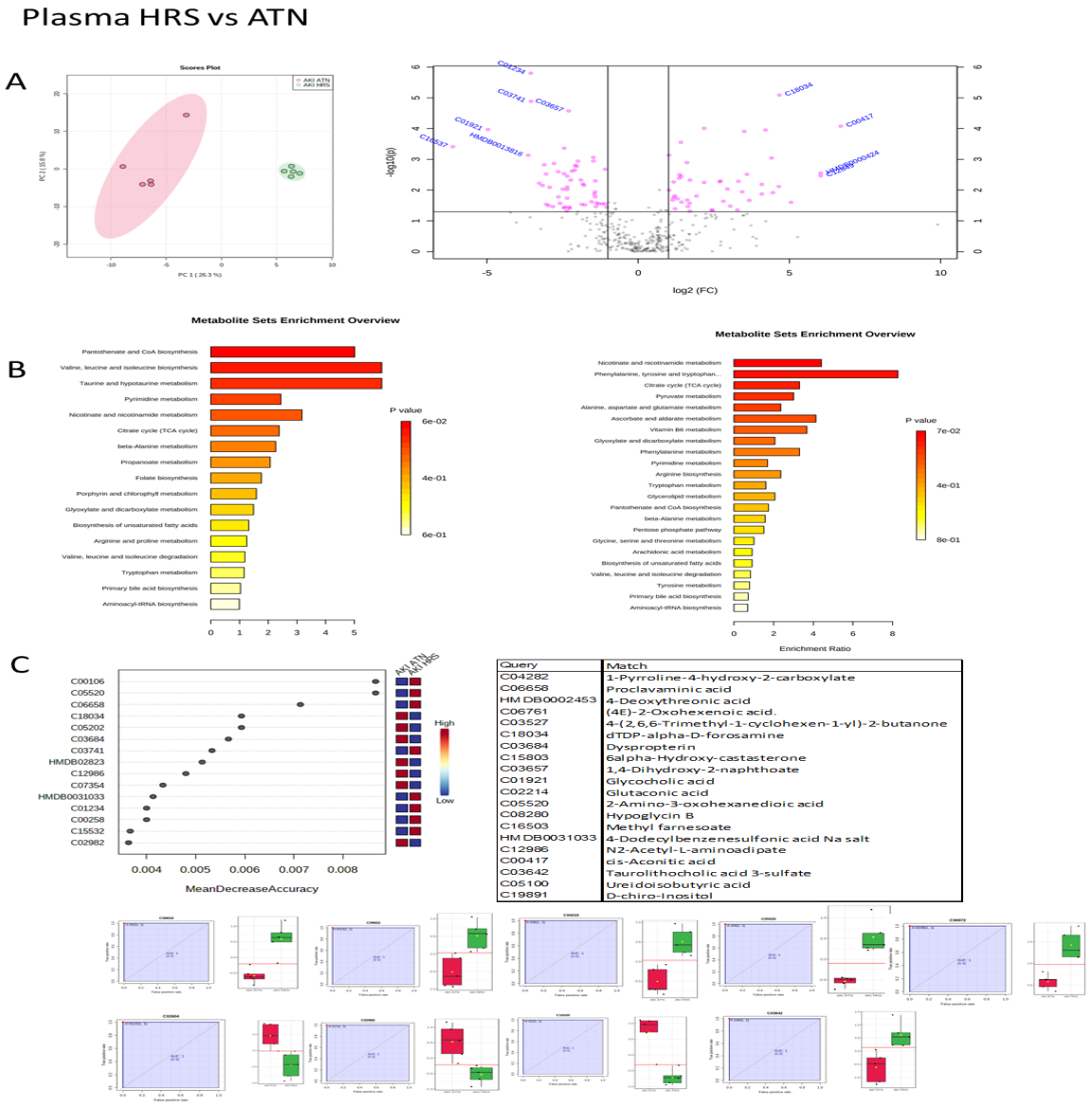


Fig6. (A). Score scatter plot of PCA model for HRS vs. ATN in plasma and volcano plot showing significant differential abundance (p value <0.001, absolute fold change >2). (B). KEGG enrichment pathways annotation based on significant different metabolites between HRS vs. ATN. The *x* axis indicates the proportion and number of metabolites annotated to the pathway, and the *y* axis indicates name of the KEGG metabolic pathway (p value <0.001). (C).MeanDecreaseAccuracy plot (random

forests) and heat map showing differential metabolite between HRS vs. ATN and AUROC box plot showing up regulated and down regulated metabolites in plasma.

