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Increased plasma levels of microparticles expressing CD39 and CD133 in acute liver injury

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Abstract

Background—We have previously demonstrated that CD133 and CD39 are expressed by hematopoietic stem cells (HSC), which are mobilized after liver injury and target sites of injury, limit vascular inflammation and boost hepatic regeneration. Plasma microparticles (MP) expressing CD39 can block endothelial activation. Here, we tested whether CD133+ MP might be shed in a CD39-dependent manner in a model of liver injury and could potentially serve as biomarkers of liver failure in the clinic.

Methods—Wild type and *Cd39* null mice were subjected to acetaminophen (APAP)-induced liver injury. Mice were sacrificed and plasma MP isolated by ultracentrifugation. HSC and CD133+ MP levels were analyzed by FACS. Patients were enrolled with acute (n = 5) and acute on chronic (n = 5) liver injury with matched controls (n = 7). Blood was collected at admission and plasma CD133+ and CD39+ MP subsets were analysed by FACS.

Results—HSC and CD133+ MP levels were significantly increased only in the plasma of wild type mice with APAP-hepatotoxicity (p<0.05). No increases in CD133+ MP were noted in *Cd39* null mice. Plasma MP increases were observed in patients with liver injury. These MP were characterized by significantly higher levels of CD39 (P< 0.05).

Conclusions—HSC and plasma CD133+ MP levels increase in a CD39-dependent manner during experimental acute liver injury. Increased levels of CD39+ MP are differentially noted in

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patients with liver injury. Further research is needed to determine whether MP fluxes are secondary to pathophysiologic insults to the liver or might reflect compensatory responses.

Keywords

CD39; CD133; microparticles; stem cells; acute liver failure

INTRODUCTION

Acute liver failure (ALF) is a significant cause of morbidity and mortality in the United States and Europe as therapeutic options for patients with ALF are limited. A variety of calculations and scores are proposed to prioritize patients for liver transplantation, but are characterized by a low sensitivity and do not reflect potential for liver regeneration [1]. Consequently, better understanding of mechanisms involved in liver injury and recovery remains imperative in the development of new therapies to facilitate survival.

Injury and recovery from experimental acetaminophen (APAP)-induced hepatotoxicity have been linked to purinergic signaling with the secondary modulations of vascular inflammation and healing responses. CD39, also known as ectonucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1) hydrolyzes pro-inflammatory extracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP) to adenosine monophosphate (AMP) to finally form adenosine via actions of CD73 [2]. In concert with previous reports demonstrating that global deletion of CD39 inhibits angiogenesis and considerably impairs liver regeneration [3, 4], mutant mice null for *Cd39* show increased APAP-induced inflammatory vascular disruption with hemorrhagic liver necrosis.

CD39 is also the dominant immune and hematopoietic stem cell (HSC) ectonucleotidase [5, 6]. Chemotaxis of HSC is regulated by CD39-dependent hydrolysis of extracellular nucleotides, leading to differential autocrine stimulation of adenosine-type receptors [6]. Distinct CD39^{high} HSC subset have been noted to be preferentially mobilized after surgical liver injury to boost regeneration in a paracrine manner by decreasing ATP-driven and interleukin-1 β (IL-1 β)-dependent vascular inflammation [6]. Given that nucleotide-dependent IL-1 β responses are required for manifestations of APAP-induced hepatotoxicity, mobilization of distinct CD39^{high} HSC might have therapeutic potential in ALF patients [7, 8].

Microparticles (MP) are submicron vesicles (< 1 μ m) that derive from well-defined parts of the plasma membranes during activation and are characterized by cell-specific cytosol and surface antigens. Stem cells secrete MP [9-11] and immune cell-derived MP have recently been suggested, by members of our group and others, as disease-specific biomarkers in inflammatory chronic liver diseases. [12, 13]. We have also shown that functionally active CD39 is incorporated into MP with proposed modulatory roles in the exchange of regulatory signals between leukocytes and vascular cells [14].

We demonstrate that increased levels of plasma CD133+ MP are present in wild type mice subject to experimental APAP-induced hepatotoxicity. In contrast, no such changes are seen in *Cd39* null mice and these mutant mice develop more pronounced injury. We note, in this pilot study differential increases in plasma levels of CD39+ functional ectonucleotidase-bearing and CD133 (prominin-1; progenitor marker) MP subsets in patients with liver injury. These data have implications for monitoring critically ill patients with hepatic dysfunction and also provide biomarkers that might help determine risk and the urgency for liver transplantation.

