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Serum bilirubin levels on ICU admission are associated with ARDS development and mortality in sepsis

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Abstract

Background—Hyperbilirubinemia is a common complication of sepsis. Elevated bilirubin may induce inflammation and apoptosis. We hypothesized that increased serum bilirubin on ICU admission contributes to sepsis-related ARDS.

Methods—Serum bilirubin on ICU admission was measured in 1006 septic patients. Serial serum bilirubin was analyzed prospectively in septic patients with ARDS for a period of 28 days. The effects of clinical factors and variants of *UGT1A1* gene on serum bilirubin levels were determined. Outcomes were ARDS risk and mortality.

Results—During 60-day follow-up, 326 septic patients developed ARDS in whom 144 died from ARDS. The hyperbilirubinemia (≥2.0 mg/dL) rate in patients with ARDS (22.4%) was higher than those without ARDS (14.1%, p = 0.002). For each 1.0 mg/dL increase in admission bilirubin, ARDS risk, 28- and 60-day ARDS mortalities were increased by 7% (OR = 1.07; p = 0.003), 20% (OR = 1.20; p = 0.002), and 18% (OR = 1.18; p = 0.004), respectively. Compared with subjects with bilirubin levels <2.0 mg/dL, patients with hyperbilirubinemia had higher risks of ARDS (OR = 2.12; p = 0.0007), 28-day (OR = 2.24; p = 0.020), and 60-day ARDS mortalities (OR = 2.09; p = 0.020). In sepsis-related ARDS, serial bilirubin levels in non-survivors were consistently higher than in survivors (p<0.0001). Clinical variables explained 29.5% of the inter-individual variation in bilirubin levels, whereas genetic variants of *UGT1A1* contributed 7.5%.

Conclusion—In sepsis, higher serum bilirubin level on ICU admission is associated with subsequent ARDS development and mortality.

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Keywords

sepsis; bilirubin; ARDS; clinical factors; *UGT1A1*

Sepsis is the second most common cause of death in non-coronary intensive care unit (ICU) and is among the top 10 causes of death for all hospitalized patients.[1] Patients with sepsis are at the highest risk of developing acute respiratory distress syndrome (ARDS).[2, 3] However, little is known about biomarkers predictive of ARDS development and mortality in patients with sepsis.

Bilirubin, the end product of heme catabolism in mammals, is generally considered a lipid-soluble waste product that needs to be excreted. However, growing evidences have suggested that bilirubin at high concentrations can induce inflammation, apoptosis, and oxidative stress.[4–8] Hyperbilirubinemia, or jaundice, is a well-known complication of sepsis or non-bacterial infection.[9] Sepsis and bacterial infection account for 20% of jaundice cases in patients of all ages in community hospital settings.[10] But there are no data from large prospective studies on the exact incidence and prognostic relevance of hyperbilirubinemia in adults with sepsis.[11] Since most physicians view hyperbilirubinemia as a late event in critical illness, low-grade hyperbilirubinemia is often overlooked in patients not presenting with clinically evident jaundice.[12–13] Although hyperbilirubinemia has been associated with overall poor outcomes in critical illness,[12–14] the associations of bilirubin with ARDS risk, and factors influencing bilirubin variations in sepsis remain largely unknown.

In humans, bilirubin is mainly metabolized by uridine diphosphase glucuronosyltransferase 1A1 (UGT1A1) that contributes to bilirubin glucuronidation and thus enhances bilirubin elimination.[15] The gene encoding for UGT1A1 is located in chromosome 2 (2q37) and spans approximately 160 kb. Individual genetic variations in the *UGT1A1* gene, such as the *-53 ~-42 $(TA)_{6–7}$* (*UGT1A1*28*, rs8175347), *-3279T>G (UGT1A1*60*, rs4124874 *), 211G>A (UGT1A1*6*, rs4148323*),* and *-3156G>A* (rs10929302) have been reported to affect *UGT1A1* gene expression, enzyme activity, and serum bilirubin levels.[16–18] However, conflicting associations with these polymorphisms have also been reported.[19–21] The apparent discrepancy suggests that single polymorphism may not be sufficient enough to define the contribution of *UGT1A1* variants to serum bilirubin levels. No systematic studies have addressed the association of overall genetic variation of the *UGT1A1* gene with circulating bilirubin levels in critically ill patients.

The aims of the present study were to evaluate, firstly, whether serum bilirubin levels on ICU admission were associated with sepsis-related ARDS risk and mortality; secondly, whether clinical factors and *UGT1A1* genetic variants contribute to inter-individual serum bilirubin variations in sepsis; and finally, whether *UGT1A1* polymorphisms were associated with ARDS risk and mortality that were consistent with their effects on bilirubin levels.

