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Prospective assessment of hepatic function and mechanisms of dysfunction in the critically ill

by

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Running title: Liver dysfunction in the critically ill

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Abstract

Liver dysfunction affects a variety of metabolic pathways in the critically ill but mechanisms remain poorly understood. We prospectively assessed markers of hepatic injury and function in sepsis and ischemia/reperfusion injury *in vivo* and molecular mechanisms in human liver tissue *ex vivo*. Markers of hepatocellular injury, synthesis, and excretion, including plasma disappearance rate of indocyanine green, were measured in 48 patients with severe sepsis. Incidence of liver dysfunction was 42% as assessed by hyper-bilirubinemia but 74% by impaired dye excretion. Conventional markers for liver injury failed to predict outcome, while dye excretion <8%/min predicted death with high sensitivity and specificity. Potential mechanisms were assessed via 1) gene expression analysis of transporter proteins for bilirubin and indocyanine green in cultured human liver tissue, and 2) monitoring uptake and excretion of the dye after ischemia/reperfusion injury in 12 patients receiving a biliary T-tube during liver transplant. *Ex vivo* gene expression of transporters was differentially affected for bilirubin and indocyanine green with up-regulation of basolateral and down-regulation of canalicular indocyanine green transporters. Consistently, patients with unfavorable course after liver transplantation displayed almost complete cessation of biliary dye excretion while uptake into the hepatocyte was reduced by only 40%.

In conclusion, standard liver tests lack the required sensitivity to assess hepatic injury and function in the critically ill. Dye excretion better reflects excretory and/or microvascular dysfunction but still underestimates impaired canalicular transport. The observed differential susceptibility of the polar surfaces of human hepatocytes has potential implications for monitoring liver function and drug-induced liver injury.

Keywords: transporter protein, basolateral, canalicular, indocyanine green, sepsis, liver

Introduction

The liver with its multitude of metabolic functions plays a key role in the host defense response but is also a target for remote organ dysfunction in systemic inflammation (1). The hepatic response to infection involves synthesis of proteins such as acute phase reactants and coagulation factors (2), as well as removal of bacterial toxins (3). Based on the kinetics of the rise in serum bilirubin, liver dysfunction has traditionally been thought to occur late in multiple organ dysfunction (4,5,6) although prospective and synoptic data on the various liver injury and function tests have not yet been reported (7). A recent analysis of the data collected by the Austrian Center for Documentation and Quality Assurance in Intensive Care Medicine indicated in a large cohort of mixed ICU patients that increased bilirubin upon admission without history of liver disease occurs in 11% of patients and is an independent risk factor for poor prognosis (8). However, serum bilirubin as well as transaminases may lack the required sensitivity to assess the complex function of this important metabolic and immune organ with its heterogeneous cellular composition.

Based on scoring systems, most notably the SOFA score using serum bilirubin to assess and define liver dysfunction, an incidence of only 1-3% of overt liver failure has been reported, reflecting a rare impairment of the liver in severe sepsis (9,10). This is at odds with a multitude of careful experimental studies highlighting the particular sensitivity of the liver with its high amount of tissue fixed macrophages (1,3) as well as a previous retrospective study by Sakka et al. using plasma disappearance rate of indocyanine green (PDR_{ICG}), an anionic dye, to evaluate functional impairment of hepatocytes under these conditions (11). The latter is a complex measure of both sinusoidal perfusion as well as hepatic cell membrane function, and thus, reflects a functional reserve of intact hepatocytes which participate in maintained nutritional perfusion (12). Although these studies suggested that PDR_{ICG} may help to assess "liver function," any retrospective study might overestimate the

sensitivity and specificity as the test is performed when liver dysfunction is already suspected.

The present study began with a prospective investigation of the development of liver dysfunction in patients admitted to an interdisciplinary ICU for severe sepsis, as reflected in a broad spectrum of conventional and auxiliary parameters as well as PDR_{ICG}. Next, as dye excretion appeared as the most sensitive marker, we aimed to further elucidate molecular mechanisms of impaired excretion in an *ex vivo* model using precision cut human liver tissue (13) cultured in the presence of stressors mimicking the hepatocellular milieu in sepsis, ischemia/reperfusion, and shock (1,3,14). Finally, as the *ex vivo* studies pointed toward a highly differential susceptibility to injury of the two polar surfaces of the human hepatocyte (basolateral and canalicular), we compared the rate of disappearance of ICG dye from the blood (a basolateral transporter function) and its appearance in the bile (a canalicular function) of patients undergoing liver transplant. Taken together, these research components provide evidence that dye excretion can add important, timely information to traditional markers to identify excretory or microvascular dysfunction in the human liver associated with ischemia, reperfusion, and infection.

Methods

Prospective assessment of liver dysfunction in severe human sepsis

After study approval by the institutional review board and written informed consent by legal representative or next of kin, 48 consecutive patients of either sex fulfilling ACCP/SCCM criteria for severe sepsis (15) were included in this single-center prospective observational study. In addition, informed consent was obtained from patients undergoing liver resection or transplant to either study ex vivo liver tissue from surgical waste or monitor bile appearance of ICG.

Acute Physiology and Chronic Health Evaluation (APACHE) II–Score (16), Multiple Organ Dysfunction (MOD)-Score (17) and Sepsis-related Organ Failure Assessment (SOFA)-Score (4) were calculated on a daily basis.

After inclusion in the study a transpulmonary thermodilution catheter (COLD system, Pulsion Medical Systems, Munich, Germany) with a fiberoptic tip to measure ICG concentrations in blood was introduced into a femoral artery and hemodynamic measurements were performed by injection of iced 5% dextrose solution with 2mg/ml ICG (Pulsion Medical Systems, Munich, Germany) at a dose of 0.3 mg/kg bodyweight into a central venous catheter. ICG is nearly exclusively eliminated by the liver without biotransformation and is not undergoing enterohepatic recirculation. PDR_{ICG} means the change of ICG concentration over time (in percent per minute) and reflects the amount of the dye which is eliminated in percent of the initial value after complete mixing (18).