RESULTS

ANIMAL STUDIES

Liver injury and vascular disruption is increased in *Cd39* null mice relative to wild type mice in APAP hepatotoxicity—With APAP-induced injury, centrilobular hepatocyte apoptosis and necrosis occurred in both wild type and mutant mice with *Cd39* null livers, which was characterized by hemorrhagic necrosis and vascular disruption. (Figure 1a) Areas of cell death were 2-fold increased in *Cd39* null mice, when compared to wild type mice at different time points ($P < 0.001$). (Figure 1b) Plasma ALT levels were significantly elevated 24 h after APAP hepatotoxicity in wild type ($P = 0.03$) as well as in *Cd39* null mice ($P = 0.01$). However, in contrast to wild type mice ALT levels remained at high levels for 72h in *Cd39* null mice ($P = 0.04$). (Figure 1c) Plasma vascular endothelial growth factor (VEGF) levels were significantly increased in wild type and *Cd39* null mice, over controls, after APAP with higher levels noted in mutant mice null for *Cd39*. (Figure 1d)

Differential HSC responses in wild type and *Cd39* null mice in APAP

hepatotoxicity—Blood levels of HSC increased over time in wild type mice with significantly increased levels noted at 72 h with 13.3% ($\pm$ SEM 4.4%; $P = 0.02$), when compared to shams with 0.6% ($\pm$ SEM 0.14%). In contrast, no significant HSC mobilization was noted in *Cd39* null mice with 0.9% ($\pm$ 0.26%) at 72 h, when compared to the baseline. Relative to wild type mice, mobilization of HSC in *Cd39* null mice was significantly decreased at 24 h ($P = 0.006$) and 72 h ($P = 0.03$). (Figure 1e)

Vascular and hepatocyte injury correlates with HSC responses in wild type

mice—Vascular injury, as indicated by plasma VEGF levels, were positively correlated with HSC levels in the bone marrow of wild type mice at 24 h ($R = 0.730$, $P = 0.02$). There was a trend towards a correlation between hepatocyte injury, as detected by areas of liver necrosis, and HSC levels in the bone marrow at 72 h in wild type mice ($R = 0.606$, $P = 0.15$, data not shown).

Differential HSC and CD133+ MP responses in wild type and *Cd39* null mice in APAP hepatotoxicity

—We could detect CD133+ MP in the plasma of sham operated wild type and *Cd39* null mice. In agreement with previous studies MP did not express CD45 [15] (data not shown). CD133+ MP levels increased over time in wild type mice with significantly higher levels at 48 h, when compared to shams ($P = 0.045$). In contrast, no increase of CD133+ MP was observed in *Cd39* null mice. CD133+ MP levels increased over time in wild type mice with significantly higher levels at 48h, when compared to *Cd39* null mice ($P = 0.036$). (Figure 1f) There was a trend towards a correlation between plasma VEGF levels and CD133+ plasma MP of wild type mice at 24 h ($R = 0.685$, $P = 0.06$).

CLINICAL STUDIES

MP responses in liver injury patients—Patients with acute liver injury and acute on chronic liver injury were enrolled in a pilot study. Plasma MP could be detected in all patients as well as in controls. (Figure 2d) Distinct MP populations were identified in liver injury patients: these were noted to be CD133 single positive, CD39 single positive or double positive for CD133 and CD39. (Figure 2a-2c) Patients with acute liver injury were characterized by 2 - 3 fold higher CD39+ MP plasma levels, when compared to controls ($P = 0.01$). Further, trends for increments in ectonucleotidase-bearing CD39+ CD133+ MP double positive subsets ($54.2\% \pm$ SEM 8.2%; $P = 0.06$) were seen in these patients, when compared to controls ($30.3\% \pm$ SEM 7.9%). (Figure 2e, 2f) Trends toward higher CD39+ MP levels ($24.0\% \pm$ SEM 6.5%, $P = 0.08$) were also seen in patients with acute on chronic

liver injury with significantly higher levels of CD39+CD133+ MP subsets in these patients when compared to controls ($74.4\% \pm \text{SEM } 8.1\%$, $P = 0.003$). (Figure 2e, 2f)