METHODS

Study subjects

Study patients were drawn from a prospectively enrolled cohort assembled for the Molecular Epidemiology of ARDS Study.[22] Consecutive admissions to the ICUs at the Massachusetts General Hospital (MGH, Boston, MA) were screened for sepsis from September 1999 to November 2006. Sepsis was diagnosed according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference.[23] Exclusion criteria included age <18 years, diffuse alveolar hemorrhage, chronic lung diseases, directive to withhold intubation, immunosuppression except if secondary to corticosteroid, and

treatment with granulocyte colony-stimulating factor (G-CSF). Alcohol abuse was defined as the history of active alcohol abuse, or diagnosis for alcohol detoxification and alcoholism in the past year. ARDS was defined according to American-European Consensus Conference (AECC) criteria.[24] Organ dysfunction was measured by the ARDS Network criteria.[25] Patients were followed daily for all-cause of 28- and 60-day mortalities. Baseline clinical and laboratory information were collected in the first 24 hrs of ICU admission. The MGH Human Subjects Committee approved the study and informed written consent was obtained from all subjects or surrogates.

Laboratory analysis

Serum total bilirubin and other biomarkers were measured on ICU admission, using the Roche Hitachi 917 analyzer with reagents from Roche Diagnostics (Indianapolis, IN). Serial serum bilirubin levels were measured from day 1 of ARDS diagnosis until ICU discharge or death, for a period of 28 days.

Selection criteria for tagging SNPs (tSNPs) of *UGT1A1* were $r^2 \geq 0.8$ and minor allele frequency >5.0% across the entire *UGT1A1* gene based on the database of the International HapMap Project (http://www.hapmap.org). DNA was extracted from whole blood using PureGene kits (Gentra Systems, Inc. Minneapolis, MN). Genotyping was determined using the Taqman assay with a ABI 7900HT sequence detector system (Applied Biosystems, Foster City, CA). The primer and probe sequences for each SNP are available on request. A total of 10% of samples were genotyped in duplicate for quality control and showed 100% concordance.

Statistical analysis

We compared baseline variables using χ^2 test, Fisher's exact test, Student's *t*-test, or Wilcoxon test, as appropriate. Univariate and stepwise multivariable linear regression models were used to test the associations of clinical factors and genetic variants with bilirubin levels. Since serum bilirubin levels had a skewed distribution, bilirubin levels were naturally log transformed and log bilirubin values were used in linear regression models as dependent variables. Multivariable logistic regression was used to evaluate associations of serum bilirubin levels and genetic variants of *UGT1A1* with ARDS development and mortality. Association with ARDS development was adjusted for covariates including age, gender, pneumonia, aspiration, multiple transfusion, diabetes, chronic liver diseases, alcohol abuse, history of steroid use, septic shock, modified APACHE III scores (excluding bilirubin component).[2] While association with ARDS mortality was adjusted for age, gender, sepsis shock, liver cirrhosis, history of alcohol use, history of steroid use, diabetes, modified APACHE III scores, organ dysfunctions (respiratory, cardiovascular, renal, and hematological), and positive end-expiratory pressure (PEEP, defined as treatment with PEEP >5 cm H_2O on ICU admission).[26] Survival probability was estimated using Kaplan and Meier log-rank test. Serial measurements were analyzed by generalized Estimating Equation (GEE) model.

Hardy-Weinberg equilibrium was determined using χ^2 test. Haplotypes were calculated using SAS macro HAPPY programs.[22] Genetic covariates were analyzed by additive (wildtype, heterozygotes, and homozygotes were coded as 0, 1, and 2, respectively) and haplotype models. False-discovery rate (FDR) was assessed to account for multiple comparison.[27] Colinearity test was performed by SAS PROC PRINCOMP procedure.

All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC). A p value less than 0.05 was considered statistically significant.

RESULTS

Characteristics of septic patients

Although individuals of all races were screened for this study, we restricted our analysis to Caucasians since 92% of ICU admissions during the study period at MGH were Caucasians. A total of 26580 consecutive ICU admissions were screened. Among them, 1931 patients meeting the criteria for sepsis and without exclusion criteria were recruited. Consent was obtained from 1224 septic patients (consent rate = 63.4%). 217 (18%) patients without bilirubin records on ICU admission and 1 patient with genotyping failure were excluded, leaving 1006 patients for analyses. Based on the sample size of 1006, the power of this study to detect a minimum OR = 1.50 (two-sided alpha = 0.05) was >80%. Of the 1006 patients with sepsis, 326 (32.4%) developed ARDS, and 144 (44.2%) of them died within 60-day follow-up. No differences were noted between patients with and without ARDS with respect to gender, prevalence of Gram-positive and Gram-negative bacteria infection, mean creatinine levels, or steroid use (table 1). Patients developed ARDS were younger, had higher APACHE III scores and lower platelet counts and lower prevalence of diabetes. Pneumonia, chronic liver disorder, and septic shock were more frequent in patients with ARDS. Serum bilirubin levels as well as hyperbilirubinemia rate were significantly higher in septic patients with ARDS than those without ARDS (table 1).