Change in plasma cytokines in ACLF with and without AKI

Increased systemic inflammatory and oxidative stress had been thought to be associated with systemic organ dysfunction in ACLF. TO further understand how the systemic inflammatory response change with development of AKI in ACLF we analysed the plasma level of 29 different pro- and anti- inflammatory cytokines[IL-2, IL-4, IL-RA, IL-13, 1L-10], anti-inflammatory [Eotaxin, IFN-2, IFN-G, IL-12P40, IL-12P70, IL-15, IL-17A, IL-1Beta, IL-3, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1Alpha, MIP-1Beta, TNF-Alpha, TNF-Beta] and growth factors [EGF, G-CSF, GM-CSF, VEGF] in 60 ACLF (NO-AKI, n=15; HRS-AKI, n=15; CN-AKI, n=15 and ATN-AKI, n=15). The diagnosis of HRS, CN and ATN was based on urine microscopy, clinical parameters, and kidney biopsy (wherever available). In comparison of patient characteristics and clinical parameters it showed a comparable age, etiology, liver severity indices between the three groups. However, incidence of mortality is highest in ATN (65%) followed by CN (35%) and HRS. In comparison to ATN, CN shows 80% of regression of AKI.

Except IL8 plasma level of all the pro-and anti-inflammatory cytokines were significantly low in ACLF patients with ATN-AKI, suggesting lowest systemic inflammatory stress in ATN-AKI. In contrast to ATN-AKI, CN-AKI ACLF patients showed significantly high (19 out of 29) systemic inflammatory stress (Figure 7A) . Further random forest classification showed significant difference in the plasma level of Eotaxin, Interleukin (IL)-15, IL3, IL-5, IL-6, IL-2, IL-4, IL1beta, Il1RA, IL17A, Tumor necrosis factor (TNF) Alpha, Monocyte inhibitory protein (MCP)-1 beta, monocyte chemoattractant protein (MCP)-1 and VEGF among the different group of ACLF (Figure-7B). In comparison to ACLF no-AKI, patients with HRS, CN and ATN AKI showed significant loss of plasma level of IL-4 and MCP-1 suggesting loss of monocyte and TH2 mediated immune response with development of AKI in ACLF. With the development of HRS-AKI there was significant increase eotaxin and TNF-alpha which further decreased with development of CN or ATN in ACLF. In comparing to No-AKI and patients with HRS and ATN-AKI , ACLP with CN-AKI showed significantly high level of IL-3, IL-6, g-CSF, MIP-1alpha, IL-5, IL-10 (Figure 7C). Altogether these data ACLF patients with **CN-AKI has highest systemic inflammatory stress which may contribute to CN-AKI in ACLF.** Recently we showed that potential therapeutic benefit of

Plasma exchange in management of acute and acute-on- chronic liver failure. As we observed significantly high level of systemic inflammatory stress in CN-AKI we check the effect of plasma exchange on dampening the inflammatory stress in CN-AKI. Post plasma exchange ACLF patents with CN-AKI showed marked reduction in (TNF-alpha, MCP-1,IFN-G, Eotaxin, IFN-A2, IL17A, IP-10, IL-6, IL-4, IL12, GM-CSF, and Mip-1beta.) with increase in resolving cytokines (IL1RA, IL10, IL-5) Figure 7D) altogether tis suggest that dampening the systemic inflammatory response by plasma

exchange increase the resolving cytokine level and may improve the regression of AKI in CN