Plasma levels of vascular cell adhesion molecule 1 (VCAM-1) and interleukin-8 (IL-8) were significantly increased in patients with acute (VCAM-1; $3800 \pm \text{SEM } 791 \text{ ng/ml}$, IL-8; $127 \pm \text{SEM } 25 \text{ pg/ml}$) and acute on chronic (VCAM-1; $4543 \pm \text{SEM } 431 \text{ ng/ml}$, IL-8; $113 \pm \text{SEM } 36 \text{ pg/mL}$) liver injury, when compared to controls (VCAM-1, $105 \pm \text{SEM } 11 \text{ ng/ml}$, $P = 0.02$, $P < 0.001$; IL-8, $4 \pm \text{SEM } 1 \text{ pg/ml}$; $P = 0.02$). Levels of VEGF were significantly decreased in patients with acute liver injury (VEGF, $29 \pm \text{SEM } 22 \text{ pg/ml}$) and acute on chronic liver injury (VEGF, $112 \pm \text{SEM } 16 \text{ pg/ml}$), when compared to controls (VEGF, $178 \pm \text{SEM } 14 \text{ pg/ml}$; $P < 0.001$, $P = 0.01$, data not shown).

DISCUSSION

We and others have proposed that HSC might serve as a novel population of immune-like cells modulating vascular inflammation at sites of injury in a paracrine manner. CD133+ bone marrow-derived HSC are known to be mobilized after partial hepatectomy in mice and surgical hepatic resections in the clinic [6]. We note that therapeutic administration of HSC enhances liver regeneration in experimental animal models and in patients after surgical resections [6, 16-18]. Our recent data indicate that HSC boost liver regeneration by exhibiting anti-inflammatory effects on the liver vasculature [6].

We here show that CD133+ and CD39+ HSC are mobilized three days after experimental APAP-intoxication at substantive levels in the blood, comparable to kinetics seen after partial hepatectomy in mice. We suggest that it is HSC cells that release CD133+ MP, which are present at high plasma levels in this model. Acute liver injury is further associated with increased levels of CD133+ MP in the blood, and these are likely to be derived from HSC. In the setting of APAP intoxication, *Cd39* null mice, which exhibit severe liver injury, do not exhibit comparable HSC mobilization responses suggesting that purinergic signals might dictate liver injury and/or the processing of MP.

We have also examined plasma MP levels in patients with liver injury admitted to the Emergency Department (ED). These MP are characterized by significantly higher levels of CD39 with acute liver insults ($p=0.01$). We further note that double positive CD39+CD133+ MP are elevated in acute on chronic liver decompensation ($p=0.003$). These early results in this pilot study suggest potential roles of MP as biomarkers in acute liver insults and/or the acute deterioration of liver function in patients with chronic liver dysfunction. Further work in larger patient cohorts is required to validate the proposed use of MP as biomarkers in acute liver decompensation per se.

We have recently shown that subsets of HSC migrate towards VEGF, in vitro; this process is triggered by regulated hydrolysis of ATP by CD39 and autocrine purinergic stimulation of adenosine sensitive P1-receptors [6]. HSC are likely mobilized in response to VEGF, in vivo, which is released in response to vascular injury in ALF [19, 20]. We note impaired VEGF-dependent HSC migration and further that mutant mice null for *Cd39* fail to mobilize HSC despite increased levels of VEGF, unlike wild type mice subjected to APAP. These data suggest VEGF resistance in *Cd39* null mice with acute liver dysfunction secondary to APAP, comparable to what has been noted in partial hepatectomy models [3, 6].

Cd39 null mice show heightened vascular inflammation in both surgical and pharmacological liver injury models [3, 6, 21, 22]. Loss of both vascular and stem cell-bound CD39 is accompanied by impaired scavenging of pro-inflammatory nucleotides leading to increased ATP-stimulated IL-1 β release at sites of injury [3, 6, 22]. Consequently,

CD39 appears to be involved in the dampening of vascular insult in acute models of liver injury and in regeneration.

We have previously shown that plasma MP bear functional active CD39 and that vascular inflammation is modulated by plasma MP in a CD39-dependent manner, *in vitro*. It remains speculative, so far, whether HSC can also modulate vascular inflammation and consequently APAP-induced ALF by rapid shedding of ectonucleotidase-bearing CD133+ MP *in vivo*. However, given paracrine effects involved in HSC-dependent liver protection, plasma CD133+ MP might well be involved in the amelioration of vascular insults in this acute model of liver injury.