In addition to routine laboratory markers, blood samples were obtained from day 1 to 4 to assess concentrations of the following static liver function tests and markers for disease severity/ liver injury: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), γ-glutamyltranspeptidase (γ-GT), bilirubin, cholinesterase, albumin, prothrombin time, fibrinogen, antithrombin, C-reactive protein, α_2-macroglobulin, interleukin 6 (IL6) in serum or plasma, respectively.

Gene expression of hepatocyte transporter proteins

To further probe molecular mechanisms of impaired liver function, we next subjected human liver tissue *ex vivo* to glutathione depletion or cytokine stimulation, reflecting prototypical injury mechanisms in ischemia/reperfusion and shock. Three slices from each of 3 individual patients undergoing liver resection for metastases of colorectal carcinomas were precision-cut from tumor-free margins of surgical waste material using a Krumdieck slicer (MD4000-01, Alabama R&D, Munford, AL) and brought into primary culture for 24 hours as previously

described in detail (13). Slices of 200µm thickness were incubated in conventional Williams E medium for 2 hours. Two series of stimulation experiments were performed by subsequent change of medium to Williams E containing either: mixed inflammatory mediators (i.e. TNFα 50ng/ml, IFNγ 10ng/ml, IL1β 10ng/ml, and lipopolysaccharide 100 ng/ml at final concentrations) mimicking the inflammatory milieu in sepsis (14), or 2,6-dimethyl-2,5-heptadien-4-one (phorone; 0.2 or 1 mM) to achieve depletion of the cellular glutathione content (19).

Expressions of transcripts encoding for basolateral and canalicular transporters were assessed at 6 and 24 hours after onset of cellular stress. Incubated slices were harvested to prepare total RNA. First strand cDNA synthesis for real time PCR was performed using the Reverse Transcription System, Promega Corporation Superscript TM II RNase H, Reverse Transcriptase (Invitrogen) according to manufacturer instructions. Real-time PCR was run on an iCycler™ (Biorad) on 20µl of reaction mixtures containing 10µL 2x iQSybr®Green Supermix, 3µL Primer mix (1.67 µM concentration of up- and down stream primers for the various transporter proteins; for specification of studied transporters and respective primers see table 1), 5µL of nuclease free water and 2µL of the probe at a final concentration of 12.5 ng/µL. The melting protocol ranged from 59°C to 100°C after a stepwise increment of 0.5°C held for 30 seconds. Hypoxanthine ribosyl transferase (HPRT) served as housekeeping gene.

Kinetics of basolateral and canalicular transport of ICG in liver transplant recipients

The third component of this study was to provide support for the concept that discriminate susceptibility of the two polar surfaces exists in the human liver exposed to stress. For this, we simultaneously monitored the disappearance of ICG from the blood and its appearance in the bile in 12 patients undergoing liver transplant. Patients were divided in two groups: i) those with good graft function in the first day and without remote organ failure (n=7), and ii) those patients presenting with a complicated course defined as primary graft dysfunction and/

or multiple organ dysfunction (n=5). Within the first 24 hours after liver transplantation PDR_{ICG} was measured non-invasively transcutaneous using a LiMON monitor and a pulse densitometry finger clip sensor (Pulsion Medical Systems; Munich, Germany). To assess the biliary concentration of ICG, we collected bile before ICG was given and then over 5-minute intervals during the first hour after injection of ICG. Samples were kept frozen until measurement. The bile collected before ICG injection was used to draw up standard c urves. Therefore ICG was added to the bile at final concentrations of 500, 250, 125, 62.5 and 31.25 ng/µl, respectively. Spectrophotometric assessment of the concentration of ICG (wave length 800 nm) was read using a Nanodrop™ spectrophotometer (Nanodrop Technologies, Wilmington, USA).

Statistical analysis

Numerical values are given as median and interquartile range. Box and whisker plots indicate median, quartiles, 10 and 90% quantiles and outliers. Data at inclusion were evaluated with unpaired t-test or Mann-Whitney rank sum test for continuous variables or Fisher's exact test for categorical variables. For serial measurements analysis of variance (ANOVA) was used. For the areas under the curve for the receiver operating characteristics (AUC), standard error and 95% confidence intervals were calculated. Furthermore, specificity and sensitivity to predict survival were assessed. Correlational analyses were performed with Pearson product moment correlation or Spearman rank order correlation respectively and correlation coefficients and p-values are given. Sigma Stat for Windows 2.03 and SPSS for Windows 11.0.1 (SPSS Inc., Chicago, Illinois) were used for the analysis. All p-values are two-tailed and were considered significant if less than 0.05.

Results

Markers for liver injury, synthesis and excretion

The 48 patients fulfilling criteria of severe sepsis included in the prospective observational segment of this study had a mortality rate of 38%. Non-survivors were significantly older than survivors and had higher APACHE II-, MOD- and SOFA-scores at time of inclusion. However, no significant differences were observed at this time for SIRS-criteria, hemodynamic parameters, oxygenation index, inotropic and vasopressor support (table 2).

Differences between survivors and patients ultimately succumbing to sepsis regarding laboratory tests were observed at inclusion for AST, creatinine, urea, cholinesterase and lactate while all other tests, most notably those traditionally used to assess liver injury and metabolic function (ALT, AP, γ-GT, albumin, prothrombin ratio, antithrombin, fibrinogen, α_2-macroglobulin, C-reactive protein, white blood count, platelet count and IL6 levels) were comparable between survivors and non-survivors (tables 2 and 3).