Associations of serum total bilirubin levels with ARDS development and mortality

Among 1006 septic patients, 169 (16.8%) had admission bilirubin ≥2.0 mg/dL (hyperbilirubinemia), and the remaining 837 patients had bilirubin <2.0 mg/dL. Higher serum bilirubin levels on ICU admission were significantly associated with increased ARDS incidence (p_{trend} <0.0001) and ARDS 60-day mortality (p_{trend} = 0.0002). Interestingly, the incidence of ARDS in patients with modest bilirubin levels (≥1.0 and <2.0 mg/dL) was significantly higher than those with bilirubin <1.0 mg/dL (p = 0.037) (fig 1). For each 1.0 mg/dL increase in admission bilirubin, ARDS risk increased by 7% (OR = 1.07; 95% CI, 1.03–1.13; p = 0.003), ARDS 28-day mortality increased by 20% (OR = 1.20; 95% CI 1.07–1.35; p = 0.002) and ARDS 60-day mortality increased by 18% (OR = 1.18; 95% CI 1.05–1.31; p = 0.004). Similarly, log bilirubin values were significantly associated with ARDS development and mortalities (table 2). Bilirubin levels on ARDS diagnosis were also associated with increased mortality of sepsis-related ARDS. In sensitivity analyses by restricting the analysis to subjects without history of chronic liver disorder (n = 948) and patients who did not have ARDS on ICU admission (n = 867), the associations of admission bilirubin levels with sepsis-related ARDS risk and mortality remained unchanged (data not presented).

Among patients with sepsis-related ARDS, serial daily serum bilirubin levels in non-survivors were persistently higher than that in survivors from day 1 of ARDS diagnosis to the end of the entire observation period (fig 2. GEE test, p<0.0001). Kaplan-Meier survival analysis showed that patients with hyperbilirubinemia had lower survival rates than patients with admission bilirubin ≤ 2.0 mg/dL (p = 0.002) (fig 3).

Clinical correlates of serum total bilirubin levels on ICU admission in patients with sepsis

In univariate linear regression analysis, age, gender, diabetes, chronic liver disorder, pneumonia, multiple transfusion, alcohol abuse, and respiratory/renal/hematological dysfunctions were significantly associated with serum bilirubin levels in patients with sepsis (table 3). In stepwise multivariable linear regression analysis, chronic liver diseases, male gender, respiratory/renal/hematological dysfunctions were positively associated with ICU admission bilirubin levels, while diabetes and pneumonia were inversely related to ICU admission bilirubin levels. Clinical correlates explained 29.5% of the inter-individual variation (R^2 = 0.295) in serum bilirubin levels. Colinearity test suggested that colinearity among clinical

factors was weak (condition number = 7.48) When analysis was restricted to patients without history of chronic liver disorders, the direction and magnitude of associations were virtually identical to results reported above (data not presented).

Associations of *UGT1A1* variants with serum total bilirubin levels in septic patients

The genotyping success rates for the 10 tSNPs ranged from 99.0% to 99.5%. All tSNPs in this study population were consistent with Hardy-Weinberg equilibrium ($p>0.05$, χ^2 goodness-of-fit). The LD pattern across the *UGT1A1* locus is shown in fig S1. Three regions of strong LD were identified: block 1 (~8 kbp), block 2 (~kbp), and block 3 (~2 kbp). Compared with the major alleles, minor alleles of rs3755319, rs887829, and rs6742078 in LD block 1 significantly associated with higher serum bilirubin levels (FDR p = 0.005, 0.0005 and 0.0005, respectively; table 4). Minor alleles at rs17864705, and rs1018124 in LD block 1 also showed a trend of association with higher levels of bilirubin, but these associations did not reach statistically significance. The tSNPs explained 7.5 % of the serum bilirubin variation ($R^2 = 0.075$) in models that included all clinical covariates. No associations between serum bilirubin and tSNPs in LD block 2 and block 3 were observed.

In LD block 1, 4 common haplotypes with frequencies ≥5% were reconstructed (table 5). Using the most common haplotype *ATTTTG* (52.8%) as the reference, haplotype *CCGTTG* (32.8%) that harbored the minor alleles of rs3755319, rs887829, and rs6742078 was significantly associated with higher bilirubin levels (adjusted p = 0.0002; global test $p<0.0001$). No haplotype in LD block 3 was associated with bilirubin levels.

Association of *UGT1A1* variants with the development and mortality of sepsis-related ARDS

Because *UGT1A1* variants were strongly associated with higher serum bilirubin levels, and higher bilirubin levels were associated with increased ARDS development and mortality, we evaluated whether *UGT1A1* tSNPs or haplotypes are associated with ARDS development or mortality. The distributions of *UGT1A1* tSNP genotypes were not significantly different between patients with and without ARDS (all p values >0.05). In logistic regression using the major allele homozygotes as referent genotypes, the rs17864705 and rs1018124 (in LD block 1) were marginally associated with increased risk of ARDS ($OR_{adj} = 1.52$; p = 0.037 for rs17864705. $OR_{adj} = 1.48$; p = 0.058 for rs1018124). But these associations were no longer significant in FDR analysis (table S3). Similarly, two haplotypes were marginally associated with increased ARDS risk in overall analysis (table S3). No significant associations were detected between any *UGT1A1* tSNPs or haplotypes and ARDS mortalities.

DISCUSSION

This study shows that higher bilirubin on ICU admission is associated with subsequent sepsis-related ARDS development and mortality. Furthermore, our study shows that serum bilirubin levels in sepsis are mainly influenced by clinical factors and partly by genetic variants of *UGT1A1*.