In concordance with data from experimental models and as noted above, distinct patterns of plasma MP responses were noted in patients with liver injury. MP appear to impact intercellular communication, by transferring gene products which modulate inflammation and impact regeneration [23, 24]. Whether CD133+ MP might exhibit distinct functions at sites of injury in these patients according to the alterations in ability to hydrolyze ATP remains uncertain. Potential roles of CD39 within the CD133+ MP and the proposed use as biomarkers needs confirmation in larger patient cohorts.

CONCLUSIONS

After APAP-induced ALF, there is increased mobilization and increases in CD133+ MP, which might be involved in modulation of vascular inflammation. APAP-induced ALF is exacerbated in *Cd39* null mice, when compared to wild type mice. These mutant mice do not show increased levels of CD133+ MP in plasma. CD39-mediated anti-inflammatory effects within MP might be involved in dampening vascular injury and limiting hepatic necrosis. CD39+ and double positive CD39+CD133+ MP are increased in the blood of patients with acute liver injury and acute on chronic liver insults, respectively. Ectonucleotidase-bearing CD133+ MP might serve as potential biomarkers with prognostic import in patients with ALF and further studies are required to validate this.

METHODS

ANIMAL STUDIES

Animals—Male wild type C57Bl/6 (Taconic, Germantown, NY) and *Cd39* null mice aged 7-9 weeks were used in accordance with the guidelines from the American Association for Laboratory Animal Care. *Cd39* null mice were derived as previously described [3,15]. All animal research protocols were approved by the Beth Israel Deaconess Medical Center (BIDMC) Institutional Animal Care and Use Committee (IACUC).

Experimental acute liver failure model—Animals were allowed to acclimate for one week before experiments. Animals were then subject to a 16-hour overnight fast with ad libitum access to water. APAP (300 mg/kg) was dissolved in warm saline and administered by intra-peritoneal injection. Food was returned 3 hours after APAP administration to allow for drug metabolism prior to re-feeding. Animals were sacrificed at 24, 48 and 72 hours after APAP administration. Prior to sacrifice, animals were anesthetized with intra-peritoneal injection of Ketamine/Xylazine and were subsequently exsanguinated via cardiac puncture. Blood and liver tissue were collected and further processed, as indicated.

FACS analysis of HSC—Blood was collected in heparinized syringes and incubated with PE-conjugated anti-CD133 (clone: 30-F11, BD Biosciences, USA) and FITC-conjugated anti-CD45 (clone: 30-F11, BD Biosciences, USA) antibodies. Subsequently, red blood cells were lysed and solution was washed twice (10 min, 500 RPM, 4°C). HSC levels were

analyzed by fluorescence activated cell sorting (FACS) on a LSR II (Becton Dickinson, San Jose, CA). HSC levels are presented as %CD133+CD45+ cells of peripheral blood mononuclear cells (PB MNC) and %CD133+CD45+ cells of bone marrow mononuclear cells (BM MNC), respectively.

Isolation of HSC-derived microparticles—Blood was collected in heparinized syringes and subsequently centrifuged (15 min, 5000 RPM). Plasma was collected and stored at -80°C. Isolation of plasma MP was performed by ultracentrifugation with modifications to recent reports [12]. Shortly, plasma was thawed and 2- step ultracentrifuged at 10,000 g for 30 min followed by 100,000 g for 90 min at 5°C to gain the biologically active S100 Fraction [12]. After plasma was discarded, plasma MP were resuspended in 300 µl sterile PBS and stored at -80°C.

FACS analysis of MP—Plasma MP were incubated with PE-conjugated anti-CD133 (clone: 30-F11, BD Biosciences, USA) for 10 min on ice. FACS analysis of plasma MP was performed on a LSR II (Becton Dickinson, San Jose, CA), as previously described [12]. MP were gated on forward and sideward scatter acquired from runs including microbeads [14]. Unbound antibody solved in PBS was run prior to measurements to separate real events from background. The number of CD133+ MP was calculated relative to the number of all gated plasma MP.

Histology—Liver tissue was harvested, fixed with formalin 10% and paraffin-embedded. Hematoxylin-Eosin (H/E) staining was performed as previously described [3]. The degree of necrosis was determined by measuring the area of necrosis as percentage of total area in representative low power (10x) fields using ImageJ software, NIH.