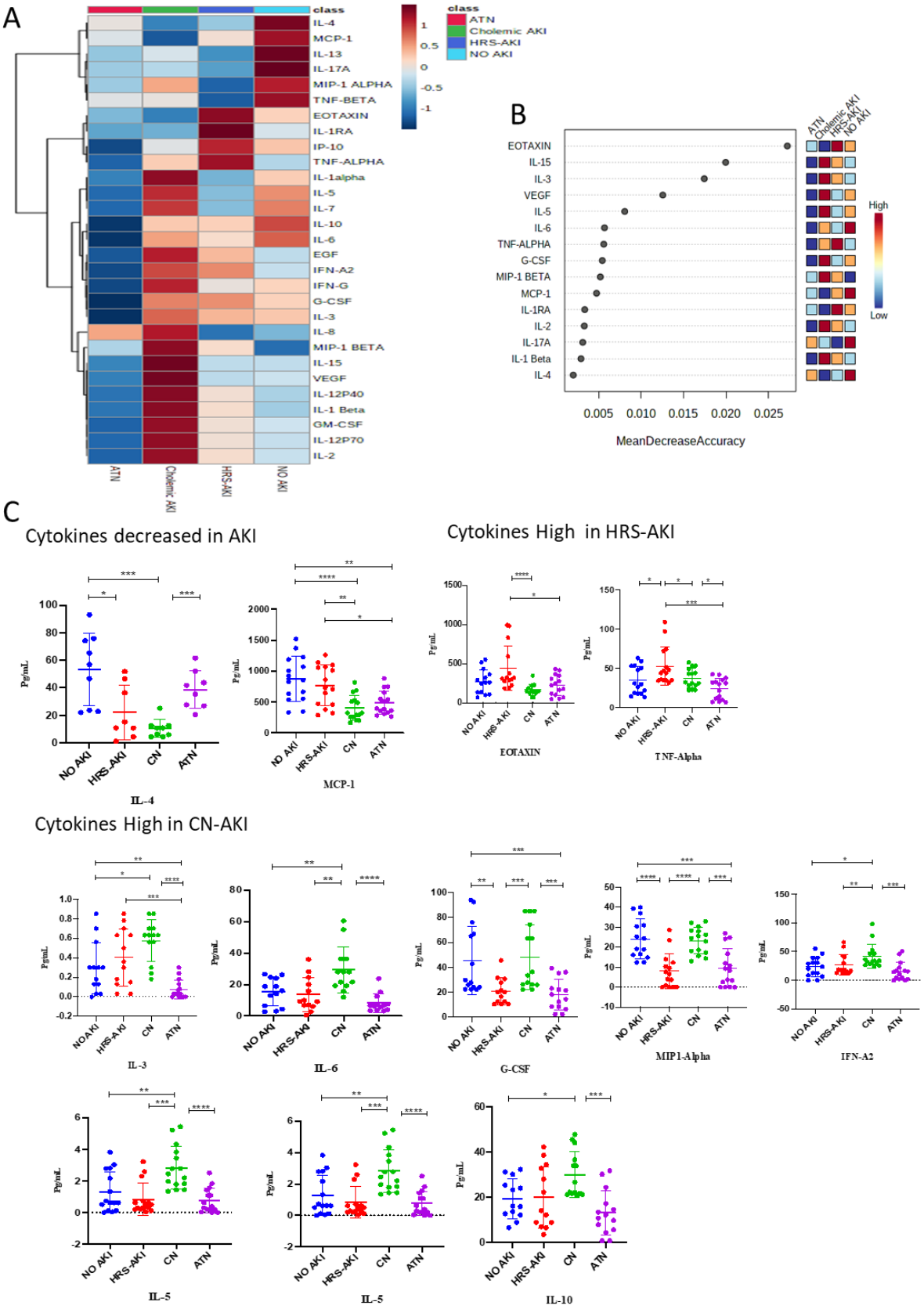


Fig.7.(A)Heat map showing differential cytokines between ATN, Cholemic AKI, HRS–AKI and NO AKI.(B) MeanDecreaseAccuracy plot showing cytokines expression (random forests).(C) Dot plot showing cytokines decreased in AKI, cytokines high in HRS-AKI and cytokines high in CN-AKI

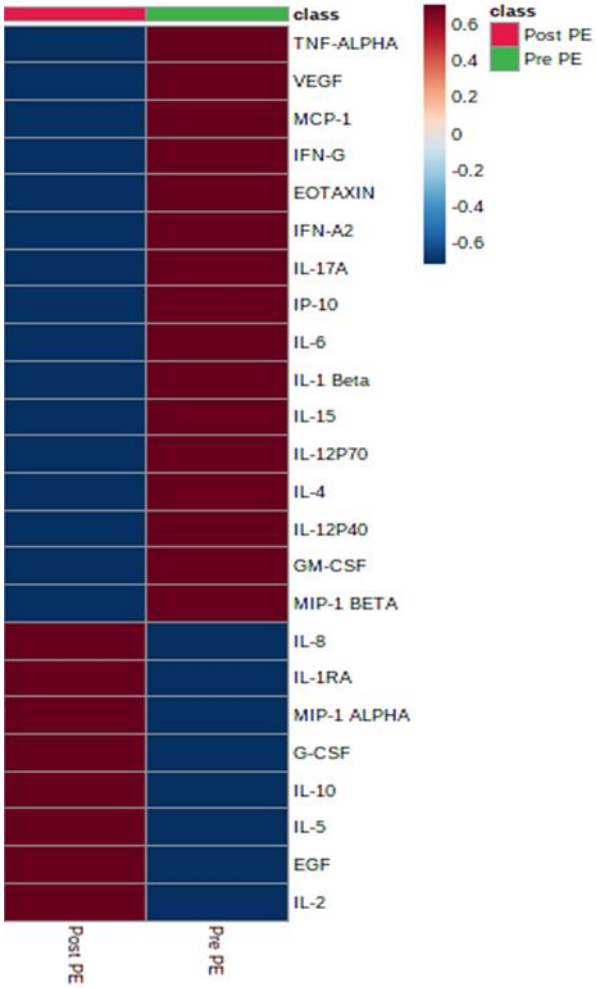


Fig.7.(D) Heat map showing change in plasma level of different cytokines with plasma exchange in CN-AKI.