Although sepsis is a major risk for ARDS,[28] no prior study has described predictive biomarkers for ARDS development in septic patients. Our results showed that a slight increase in bilirubin on ICU admission was associated with marked increase of ARDS risk and mortality in septic patients, suggesting that serum bilirubin is an early and sensitive biomarker of sepsis-related ARDS. The fact that adjustments for multiple covariates did not change these associations further suggested that bilirubin is an independent predictor of sepsis-related ARDS. Previous studies on prognostic value of bilirubin in sepsis have focused mainly on survival as the major outcome measure.[29, 30] Although the ultimate outcome measure for any patient is survival, death is not the only outcome measure of ICU treatment. In critically ill patients, identification

of risk biomarkers for ARDS development may help clinicians in both diagnostic evaluation and management. For instance, hyperbilirubinemia has been reported to predispose surgical ICU patients to infection, indicating a need for higher level of vigilance for infection in ICU patients with hyperbilirubinemia.[12, 31]

Our results from a large study population confirmed the findings in previous small studies that hyperbilirubinemia is associated with worse survival in ARDS patients.[29, 30] Unlike previous reports, ARDS diagnosis in our study was determined prospectively using the widely accepted AECC definition, and therefore, phenotype misclassification was minimized. The use of individual phenotype rather than critical illness from mixture causes reduces confounding from any possible associations between the various causes and outcomes. Moreover, the outcomes of ARDS were measured for 60-day mortality, therefore bias in survival calculation due to incomplete observation was minimized.

Although physiological levels of bilirubin are considered a potent antioxidant,[32] increasing evidences have shown that bilirubin at high concentrations may be an active participant in the disease process. Bilirubin in the blood can induce cell lysis of erythrocytes.[33] Elevated Bilirubin can stimulate oxidative stress and decrease cell survival [34–35]. Bilirubin promotes apoptosis in cultured cells.[5] In addition, bilirubin can also induce inflammatory response, which is further increased when cells are simultaneously exposed to lipopolysaccharide.

Consistent with a previous report in critically ill patients,[13] we observed that several clinical factors including pneumonia, pre-admission steroid use, diabetes history, and organ dysfunctions (respiratory, renal and hematological) were associated with serum bilirubin levels. In contrast to previous study using univariate analysis, we defined the contributions of clinical factors to bilirubin variations by multivariate regression models and demonstrated that clinical factors explained 29.5 % of the overall variation of serum bilirubin levels in septic patients. It should be pointed out that although chronic liver disease is strongly associated with increased serum bilirubin levels, hepatic dysfunction is not the only organ dysfunction associated with hyperbilirubinemia in sepsis. Cholestasis, circulating endotoxin and hemolysis may also play important roles in the etiology of hyperbilirubinemia in sepsis.[11] In subjects without chronic liver disorders, we found that other clinical factors were also significantly associated with serum bilirubin levels. It has been reported that elevated bilirubin was often seen in sepsis and septic shock patients in the absence of primary liver or biliary disease.[31]

Despite the evidence that genetic variants of *UGT1A1* were significantly associated with serum bilirubin levels, the overall contribution of these variants was small compared with clinical factors. Similar to the moderate impact of genetic variants of *UGT1A1* on serum bilirubin levels, the strengths of overall associations between *UGT1A1* polymorphisms with sepsis-related ARDS risk were also modest. Considering that less than 8% of the variance in bilirubin levels is explained by *UGT1A1* polymorphisms, it is not surprising that we did not detect a strong association of these polymorphisms with ARDS risk or mortality, although the association of serum bilirubin level with ARDS risk and mortality is relatively strong.

The large sample size, the adjustment for multiple clinical covariates, comprehensive evaluation of common variants surrounding the *UGT1A1* gene, and serial measurements of bilirubin levels are the strengths of this study. Despite these assets, our study has some limitations. First, our study design did not allow us to define whether bilirubin is an effecter molecule in the pathogenesis of ARDS or merely a marker of systemic injury. Future research is needed to elucidate the potential pathogenic role of bilirubin in ARDS. Second, this study could not exclude the possibility of medication influence on bilirubin levels. However, a recent study including 17 drugs potentially inducing hepatotoxicity has shown that medication administrations during ICU stay did not significantly contribute to serum bilirubin levels.[13]

Third, we were unable to exclude the possibility that our cohort might include subjects with existing Gilbert's syndrome because all subjects in this study were critically ill patients. Whether Gilbert's syndrome might be associated with sepsis-related ARDS requires further investigation. Fourth, 217 septic patients were excluded from analysis due to no ICU admission bilirubin records. Since most of these patients had bilirubin measurements <2.0 mg/dL analyzed before ICU admission, they were considered as patients with normal bilirubin levels and were not tested for bilirubin by ICU clinicians. Comparison analysis showed that patients without ICU admission bilirubin values had lower severity of illness and less organ failure than those with admission bilirubin and were more similar to those with bilirubin <1.0 mg/dL than those patients with bilirubin >1.0 mg/dL (table S2). Therefore, we assumed that patients without ICU admission bilirubin values were probably among subjects with bilirubin <1.0 mg/dL and exclusion of these patients from our analyses did not change the results, and probably diluted the strength of associations detected in this study.