Transfusion requirements were comparable between survivors and non-survivors for packed red blood cells, platelets, prothrombin complex and antithrombin. Fresh frozen plasma (FFP) transfusion was also comparable with respect to the number of transfused units. However, by tendency more non-survivors were transfused during the observation period (p=0.074) and significantly more non-survivors received units of FFP during the first day (survivors: 3 out of 30 patients, non-survivors: 7 of 18 patients, p=0.027). This might be the cause of the paradox observation of higher values of cholinesterase in non-survivors.

PDR_{ICG} was significantly higher in survivors on days one and three while there were no significant differences for bilirubin over time (figure 1). As shown in figure 2, receiver operating characteristics of conventional markers for liver injury (e.g. ALT: AUC 0.48, p=0.084; bilirubin: AUC 0.43, p=0.412) failed to predict poor outcome, while $PDR_{ICG} < 8\%$/ min (AUC 0.81, p=0.006) as a complex estimate of perfusion, energy metabolism and

transporter function predicted death with a sensitivity of 81% and specificity of 70%. Weak but significant correlations were obtained between PDR_{ICG} and surrogates of altered blood flow, i.e. cardiac index (r^2=0.05; $p<0.05$) and lactate (r^2=0.22; $p<0.05$), as well as between PDR_{ICG} and systemic inflammation, i.e. IL6 (r^2=0.09; $p<0.05$). Incidence of liver dysfunction was estimated to be 42 % and 74% as assessed by hyperbilirubinemia (i.e. bilirubin > 1.2 mg %) or PDR_{ICG} (< 16%/min), respectively. Consistent with previous epidemiological results from large cohorts, incidence of severe liver failure as assessed by a SOFA-subscore for the liver item of ≥ 3 (bilirubin ≥ 6 mg%) was 8%.

Gene expression of transporter proteins in cultured human liver tissue

Gene expression of basolateral and canalicular transporter proteins for the two cellular stress events, i.e. inflammatory stimulation and glutathione depletion, revealed differential changes for individual transporter genes as well as differential changes in the expression of basolateral as opposed to canalicular transporters for bilirubin and ICG (figure 3; for a comprehensive summary of all transporters studied see supplemental figure 1 in the electronic supplement). While some of the transporters displayed maintained or even increased expression despite hepatocellular oxidative or inflammatory stress, some steady state transcript concentrations were substantially decreased reflecting a possible mechanism for the observed disturbance in hepatocellular excretory function and differential susceptibility of the two polar surfaces of the human hepatocyte. Particularly sensitive proteins regarding stress were the basolateral transporter multidrug resistance-associated protein (MRP)3 as well as the canalicular transporters MRP2, multidrug resistance protein (MDR)3, bile salt export pump (BSEP) and the anion exchanger Cl^-/HCO_3^- AE2. Organic anion transporting polypeptide (OATP)1, the prototypical transporter for ICG on the basolateral membrane displayed increased expression for both stress events. Overall, the canalicular transporters seemed to be more sensitive than the basolateral transporters to either stress event.

It is particularly noteworthy that the prototypical transporters for bilirubin (OATP2 and MRP2) (20, 21, 22) and ICG (OATP1 and MDR3) (21, 23, 24) at the basolateral and canalicular membrane displayed discrepant susceptibility to stress events. The up-regulation of OATP1 along with the down-regulation of MDR3 would indicate maintained uptake mechanisms from the sinusoidal blood stream but a substantially impaired capacity to transport ICG into the bile at the canalicular pole (figure 3).

Biliary excretion of the dye in transplant recipients

Cold ischemia and reperfusion initiates a significant inflammatory response with local cytokine production (25) along with glutathione depletion (26). As both events, i.e. cytokine stimulation and glutathione depletion, had similar effects on the genes encoding basolateral and canalicular transporter proteins *ex vivo*, we hypothesized that the insult associated with transplantation differentially affects the two polar surfaces of the hepatocyte. More specifically, we expected a better preserved transport from the sinusoid into the hepatocyte compared to biliary excretion of the dye depending on the degree of injury as reflected in a complicated course. Indeed, although both plasma disappearance and peak biliary concentration of the dye were significantly affected by injury, the canalicular transport was particularly vulnerable (figure 4) with an almost complete cessation of ICG excretion into the bile in those patients with an unfavorable course after transplant.

Discussion

In the present study, we first have investigated the time course of a variety of static and dynamic parameters to assess hepatocellular function and injury, as they relate to the prognosis of patients admitted for severe sepsis. Among the various investigated parameters only the complex and dynamic PDR_{ICG} which indicates sinusoidal perfusion and hepatocellular membrane function, was able to predict outcome with reasonable sensitivity

and specificity. In contrast, traditional parameters of injury or synthetic function – including acute phase reactants - failed to predict unfavorable outcome. Moreover, a number of laboratory parameters of synthesis (e.g. cholinesterase) interfere with interventions such as transfusion of fresh frozen plasma, rendering interpretation in the ICU setting elusive. These data indicate that among the three key features defining acute liver failure, i.e. impaired excretory function, impaired synthesis, and hepatic encephalopathy, under the conditions of severe sepsis only excretory function can be used to estimate severity of liver dysfunction. Clearly, excretory function seems particularly vulnerable, while synthesis is well maintained or masked by therapeutic interventions and loss of consciousness in multiple organ dysfunction may result from various reasons or simply reflect the need for sedatives in the mechanically ventilated critically ill.

Correlational analyses between cardiac index and lactate as surrogates of perfusion and PDR_{ICG} would indicate that cardiovascular impairment at the macrocirculatory level is not an exclusive mechanism underlying the impaired PDR_{ICG} as low PDR_{ICG} values were observed under conditions of hyperdynamic circulation with normal lactate. Similarly, an r^2 of only 0.09 between IL6 and PDR_{ICG}, albeit statistically significant, argues against an exclusive role of a systemic inflammatory response in mediating sinusoidal perfusion abnormalities and/or excretory dysfunction in severely septic patients.