Change in known renal injury and repair markers in urine of ACLF with and without AKI

TO further understand the status of renal injury and repair in ACLF we analysed the panel of 18 different renal injury and repair markers{ (tubular injury(BOTI) [NGAL, Cystatin-C, Renin, Alpha-1 Microglobulin, GST-Alpha, FABP-1, IP-10, TIMP-1, KIM-1, Albumin], repair(BOTR) [EGF, Calbindin, OPN, Clusterin] and renal fibrosis(BORF) [Collagen-IV, TFF-3, Osteoactivin]} in 60 ACLF (NO-AKI, n=15; HRS-AKI, n=15; CN-AKI, n=15 and ATN-AKI, n=15). Principal component analysis (PCA) documented a clear distinction among different group of ACLF patients with and without AKI. While HRS and CN AKI are closer to no-AKI ATN-AKI sowed more separation from rest of the group (Figure 8A). Further random forest classification showed significant difference in the urine level of Lipocalin-2/NGAL, Renin, Alpha-microglobulin, Collagen-IV, GST-alpha, cystatin-c, ostopontine(OPN) calbindin, EGF, Osteo Activin, TFF-3, FABP-1. TIMP-1, IP-10 and albumin among the different group of ACLF (Figure-8B). Interestingly while all the renal injury markers (NGAL, Cystatin-c, collagen-IV, Renin, alpha-1 microclobulin) were significantly high in ATN, they showed lowest level of renal repair markers (calbindin, OPN, EGF, Osteo Activin) , suggesting increase renal injury and loss of renal repair in ATN-AKI in ACLF. Unlike ATN-AKI, In CN-AKI showed increase in both renal injury (NGAL, RENIN, collagen-IV) and renal markers (calbindin, OPN and EGF) suggesting presence of both structural injury and renal repair in CN-AKI in ACLF (Figure-8C). Urine level of calbindin progressively decrease from No-AKI to HRS to Cn and CN to ATN in ACLF. Patients with CN also showed significant elevation serum bile acids as compared to ATN and HRS (Figure-8D)

SUMMARY OF RESEARCH WORK

By our research we have shown the following

- **Metabolic stress drive progression of ATN in ACLF AKI**. Increased systemic inflammatory and oxidative stress had been thought to be associated with systemic organ dysfunction in ACLF. Acute tubular necrosis (ATN) accounts for more than 30% of AKI in ACLF. Underlying pathophysiology of ATN in ACLF is not defined. Using multiomic approach we showed that it is not the inflammatory stress rather then loss of mitochondrial respiration and tryptophan metabolism and production of NAD may drive the hypoxia induced renal injury and loss of repair in ACLF-ATN.

- **Increased systemic inflammatory stress and bile acid drive cholemic nephrosis in ACLF** . using postmortem kidney biopsy we

showed that cholemic nephrosis (CN) accounts for more that 60 of AKI in ACLF. Unlike ATN, patients with CN-AKI showed marked increased in systematic accumulation of various pro-and anti-inflammatory mediators and bile acid, Renal tissue of CN showed presence of bile cast and prominent endothelial cell injury however tubular epithelial cell injury in CN in compared to ATN is markedly low. Unlike ATN, repair mechanism in CN-AKI is protected, suggesting high chance of recovery.

Maladaptive repair drives AKI progression to CKD

We have by series of elegant experiments demonstrated the repair mechanisms are defective in patients who develop CKD after AKI

Impairment of systemic energy metabolism in Cirrhotic AKI

We observed significant impairment in mitochondrial energy metabolism and fatty acid oxidation in exfoliated urine cells of cirrhotic AKI patients. Due to technical limitations (insufficient amount of urine cells in cirrhotic AKI), we further analyze the mitochondrial energy metabolism of peripheral blood monocytes to confirm the bioenergy dysfunction in patients with cirrhosis and AKI. Analysis of mitochondrial respiration showed a significant decrease in basal and maximal mitochondrial respiration in patients with cirrhotic AKI compared to non-cirrhotic AKI (Figure-1A). Based on prior studies, we know mitochondrial respiration fuels the phagocytic function of monocytes. To further understand whether defect mitochondrial energy metabolism affects monocyte function, we compare the phagocytic process of monocyte of cirrhotic AKI with non-cirrhotic AKI and cirrhosis. In comparison, cirrhotic and non-cirrhotic AKI, cirrhotic AKI monocytes showed a significant decrease in their phagocytic function (Figure-1B). Altogether suggesting impairment of systemic energy metabolism in cirrhotic AKI.