In conclusion, we show that serum bilirubin level on ICU admission is an independent early predictor of ARDS development and mortality in septic patients. Several clinical characteristics and genetic variants of *UGT1A1* independently affect serum bilirubin levels. However, the relationship between *UGT1A1* variants and bilirubin levels did not translate largely to an associated change in ARDS outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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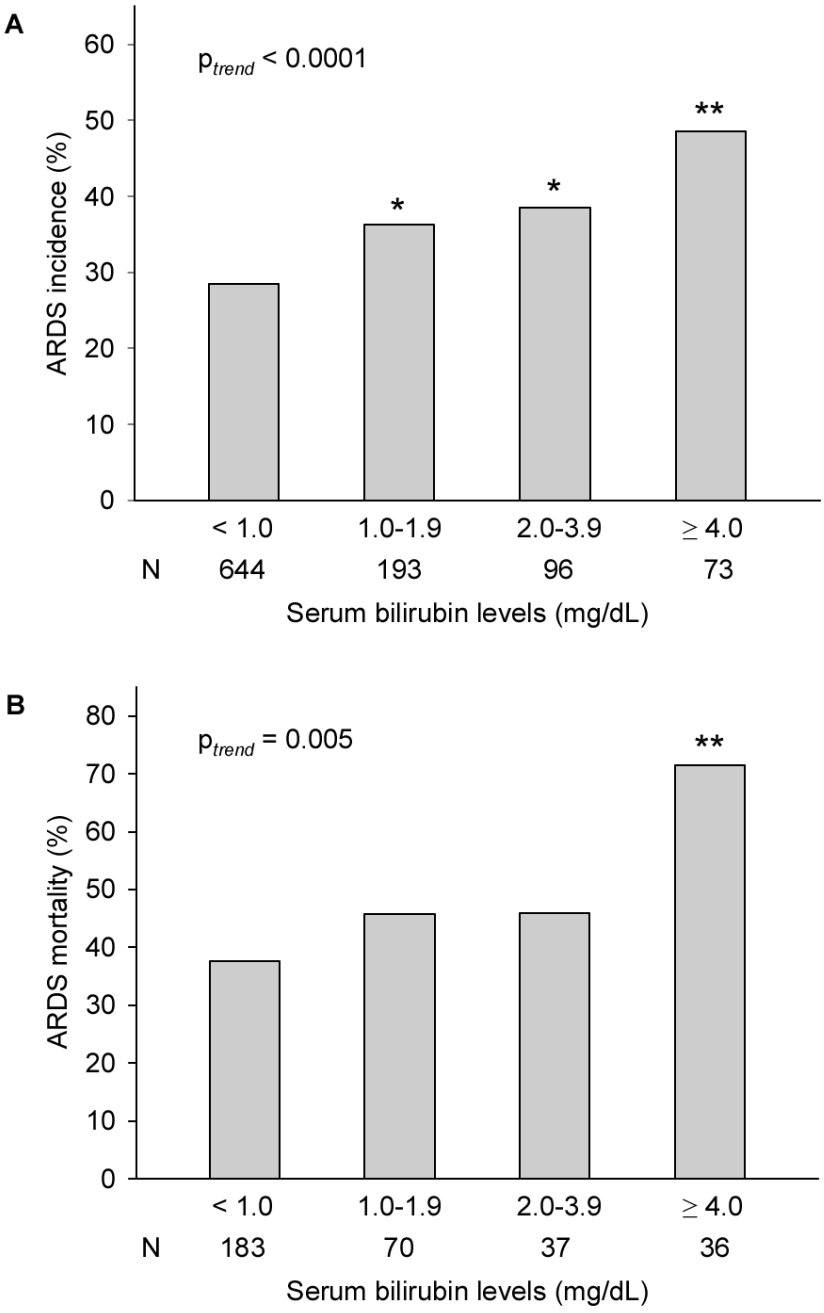

Figure 1.

Distribution of ARDS incidence (A) and ARDS 60-day mortality (B) by strata of serum bilirubin levels on ICU admission. * $p<0.05$ as compared with patients with bilirubin <1.0 mg/dL; ** $p<0.01$ as compared with patients with bilirubin <1.0 mg/dL. N (number of subjects).