However, the well maintained expression of OATP1 *ex vivo*, as the prototypical transporter for the anionic dye ICG, would be consistent with a contribution of sinusoidal perfusion failure to impaired ICG clearance. Indeed, intrinsic hepatic perfusion failure despite maintained cardiac output is a hallmark of sepsis/endotoxemia and reflects activation and release of vasoactive factors by non-parenchymal cells, including Kupffer and stellate cells in experimental sepsis (27,28,29), while its contribution to dysfunction in the human liver has not been directly demonstrated to date.

These results would suggest that various hepatic functions are highly selectively affected by the sepsis process: sinusoidal perfusion and excretory function which are both reflected in the PDR_{ICG} are substantially impaired in non-survivors. Furthermore, plasma concentration of bilirubin, which is affected by a multitude of factors other than excretion in the critically ill, lacks the required sensitivity to predict liver dysfunction as it relates to outcome or might reflect the protective role of biliverdin/ bilirubin derived from heme oxygenase activity (30,31). Thus, monitoring PDR_{ICG} is superior to serum bilirubin levels.

Traditionally, liver dysfunction under conditions of shock and sepsis is considered to be biphasic with an initial ischemic insult ("ischemic hepatitis", (32)) followed by "ICU-jaundice" (5,33) developing several days later. However, our data would argue against the simple concept of an initial ischemic insult due to septic shock followed by a somewhat delayed cholestasis reflecting a prolonged inflammatory response to the initial ischemic insult. Our data indicate that unlike previous observations in hypovolemic shock (5), a full blown "ischemic hepatitis" as reflected in transaminases above 500 U/l rarely occurs in septic shock (4 out of 48 patients) and that impaired excretory function is present as early as on the first day if PDR_{ICG} with its better sensitivity is used rather than cholestasis-indicating enzymes (AP, γ-GT) and bilirubin.

In the second part of our study we therefore evaluated the potential molecular mechanisms of impaired excretion in human liver tissue, *ex vivo*. Gene expression of various transporters was studied in precision cut liver slices cultured under conditions mimicking central cellular events associated with ischemia/reperfusion and inflammation.

While changes in gene expression of hepatocellular transporters proteins have been described for hyperbilirubinemia in chronic diseases, such as Dubin-Johnson syndrome, a cholestatic syndrome resulting from absence of MRP2, an adenosine triphosphate (ATP)-dependent conjugate export pump from the hepatocyte canalicular membrane (22), studies addressing molecular mechanisms of cholestasis in critically ill patients are sparse or absent.

In the light of recent animal data indicating a maintained basolateral uptake of ICG while its excretion in bile was substantially impaired in the first hours after endotoxin challenge in pigs (34), we determined steady state transcript levels of a broad spectrum of transporters in an *ex vivo* human model. These experiments displayed complex and divergent changes with basolateral transporters being up-regulated (i.e. OATP1, the rate limiting transport system for ICG uptake from the sinusoid (21,23)) and canalicular transporters being down-regulated (i.e. MDR3, the homolog of rodent MDR2, i.e. the essential canalicular export system for ICG (21,24)). Interestingly, the various inflammatory stimuli led to directed changes in cultured human liver slices regarding steady state transcripts for the various transporters despite substantial variability among the various donors, consistent with described polymorphisms in human canalicular ABC-transporter proteins (35).

In the third part of our study we tested these *ex vivo* findings that support the above mentioned animal data (34) in human liver transplant recipients as a model of ischemia/reperfusion and inflammation. Indeed, we could demonstrate a differential susceptibility of the two polar transporter systems for ICG *in vivo* in patients with organ dysfunction. While ICG excretion into bile almost cessates hepatocellular uptake of ICG is reduced by only 40%. However, impaired intracellular transport of the dye could not be ruled out and would lead to similar findings.

It is obvious and supported by the weak correlations between PDR_{ICG} and surrogates for perfusion as well as inflammation, that the net effect of an altered transporter pattern will be complicated and overshadowed in the setting of critically ill septic patients by changes in perfusion and hepatocellular ATP content, as many of these transporters require active transport processes and are characterized by an ATP-Binding-Cassette (ABC)-motif (35). Nevertheless, our present findings in patients admitted for severe sepsis lend support to the concept that the combined information obtained by measuring PDR_{ICG} reflecting both perfusion and basolateral transporter function is superior to the other studied parameters to

detect hepatocellular dysfunction in the septic patient as it relates to outcome. Moreover, impaired PDR_{ICG} in our patient population despite maintained or even increased cardiac output along with over-expression of the basolateral transporter OATP1 *ex vivo* would be consistent with experimental studies indicating that microcirculatory shunting which is mediated by intrinsic mechanisms on the level of the sinusoid reflects the hallmark of early hepatic dysfunction in sepsis (12,27,28,29,36,37). Thus, dye excretion might be impaired due to functional shunting even if overall blood flow at the level of hepatic artery and portal vein is maintained.

Conclusion

In summary, our data indicate that among the various functions of the liver, excretory function is particularly sensitive to noxious stimuli in the septic patient while other functions such as synthesis are well preserved. Our present data, in concert with previous reports by Kimura (38) and Sakka (11), suggest that impaired excretory function and/or altered microcirculation play a central role, since in all studies impaired PDR_{ICG} correlated well with a poor prognosis. However, the differential susceptibility of the two polar surface domains of hepatocytes was unexpected and is underestimated by PDR_{ICG}. Overall, it has to be assumed that pharmacokinetics of a multitude of drugs administered in the critically ill that are subject to hepatic metabolism will be altered substantially in severe sepsis or ischemia/reperfusion, including the mechanism of altered transporter expression observed in the present study. As excretory function is important for biotransformation of xenobiotics, this may be a hitherto underestimated problem in the context of a pharmacotherapy in patients presenting with multiple organ dysfunction.