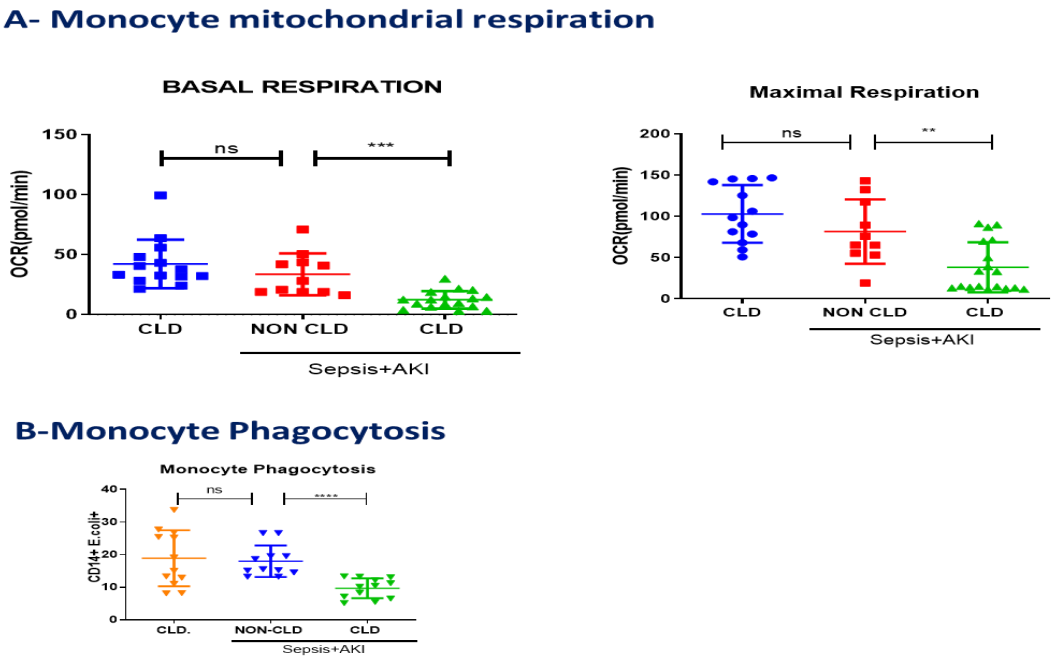


Figure-1. Dot plot showing (A) **basal respiration and maximal respiration** were significantly higher in non-cirrhotics with sepsis and AKI as compared to those of cirrhotic patients,furthermore, (B) **Monocytic phagocytosis** was significantly elevated in non-cirrhotics with sepsis and AKI with respect to cirrhotics with sepsis and AKI.

and anti-inflammatory cytokines and growth factors in cirrhotics without AKI, cirrhotics with sepsis and AKI and non-cirrhotics (acute pancreatitis) with sepsis and AKI.

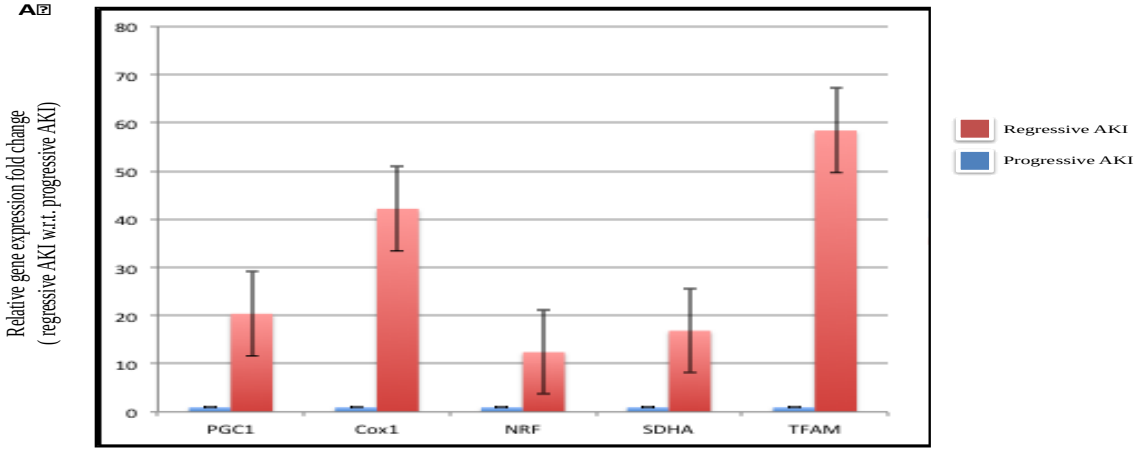
Impaired mitochondrial biogenesis and defective fatty acid oxidation leads to maladaptive repair and development of CKD in cirrhotic acute kidney injury (AKI)

The persistent pro-inflammatory state is associated with worse organ dysfunction and sepsis outcome. An inability to switch metabolism from aerobic glycolysis to OXPHOS and thus ‘turn off’ inflammation is related to chronic inflammation and impaired organ