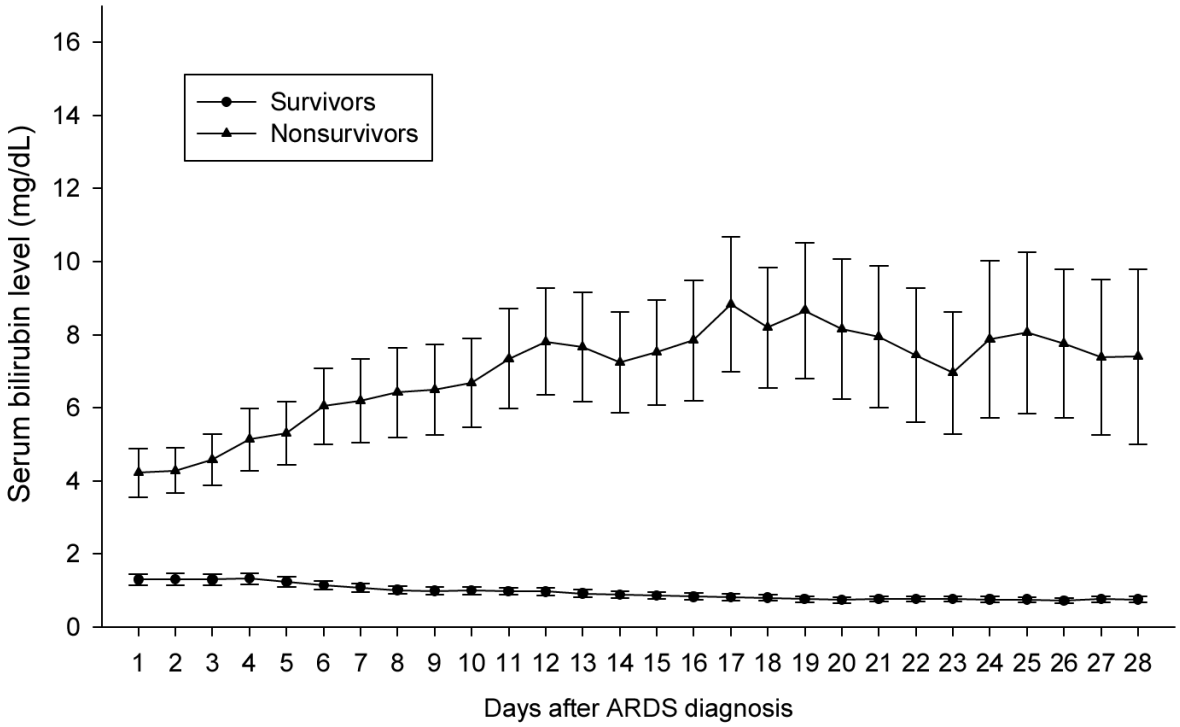

Figure 2.
Serial mean serum bilirubin levels after ARDS diagnosis over the observation period between survivors (lower) and non-survivors (upper) in patients with sepsis-related ARDS (n = 326) ($p<0.0001$, GEE analysis). Day 1 represents the day of ARDS diagnosis. Error bars are SE.

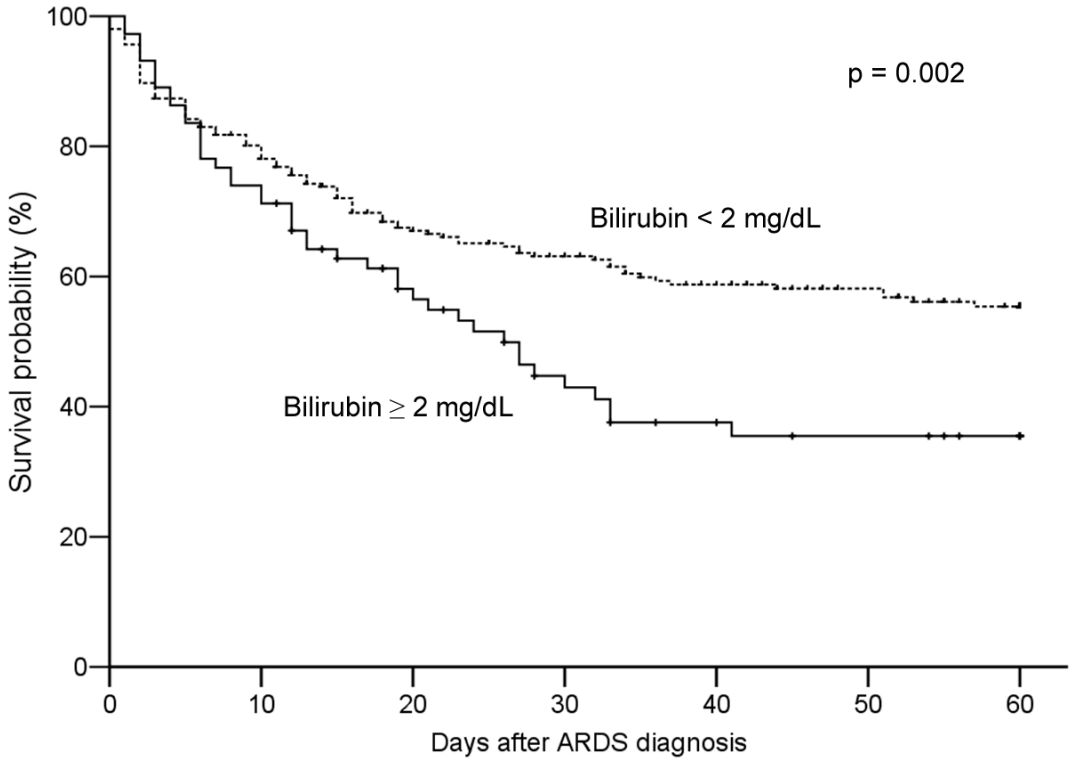

Figure 3.
Estimated survival probability in patients with sepsis-related ARDS by admission bilirubin levels.