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Table 1: Sequences of primers used to amplify transcripts encoding for basolateral and canalicular transporters and the housekeeping gene hypoxanthine ribosyl transferase

basolateral transporters	oligonucleotide sequence for PCR (5´→3´)
OATP1	forward: AAGACCAACGCAGGATCCAT
	reverse: GAGTTTCACCCATTCCACGTACA
OATP2	forward: AAGCCACTTCTGCTTCTGTGTTT
	reverse:AATTCTTAGTGAAAGGACCAGGAACT
OATP8	forward: GTCCAGTCATTGGCTTTGCA
	reverse:CAACCCAACGAGAGTCCTTAGG
NTCP	forward: GGGACATGAACCTCAGCATT
	reverse: TCCTTCAGGTCCCCATCATA
MRP3	forward: GGACTTCCAGTGCTCAGAGG
	reverse: AGCTGTGGCCTCGTCTAAAA
canalicular transporters	oligonucleotide sequence for PCR (5´→3´)
MDR1	forward: GGCAAAGAAATAAAGCGACTGAA
	reverse: GGCTGTTGTCTCCATAGGCAAT
MDR3	forward: TCAATGGCTTTTAAAGCAATGCTA
	reverse: TGCAATTAAAGCCAACCTGGTT
BCRP	forward: CAGGTGGAGGCAAATCTTCGT
	reverse: ACACACCACGGATAAACTGA
MRP2	forward: TGCAGCCTCCATAACCATGAG
	reverse: CTTCGTCTTCCTTCAGGCTATTCA
BSEP	forward: ACGTTGTGGGTTGCTGAACAT
	reverse: GCAGTCAAACCACCCTATTTCC
AE2	forward: CTCTCATGGGAGGTGTTCCT
	reverse: AATCTTCTCCAGCAGTTTCAGC
Housekeeping gene	
HPRT	forward: CCTGGCGTCGTGATTAGTGAT
	reverse: CCTGGCGTCGTGATTAGTGAT

OATP: Organic Anion Transporting Polypeptide, MRP: Multidrug Resistance-associated Protein, NTCP: Na^+-taurocholate Cotransporting Polypeptide, BCRP: Breast Cancer Resistance Protein, MDR: Multidrug Resistance Protein, BSEP: Bile Salt Export Pump, AE-2: Cl^-/HCO_3^- Anion Exchange, HPRT: Hypoxanthine ribosyl transferase

Table 2: Baseline characteristics of surviving and non-surviving patients fulfilling criteria for severe sepsis

	survivors	non-survivors
age [years]	56 (46/69)	68 (60/73) [a]
sex [female/male]	12/18	9/9
weight [kg]	76 (63/85)	73 (60/90)
APACHE II-score	27 (23/33)	31 (28/41) [a]

SOFA-score	12 (10/13)	14 (11/17) [a]
MOF-score	10 (9/11)	10 (9/12)
MOD-score	6 (5/8)	9 (6/11) [a]
heart rate [beats/min]	111 (103/124)	111 (104/128)
mean arterial pressure [mmHg]	86 (73/91)	82 (77/83)
cardiac index [l/min/m²]	3.8 (3.3/5.3)	3.2 (3.1/3.9)
systemic vascular resistance [dyn*s/cm^5]	802 (643/941)	925 (799/1052)
oxygenation index [mmHg]	224 (172/295)	212 (164/230)
creatinine [mg/dl]	0.8 (0.7/1.5)	1.4 (1.0/2.7) [a]
urea [mg/dl]	47 (29/79)	83 (53/117) [a]
glucose [mg/dl]	146 (120/185)	145 (126/176)
lactate [mmol/l]	1.6 (1.3/2.5)	3.8 (2.5/6.5)[a]
hematocrit [%]	31 (28/36)	30 (29/36)
white blood count [10^3/µl]	18.4 (13.9/26.9)	19.4 (8.6/24.2)
platelet count [10^3/µl]	228 (166/397)	109 (71/223) [a]
body temperature [°C]	37.9 (37.4/38.7)	38.3 (37.6/38.8)

Values are absolute numbers (sex) or median (interquartile range); [a] indicates $p < .05$ for comparison between survivors and non-survivors

Table 3: Time course of markers for liver injury and function in patients with severe sepsis stratified according to 28 day survival

	day 1	day 2	day 3	day 4
aspartate aminotransferase [U/l]				
survivors	15 (8/37)	15 (8/48)	12 (8/36)	15 (9/27)
non-survivors	35 (25/63)	35 (19/56)[a]	20 (9/42)	23 (9/75)
alanine aminotransferase [U/l]				
survivors	13 (5/28)	14 (6/36)	10 (6/32)	13 (6/32)
non-survivors	17 (11/44)	16 (9/41)	13 (7/54)	12 (6/62)
γ-glutamyltranspeptidase [U/l]				
survivors	17 (8/47)	21 (9/34)	22 (13/47)	25 (17/66)
non-survivors	29 (12/48)	27 (17/45)	25 (21/39)	39 (19/51)
alkaline phosphatase [U/l]				
survivors	142 (58/207)	125 (96/187)	128 (106/201)	147 (109/240)
non-survivor	151 (65/223)	169 (92/406)	131 (101/304)	147.5 (103/271)
prothrombine time [%]				
survivors	74 (66/87)	80 (70/960)	86 (68/100)	87 (73/99)
non-survivors	73 (48/96)	74 (62/98)	88 (62/100)	88 (62/100)
antithrombin [%]				
survivors	64 (53/73)	60 (42/71)	55 (48/65)	63 (50/71)
non-survivors	48 (37/63)	56 (43/64)	62 (46/69)	65 (44/68)
fibrinogen [mg/dl]				
survivors	470 (371/655)	519 (345/747)	452 (386/702)	480 (416/638)
non-survivors	480 (406/563)	538 (456/619)	512 (450/572)	490 (387/534)
cholinesterase [kU/l]				
survivors	0.96 (0.65/1.94)	0.93 (0.61/1.59)	0.94 (0.58/1.57)	0.99 (0.64/1.48)
non-survivors	2.02 (1.20/2.49)[a]	1.16 (0.96/2.28)[a]	1.15 (1.01/1.79)	1.60(1.17/2.15)[a]
C-reactive protein [mg/l]				
survivors	234 (167/319)	257 (197/355)	199 (144/248)	168 (116/196)
non-survivors	226 (144/304)	209 (168/325)	172 (136/251)	154 (119/204)
albumin [g/l]				
survivors	16 (13/21)	17 (13/19)	17 (14/19)	17 (16/20)
non-survivors	19 (14/22)	18 (15/20)	18 (13/20)	19 (16/21)