recovery. In an animal study, defective fatty acid oxidation in renal tubular epithelial cells was associated with maladaptive repair and development of kidney fibrosis after AKI. There is emerging evidence suggesting the role of renal tubular epitheial cell (RTEC) in driving tubulointerstitial inflammation and fibrosis after renal injury. The presence of tubule epithelial cells in urinalysis indicates a prolonged maladaptive repair process. It also signifies ongoing inflammation, which in turn is a risk factor for renal fibrosis. The crosstalk between the RTEC and renal interstitium, the inflammatory cells determines recovery after an insult to the kidneys. There is emerging evidence suggesting the role of RTEC in driving tubulointerstitial inflammation and fibrosis after renal injury. Acute kidney injury leads to mitochondrial dysfunction, and an increase in mitochondrial biogenesis is required to resolve the damage. Our proteomic data showed impairment of fatty acid oxidation and mitochondrial respiration in cirrhotic AKI. We did additional experiments to understand whether impairment in mitochondrial biogenesis is associated with repair failure and non-resolution of AKI. We analyze the expression of various genes related to mitochondrial biogenesis [*peroxisome proliferator-actiavted receptor-gamma coactivator* (*PGC1), cyclooxygenase-1(Cox1), nicotinamide riboside (NR), succinate dehydrogenase (SDH), transcription factor A mitochondrial (TFAM*)] in exfoliated urine epithelial cells at the baseline. Interestingly, exfoliated urine cells of cirrhotic patients who had AKI regression showed a more than five-fold increase in *PGC1, Cox1, NR, SDH, TFAM* compared to progressive cirrhotic AKI (Figure-3A). These findings suggest defective mitobiogenesis in renal epithelial cells might contribute to the failure of resolution of AKI in cirrhotic.

To further understand the pathophysiology of renal injury in progressive AKI in cirrhotic, we analyze postmortem biopsy of cirrhotic AKI patients (n=15). Most biopsies

showed mild interstitial edema in 4 (27%) or fibrosis and collagen deposition in the interstitium in 11 (73%). In most of these cases, 8 (73%), we observed interstitial involvement by the fibrous tissue (varying from less than 10% to 20%). At the same time, the remaining patients showed severe interstitial fibrosis, suggesting the progression of AKI to CKD in progressive cirrhotic AKI. We confirmed the presence of fibrosis by the collagen stains such as Masson trichrome and Picrosirius. We further corroborated the presence of fibrosis by collagen immunohistochemistry for the essential collagens. Most of the cases (53%) showed collagen one: collagen three ratios more than one, and the remaining patients showed an equal degree of intensity for both of them. Interstitial inflammatory cell infiltrate was recorded in less than 20% of the cases. None of them revealed interstitial neutrophils. Macrophages and lymphocytes were the predominant cellular infiltrate, varying from focal to diffuse and mild-moderate infiltration. Most of the patients showed mild to moderate myofibroblast proliferation and increased vessel and capillary density. We further confirmed these observations by α-SMA and CD31 immunohistochemistry (IHC). The CD31 staining was mild focal or diffuse in 7 (47%) patients, and in 4 (27%) was moderate to marked and diffuse (Figure-3B). Altogether these data suggest that impairment in mitochondrial biogenesis might lead to defective mitochondrial respiration and fatty acid oxidation and development of CKD in cirrhotic AKI.



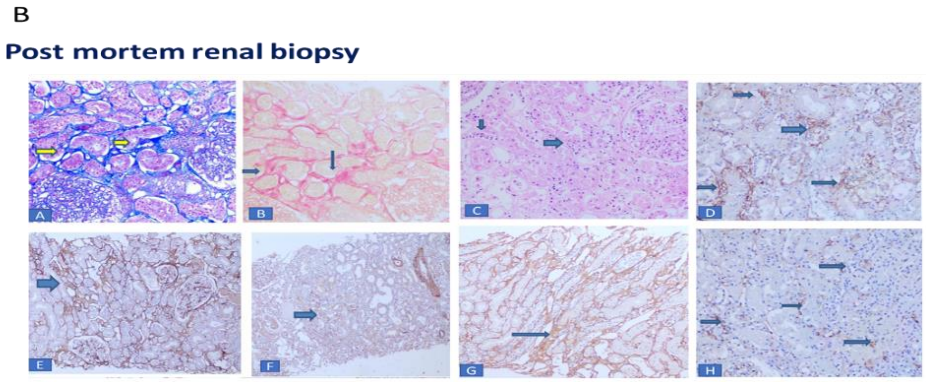


Figure-3. (**A)** Graph showing relative expression of mitochondrial biogenesis associated genes in regressive AKI with respect to progressive AKI in cirrhotics. **(B)** Representative images of post morterm kidney biopsies showing **(a)** grade 2 interstitial fibrosis on metallothinein stain **(b)** interstitial fibrosis on picrosirius stain **(c)** IHC demonstrating mild inflammatory infiltrates mainly macrophages (HE, 200x) magnification **(d)** IHC for alpha smooth muscle actin (α-SMA) by labeled streptavidin biotin method (LSAB method) displays myofibroblasts **(e) and (f)** IHC showing collagen (col) 1 and collagen 3 by LSAB method displaying collagen 1

>collagen 3 (100x) magnification **(g)** Another case with col1 (C) equal to col 3 H (100x) magnification. **(h)** IHC for CD31 highlights several vessels and capillaries. (200x magnification)

Increased vascular injury in progressive cirrhotic AKI

Our proteomic data observed increased shear stress and vascular contraction in cirrhotic even in the absence of AKI. To further understand the vascular dysfunction in cirrhotic AKI, we compared the plasma levels of vascular injury [endothelin-1(EDN-1), von Willebrand factor(vWF)] and repair marker [Angiopoietin-2 (ANGPT2), a disintegrin and metaloproteinase with thrombospondin motifs (ADAMTS)] in progressive and regressive cirrhotic AKI. In comparison to patients with AKI Progression, patients with regressive AKI showed a significant increase in plasma level of ANGPT2, ADAMTS and decreased in endothelin-1 and vWF, suggesting increased vascular stress and injury in progressive cirrhotic AKI (Figure -4).