Table 1

Characteristics of 1006 patients admitted to ICU with sepsis

Characteristics	All patients (n = 1006)	Patients developed ARDS(n = 326)	Patients did not develop ARDS (n = 680)	p value
Age, yrs	62.3 ± 17.1	59.8 ± 17.7	63.5 ± 16.7	0.001
Female	402 (40.0%)	134 (41.1%)	268 (39.4%)	0.631
APACHE III $_{score}$	74.8 ± 23.8	82.6 ± 23.2	71.0 ± 23.3	<0.001
History of alcohol abuse	127 (12.6%)	51 (15.6%)	76 (11.2%)	0.054
History of steroid use	97 (9.7%)	33 (10.1%)	64 (9.4%)	0.733
Serum bilirubin (mg/dL)	0.7 (0.4–1.3)	0.9 (0.5–1.8)	0.6 (0.4–1.2)	<0.0001[a]
Bilirubin ≥2.0 mg/dl	169 (16.8%)	73 (22.4%)	96 (14.1%)	0.002
Serum creatinine (mg/dL)	1.4 (0.9–2.4)	1.4 (0.9–2.4)	1.3 (0.9–2.4)	0.525[a]
Platelets (×1000/μL)	215.8 ± 133.5	205.0 ± 145.2	220.9 ± 127.3	0.005
Sepsis shock	612 (60.8%)	234 (71.8%)	378 (55.6%)	<0.001
Pneumonia	593 (59.0%)	249 (76.4%)	344 (50.6%)	<0.001
Multiple transfusion	35 (3.5%)	11 (3.4%)	24 (3.5%)	1.00
End-stage renal disease	64 (6.4%)	23 (7.1%)	41 (6.0%)	0.540
Trauma	9 (0.9%)	5 (1.6%)	4 (0.6%)	0.159
Diabetes history	254 (25.4%)	63 (19.3%)	191 (28.2%)	0.003
Liver cirrhosis/failure	58 (5.8%)	26 (8.0%)	32 (4.7%)	0.043
PEEP >5 cm H_2O	313 (31.1%)	176 (54.0%)	137 (20.5%)	<0.0001
Microorganisms				
Gram-positive	432 (42.9%)	130 (39.9%)	302 (44.4%)	0.174
Gram-negative	317 (31.5%)	110 (33.7%)	207 (30.4%)	0.292
Anaerobic	47 (4.7%)	9 (2.8%)	38 (5.6%)	0.047
Fungus	53 (5.3%)	25 (7.7%)	28 (4.1%)	0.018
Virus	12 (1.2%)	2 (0.6%)	10 (1.5%)	0.241
Unknown	336 (33.4%)	122 (37.4%)	214 (31.5%)	0.061

Data are presented as n (%), mean ± SD, or median (lower quartile-upper quartile);

[a] Wilcoxon test

Table 2

Associations of serum total bilirubin levels with sepsis-related ARDS risk and mortality: Multivariate logistic regression analysis

	ARDS development		ARDS 28-day mortality		ARDS 60-day mortality	
Serum bilirubin levels	**OR[a] (95% CI)**	**p value**	**OR[b] (95% CI)**	**p value**	**OR[b] (95% CI)**	**p value**
On ICU admission						
Bilirubin	1.07 (1.03–1.13)	0.003	1.20 (1.07–1.35)	0.002	1.18 (1.05–1.29)	0.004
Log-bilirubin	2.17 (1.46–3.25)	0.0001	2.86 (1.36–6.01)	0.005	2.70 (1.30–5.61)	0.008
Bilirubin ≥2.0 mg/dL	2.12 (1.37–3.27)	0.0007	2.24 (1.14–4.38)	0.019	2.09 (1.12–3.90)	0.020
On ARDS diagnosis						
Bilirubin	-	-	1.19 (1.06–1.32)	0.002	1.16 (1.05–1.28)	0.003
Log-bilirubin	-	-	3.32 (1.57–7.06)	0.002	3.29 (1.57–6.89)	0.002
Bilirubin ≥2.0 mg/dL	-	-	2.50 (1.23–5.08)	0.012	2.54 (1.33–4.87)	0.005

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Adjustment for age, gender, clinical risks for ARDS (trauma, pneumonia, aspiration, multiple transfusions), modified APACHE III scores, sepsis shock, chronic liver diseases, history of alcohol use, history of steroid use, and diabetes.

[b] Adjustment for age, gender, sepsis shock, chronic liver diseases, history of alcohol use, history of steroid use, diabetes, modified APACHE III scores, PEEP, and organ dysfunctions (respiratory, cardiovascular, renal, and hematological).

Table 3

Clinical correlates of serum total bilirubin levels in 1006 patients with sepsis: Linear regression analysis