α_2-macroglobulin [mg/dl]				
survivors	70 (53 /89)	71 (64/91)	80 (59/109)	88 (75/102)
non-survivors	88 (77/110)	100 (83/119)[a]	101 (64/116)	102 (71/126)
interleukin 6 [pg/dl]				
survivors	198 (82/367)	111 (70/350)	172 (58/336)	67 (50/192)
non-survivors	129 (79/301)	380 (86/777)	59 (46/989)	95 (65/389)

all values are median (interquartile range); [a] indicates $p < .05$ for comparison between survivors and non-survivors on that day

Figure legends

Figure 1. Time course of excretory dysfunction in severe human sepsis as assessed by plasma bilirubin values (upper panel) or plasma disappearance rate of the anionic dye indocyanine green (lower panel).

Open bars reflect patients ultimately surviving while solid bars indicate those succumbing to sepsis. * indicates $p < .05$ as compared to survivors; # indicates $p < .05$ as compared to day 1.

Figure 2. Relation of parameters indicative of (A) hepatocellular injury (AST, ALT), (B) synthetic function (cholinesterase, prothrombin ratio), (C) acute phase response (C-reactive protein, albumin) and (D) excretory function (bilirubin, PDR_{ICG}) to outcome as assessed by Receiver Operating Characteristics.

AST - aspartate aminotransferase, ALT – alanine aminotransferase

Figure 3. Steady state transcript concentrations of transporter proteins for bilirubin and the anionic dye indocyanine green located on the two polar surfaces of hepatocytes in precision cut human liver slices. The horizontal line at 1 represents no change, above it is up-regulation, below it is down-regulation compared to unchallenged time-matched controls. OATP: organic anion transporting polypeptide, MRP: Multidrug Resistance-associated Protein, MDR: Multidrug Resistance Protein. * indicates $p<0.05$ for differential gene expression of the bracketed transporters for ICG versus bilirubin.

Figure 4. Differential susceptibility of basolateral and canalicular transport of indocyanine green (ICG) to injury.

Medians and interquartile range are given for patients with uncomplicated course (open symbols) and those with primary graft dysfunction/retransplantation with or without remote organ failure (filled symbols). * indicates $p<0.05$ for PDR_{ICG} and # indicates $p<0.05$ for peak biliary concentration for patients with primary dysfunction or remote organ failure compared to patients with an uncomplicated course. Correlation is shown for plasma disappearance rate and peak biliary concentration of the dye.

Supplemental Figure 1. Steady state transcript concentrations of various transporter proteins located on the two polar surfaces of hepatocytes in precision cut human liver slices. OATP - organic anion transporting polypeptide; MRP - Multidrug Resistance-associated Protein; MDR - Multidrug Resistance Protein; NTCP - Na+-taurocholate Cotransporting Polypeptide; BRCP - Breast Cancer Resistance Protein; BSEP - Bile Salt Export Pump; AE2 - Anion Exchanger Cl/HCO3