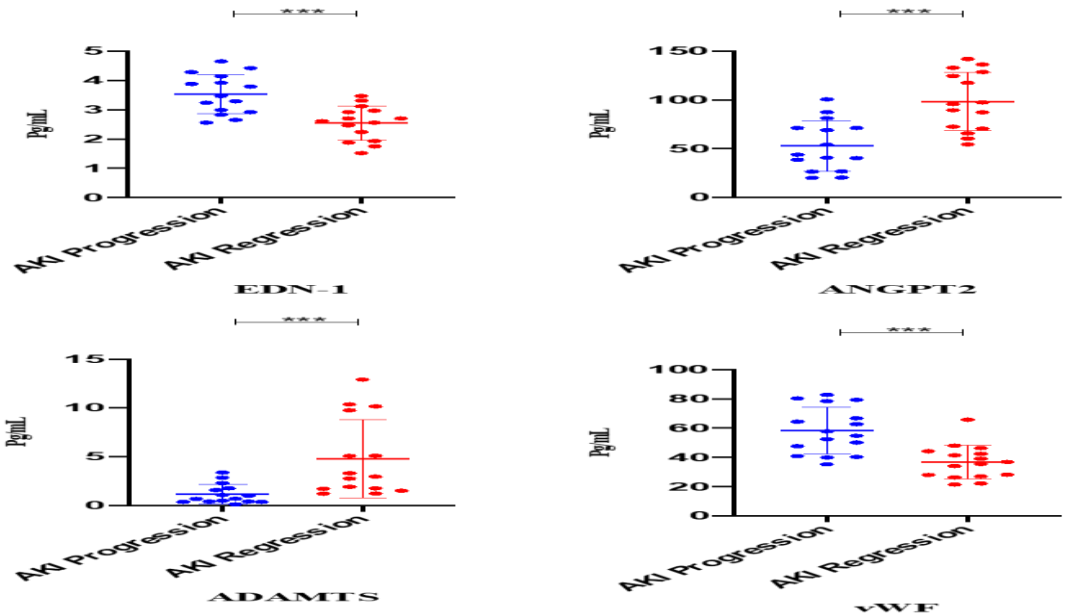


Figure -4. Dot plot showing the plasma levels of vascular injury (**EDN-1, vWF**) and repair marker (**ANGPT2, ADAMTS**) in progressive and regressive cirrhotic AKI.

Increased urine NGAL, RENIN, collagen-IV Kim-1, and decrease in OPN can predict the development of progressive AKI in cirrhotic.

Currently, there is a lack of studies on biomarkers of AKI progression in patients with cirrhosis and sepsis admitted to the intensive care unit. The results of our proteomic data performed in the urine supernatant had demonstrated dysregulation of the renin-angiotensin system in cirrhotic AKI. We, therefore, analyzed a panel of seventeen known renal injury and repair markers in patients with progressive or regressive AKI. Principal component analysis (PCA) documented a clear distinction between developed and regressive AKI in cirrhotic

AKI stratified by the course (Figure-5A). Cirrhotic patients with progressive AKI showed a significant increase in the urine levels of urine neutrophil gelatinase-associated lipocalin (NGAL), Cystatin-C, Renin, Collagen-IV, kidney-injury molecule -1 (KIM-1). A decreased levels of Calbindin, osteopontin (OPN), and epidermal growth factor (EGF) (Table-3, Figure 5B). We performed the area under the receiver operating curve (AUROC) analysis. We found urine NGAL, renin, OPN, COLLAGEN-IV, and Kim-1 were the most significant biomarkers, which showed AUROC of more than 0.95 for discriminating progressive from regressive AKI. Hence, an increase in the baseline urine NGAL, RENIN, Collagen-IV, Kim-1 and decrease in OPN can be used as an effective urinary marker to distinguish cirrhotic AKI patients likely to resolve or progress to CKD.