TUNEL—DNA fragmentation in the liver was determined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) as described [3]. TUNEL was performed on 10% formalin-fixed and subsequently paraffin-embedded liver sections and analyzed by measuring the area of apoptosis as percentage of total area in representative low power (10x) fields using ImageJ software, NIH.

ELISA—Plasma VEGF was determined using commercial ELISA kits (R&D Systems, USA) following manufacturers' instructions.

ALT assay—Plasma levels of ALT were determined using commercial ALT reagents (Thermo Scientific, USA) following manufacturers' instructions.

CLINICAL STUDIES

Patients—Patients with acute ($n = 5$; $\bar{x} = 3$; $\sigma = 2$; age 64 ± 19) and acute on chronic ($n = 5$, $\bar{x} = 2$; $\sigma = 3$; age 53 ± 13) liver injury admitted to Beth Israel Deaconess Medical Center (BIDMC), Boston, MA, were included in this pilot study. Seven matched patients admitted with minor injuries and without history of liver disease served as controls. Acute liver injury was defined by elevated transaminases ($\text{ALT} > 250$). Acute on chronic liver disease was defined by acute deterioration of the liver in patients with known preexisting liver cirrhosis. Patient characteristics are described in Table 1.

EDTA blood was collected at enrollment and further processed, as indicated. Written informed consent was obtained from all and the study was approved by the institutional review board (IRB) for clinical research.

Isolation of plasma microparticles—Isolation of plasma MP from human plasma was performed by 2-step ultracentrifugation according to animal studies (see above).

Fluorescence activated cell sorting (FACS)—FACS analysis of plasma MP was performed on a LSR II (Becton Dickinson, San Jose, CA) according to animal studies (see above). Antibodies were used as following: anti-CD133-PE (clone: 293C3, Miltenyi Biotec, Germany) and CD39-FITC (eBioscience, USA).

ELISA—Plasma levels of E-Selectin, VCAM, ICAM, IL-6, TNF α , VEGF, IL-1ra, IL-8 and IL-10 were determined by enzyme-linked immunosorbent assay (ELISA) using commercial ELISA kits (Millipore, USA) according to manufacturer's specifications.

HPLC—Plasma levels of thiamine were determined by HPLC, as a component of this study as previously described [25].

Statistics—Results are reported as mean $\pm$ standard error (SEM). Student's t test was used to test significance. Values were considered significant when $P < 0.05$.

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Appendix

Moritz Schmelzle: Conception and design of the study. Performance of the research, analysis and interpretation of data. Drafting and critical revision of the manuscript.

Katrin Splith: Design of the study. Analysis and interpretation of data. Drafting and critical revision of the manuscript.

Lars W. Andersen: Interpretation of data and critical revision of the manuscript.

Mirosław Kornek: Performance of the research, analysis of data and critical revision of the manuscript.

Detlef Schuppan: Analysis of data and critical revision of the manuscript.

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Simon C. Robson: Conception and design of the study. Analysis and interpretation of data. Drafting and critical revision of the manuscript.

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Abbreviations

ALF	Acute liver failure
ACLF	Acute on chronic liver failure
ALT	Alanine transaminase
APACHE	Acute Physiology and Chronic Health Evaluation
APAP	N-acetyl-p-aminophenol
CAD	Coronary Artery Disease
CHF	Chronic Heart Failure
ELISA	Enzyme-linked Immunosorbent Assay
FACS	Fluorescence activated cell sorting
HSC	Hematopoietic stem cells
HTN	Hypertension
ICU	Intensive Care Unit
IDDM	Insulin Dependend Diabetes Mellitus
INR	International Normalized Ratio
MI	Myocardial Infarction
MP	Microparticles

PLT	Platelet count
PTT	Partial Thromboplastin Time
PT	Prothrombin time
PVD	Peripheral Artery Disease
SOFA	Sequential Organ Failure Assessment
TUNEL	TdT-mediated dUTP-biotin nick end labeling
WBC	White blood cells

Figure 1.