FIGURE 1

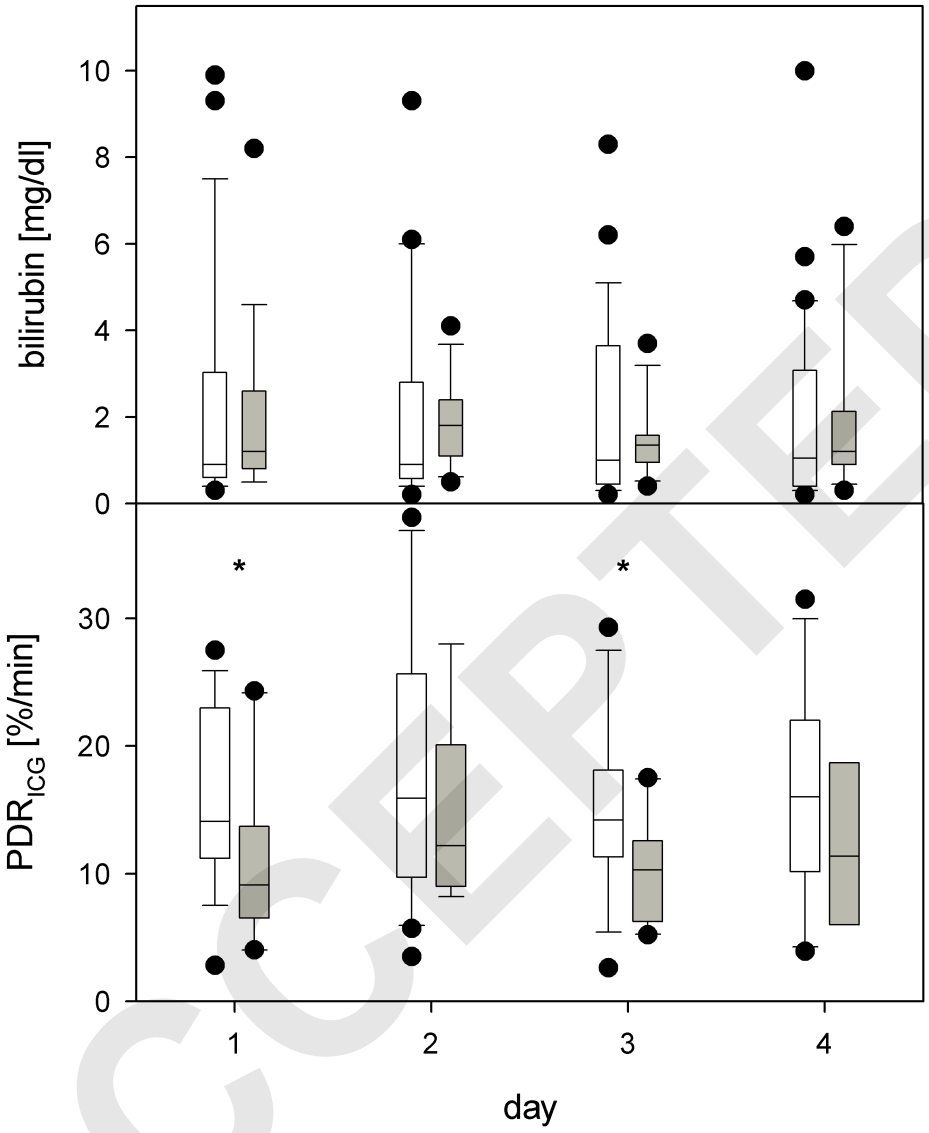


FIGURE 2

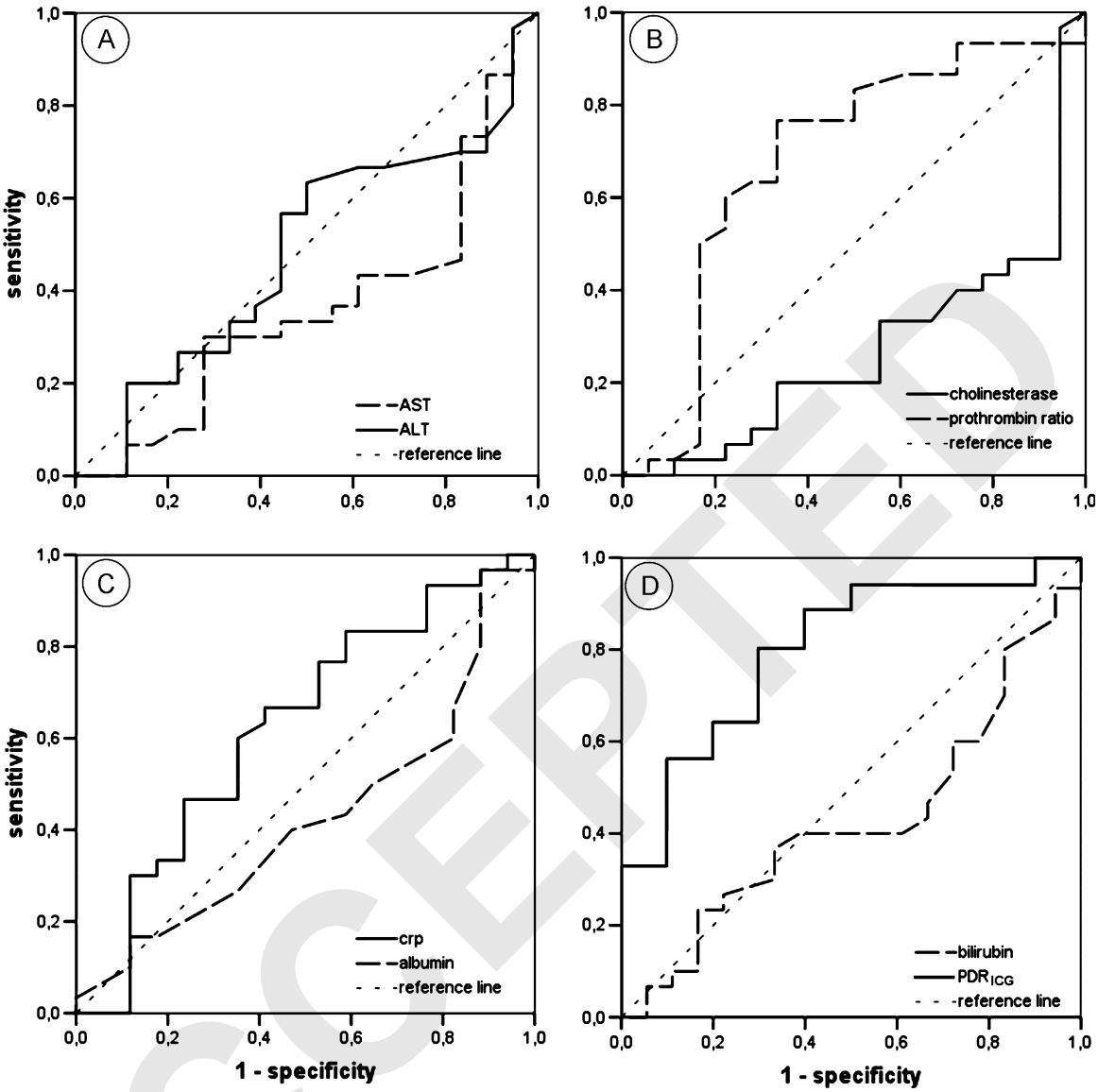


FIGURE 3