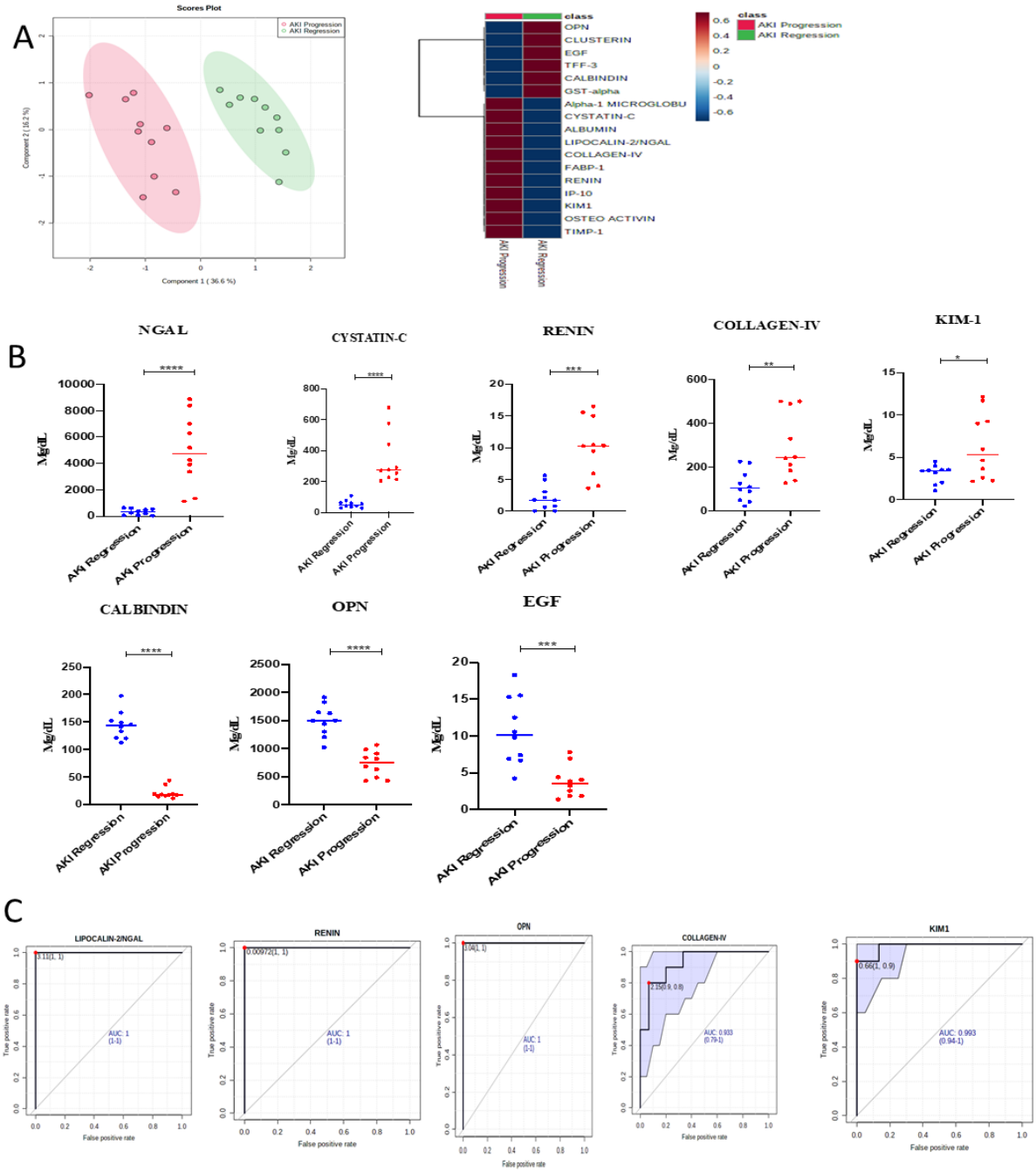


Figure-5. (A) Statistical representation showing PLS score 2D plot and Heatmap showing the components between regressive and progressive AKI in cirrhotics. (B) Dot plot showing injury markers (**NGAL, Cystatin-C, Renin, Collagen-IV and KIM-1**) were significantly elevated in progressive AKI as compared to regressive AKI. However, repair markers (**Calbindin, OPN and EGF**) were significantly increased in

regressive AKI with respect to progressive AKI. (C) Graphs showing AUROC levels of significant markers in cirrhotics with AKI.

We elegantly demonstrated a maladaptive repair process with histological specimens. Injury to the proximal tubules is a hallmark of inflammation-related AKI in the context of critically ill. The lack of neutrophil infiltration and predominance of monocyte-macrophage in the kidney biopsies suggests the pathogenic role of macrophages in renal fibrosis in the context of inflammation-related AKI in critically ill cirrhotics. This correlated with severity of endothelial dysfunction, defective mitochondrial dynamics and renal biomarkers. Future studies exploring the macrophage dynamics in chronic kidney disease (CKD) would be helpful in the development of targeted therapeutic strategies. We also propose studying the proximal tubules' molecular targeting for preventing fibrosis progression and CKD after AKI in CICs.

Strategies targeting systemic inflammation, sepsis, and endothelial dysfunction and ameliorating the impaired mitochondrial function could improve the outcomes of AKI in critically ill cirrhotics. Further, early risk stratification and appropriate therapeutic intervention with the help of protein biomarkers could improve outcomes of AKI in sick patients with cirrhosis admitted to the intensive care unit..

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