	Univariate analysis		Stepwise multivariate analysis	
Clinical variables	**Estimates (*SE*)**	**p value**	**Estimates (*SE*)**	**p value**
Age	−0.0023 (0.0008)	0.004	-	NS
Sex, female vs. male	−0.1314 (0.0278)	<0.0001	−0.0920 (0.0244)	<0.0001
Septic shock	0.0539 (0.0281)	0.055	-	NS
Diabetes	−0.1033 (0.0315)	0.001	−0.1118 (0.0275)	0.0002
Chronic liver disorder	0.7569 (0.0538)	<0.0001	0.5780 (0.0543)	<0.0001
Pneumonia	−0.1362 (0.0276)	<0.0001	−0.1241 (0.0248)	<0.0001
Aspiration	−0.0704 (0.0491)	0.152	-	NS
Multiple transfusions	0.3756 (0.0741)	<0.0001	-	NS
Trauma	−0.0512 (0.1460)	0.700	-	NS
History of alcohol abuse	0.2591 (0.0405)	<0.0001	-	NS
History of steroid use	−0.0824 (0.0465)	0.077	-	NS
PEEP >5 cm H_2O	0.0579 (0.0296)	0.051	-	NS
Organ dysfunction				NS
Respiratory	0.1000 (0.0294)	0.0007	0.0928 (0.0257)	<0.0001
Cardiovascular	0.0638 (0.0313)	0.042	-	NS
Renal	0.1117 (0.0289)	0.0001	0.0619 (0.0256)	0.022
Hematological	0.4932(0.0398)	<0.0001	0.3018 (0.0400)	<0.0001

Abbreviations: SE, standard error; NS, not significant.

Table 4

Associations of *UGT1A1* tagging SNPs with serum total bilirubin levels in septic patients: Linear regression analysis

LD block	Tagging SNPs	Bilirubin levels by genotypes[a]			Univariate analysis			Stepwise multivariate analysis		
		0	1	2	Estimates (*SE*)	p value	FDR p	Estimates (*SE*)	p value	FDR p
	rs3755319	282	513	204						
		1.64 ± 0.26	1.62 ± 0.16	1.86 ± 0.24	0.0534 (0.0199)	0.0073	0.024	0.0493 (0.0171)	0.0043	0.005
	rs887829	438	455	108						
		1.60 ± 0.19	1.60 ± 0.18	2.26 ± 0.37	0.0694 (0.0208)	0.0009	0.0045	0.0609 (0.0178)	0.0001	0.0005
	rs6742078	436	456	109						
		1.58 ± 0.18	1.60 ± 0.18	2.18 ± 0.37	0.0709 (0.0207)	0.0006	0.0045	0.0696 (0.0178)	0.0001	0.0005
	rs3771342	757	227	21				-		
		1.75 ± 0.15	1.36 ± 0.13	2.02 ± 1.29	−0.0220 (0.0282)	0.436	0.110	-	NS	-
	rs17864705	870	122	8				-		
		1.64 ± 0.13	1.72 ± 0.30	4.61 ± 3.34	0.0608 (0.0375)	0.105	0.210	-	NS	-
	rs1018124	870	114	7				-		
Block 1		1.68 ± 0.13	1.43 ± 0.19	4.50 ± 3.85	0.0172 (0.0390)	0.660	0.943	-	NS	-
Block 2	rs11888492	773	218	13				-		
		1.67 ± 0.14	1.58 ± 0.27	3.06 ± 1.70	−0.0002 (0.0300)	0.884	0.982	-	NS	-
	rs8330	601	328	73				-		
		1.75 ± 0.17	1.41 ± 0.15	2.02 ± 0.58	− 0.0103 (0.0218)	0.637	0.943	-	NS	-
	rs1500482	620	335	49				-		
		1.71 ± 0.17	1.45 ± 0.15	2.55 ± 0.84	0.0003 (0.0230)	0.989	0.989	-	NS	-
	rs4663972	610	341	55				-		
Block 3		1.76 ± 0.17	1.35 ± 0.14	2.62 ± 0.77	−0.0070 (0.0230)	0.761	0.951	-	NS	-

Abbreviations: LD, linkage disequilibrium; SE, standard error; FDR, false discovery rate.

[a]Data are n or mean ± SD; genotype 0, 1, 2 represent the wildtype, heterozygotes, and homozygotes, respectively. Missing genotyping is not included.

Table 5

Associations of *UGT1A1* haplotypes with serum total bilirubin levels: Linear regression analysis

LD block	Haplotype	Haplotype frequency	Univariate analysis		Multivariate analysis[c]	
			Estimates (*SE*)	p value	Estimates (*SE*)	p value
Block 1[a]	*ATTTTG*	52.8%	1.0	-	1.0	-
	CCGTTG	32.8%	0.0698 (0.0215)	0.0012	0.0712 (0.0189)	0.0002
	CTTGTG	5.7%	−0.0169 (0.0422)	0.689	−0.0089 (0.0368)	0.807
	CTTGGA	5.6%	0.0737 (0.0410)	0.073	0.0321 (0.0356)	0.380
	Others	3.1%	-	-	-	-
Block 3[b]	*GTT*	73.6%	1.0	-	1.0	-
	CCC	20.4%	−0.0153 (0.0235)	0.593	−0.0240 (0.0206)	0.244
	CTT	2.53%	−0.0313 (0.0585)	0.581	− 0.0179 (0.0513)	0.727
	Others	3.47%	-	-	-	-

Abbreviations: LD, linkage disequilibrium; SE, standard error.

[a] Polymorphisms are in the order of: rs3755319-rs887829-rs6742078-rs3771342-rs17864705-rs1018124.

[b] Polymorphisms are in the order of: rs8330-rs1500482-rs4663972.

[c] Adjustment for age, gender, sepsis shock, chronic liver diseases, history of alcohol use, history of steroid use, diabetes, modified APACHE III scores, and organ dysfunctions (respiratory, cardiovascular, renal, and hematological).