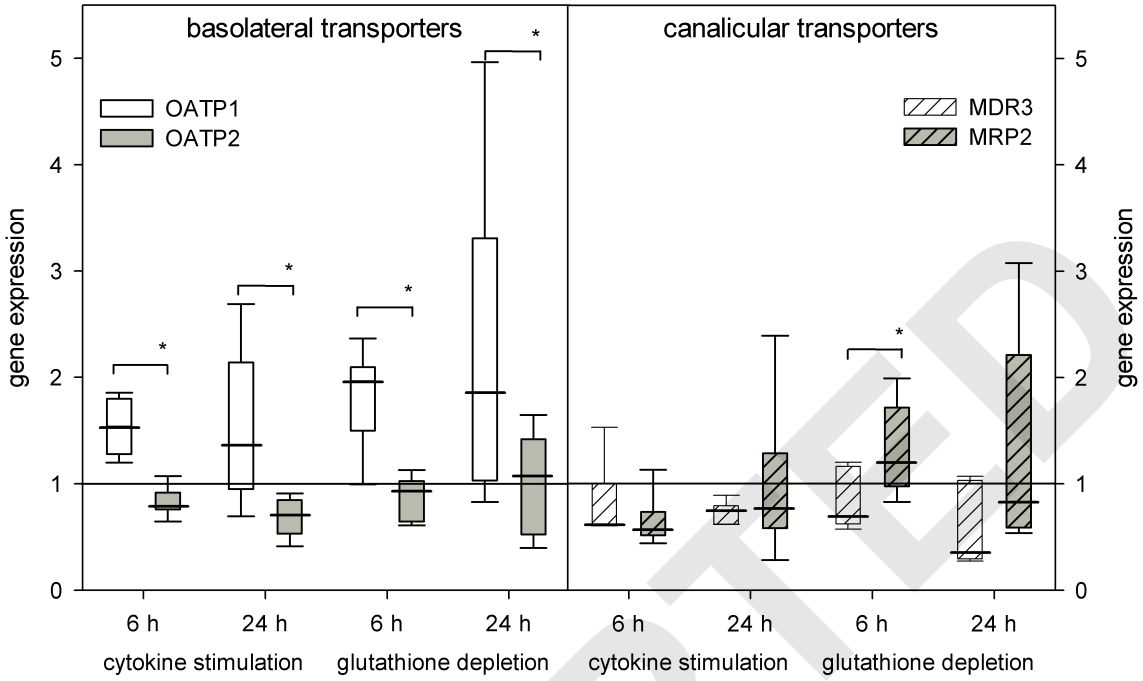
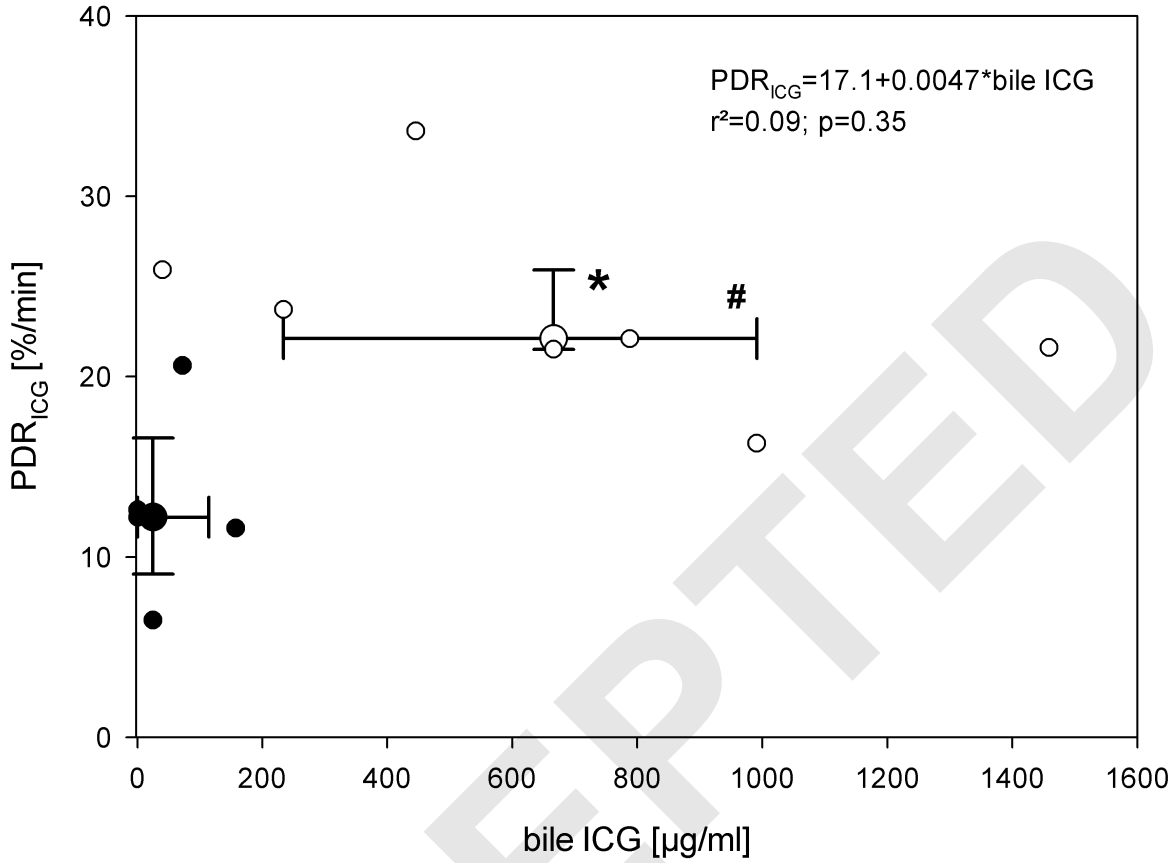


FIGURE 4



SUPPLEMENTAL FIGURE 1