Liver injury and vascular disruption after APAP intoxication in mice. Animals were treated with 300 mg/kg APAP. (a) Representative H&E-stained liver sections ($\times 10$) are shown for wild type and *Cd39* null mice 24 h after APAP intoxication. (b) Areas of cell death at 24 h after APAP intoxication in wild type and *Cd39* null mice. (c) ALT levels in are shown for wild type and *Cd39* null mice 24 h, 48 h and 72 h after APAP intoxication. (d) Vascular injury indicated by the serum VEGF levels in wild type and *Cd39* null mice 24 h after APAP intoxication. (e) HSC levels after 48 h and 72 h in the blood of APAP-intoxicated wild type and *Cd39* null mice. (f) CD133+ MP levels after 48 h and 72 h in the blood of APAP-intoxicated wild type and *Cd39* null mice. Error bars represent SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2.

Patient's characteristics and analyses of plasma MP. Plasma MP were obtained by a 2-step ultracentrifugation. FACS analysis of MP. **(a)** Single populations of microspheres (1 μ m), antibody alone and targeted microparticles. **(b)** Populations of unstained and CD133-PE stained plasma MP. **(c)** Populations of CD133-PE and CD39-FITC stained plasma MP. **(d)** The black arrow indicates the MP pellet. **(e)** The % of CD39+ MP of all plasma MP as determined by FACS in patients with acute (ALF) and acute on chronic liver injury (ACLF) **(f)** and % CD39+ MP as a fraction of all CD133+ MP determined by FACS. Error bars represent SEM, *P < 0.05.

Table 1

Clinical characteristics of the patients

Patient	Acute liver injury	Acute on chronic liver injury
Patients (n)	5	5
Age (Mean $\pm$ SD)	64 $\pm$ 12	53 $\pm$ 8
Gender (n)		
Male/ Female	2/3	3/2
BMI (Mean $\pm$ SD)	29.8 $\pm$ 3.4	30.8 $\pm$ 5.9
Race (n)		
White/Asian/Black	4/1/0	4/0/1
Ethnicity (n)		
Hispanic/ Not Hispanic	0/5	1/4
Comorbidities* (n)		
No/CAD/ MI/ CHF/ IDDM/	0/3/2/3/2/	2/0/0/0/1
PVD/HTN/cancer/alcohol	1/1/2/1	0/3/0/2
Viral Hepatitis (n)		
None/C/B,D/alcoholic	5/0/0/0	1/1/1/2
EtOH (n)		
Active/Non-active	1/4	2/3
Dialysis (n)		
Yes/No	2/3	0/5
Vasopressor (n)		
Yes/No	3/2	2/3
Mechanical Ventilation (n)		
Yes/No	2/3	3/2
Outcome (n)		
Discharged/ Expired	2/3	3/2
Scores (Mean $\pm$ SD)		
MELD	23 $\pm$ 7	29 $\pm$ 10
SOFA	10 $\pm$ 5	9 $\pm$ 8
APACHE	25 $\pm$ 11	18 $\pm$ 14
Hospital stay (Mean $\pm$ SD)		
Total days	12.2 $\pm$ 10.1	10.6 $\pm$ 9.3
ICU days	9.6 $\pm$ 7.8	4.8 $\pm$ 4.5
Death (n)		
Yes/No	3/2	2/3
Coagulopathy (Mean $\pm$ SD)		
INR	2.2 $\pm$ 1.5	2.3 $\pm$ 1.0
PT [s]	22.5 $\pm$ 12.8	24.8 $\pm$ 8.4
Liver (Mean $\pm$ SD)		
Bilirubin [μ mol/L]	41.0 $\pm$ 36.2	376.5 $\pm$ 235.7
ALT [IU/L]	1746.4 $\pm$ 1686.1	133.6 $\pm$ 106.7

Patient	Acute liver injury	Acute on chronic liver injury
AST [IU/L]	2820.2 ± 3364.8	170.8 ± 213.2
Kidney (Mean ± SD)		
Creatinine [mg/dL]	2.3 ± 1.0	1.3 ± 1.0
Blood (Mean ± SD)		
plt [/μL]	108000 ± 87700	98700 ± 93600
wbc [/μL]	11200 ± 6000	7700 ± 3100
Thiamine(Mean ± SD) [nmol/L]	51.2 ± 40.3	42.0 ± 38.7

* several selections possible; CAD = coronary artery disease, MI = Myocardial infarction, CHF = Chronic heart failure, IDDM = Insulin depended diabetes mellitus, PVD = Peripheral artery disease; HTN = Hypertension