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*Original Article*

# Direct bilirubin as a prognostic biomarker in enteric fistula patients complicated with sepsis: a case-control study

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**Abstract:** Objective: The objective of this study was to evaluate the predictive value of serial bilirubin determinations for mortality in enteric fistula (EF) patients complicated with sepsis. Methods: From January 1st, 2012 to January 13rd, 2013, a prospective study enrolling 162 patients was performed. Patients were divided into the survivors group (n = 119) and non-survivors group (n = 43) according to 28-day outcomes. Laboratory variables on day 0, day 3 and day 7 after admission were recorded. DB0 was defined as serum direct bilirubin (DB) value in admission, while ΔDB3 as the changes from DB3 to DB0. The definition applied to other parameters. The results were validated in an independent cohort of 116 patients. Results: Compared with survivors, non-survivors had significantly higher DB7 (23.1 ± 10.6 vs. 11.2 ± 1.1, P < 0.001) and procalcitonin ($PCT_7$) (5.2 ± 2.8 vs.1.7 ± 0.3 P = 0.006). ROC analysis showed that $DB_7$ > 12.8 μmol/L and $\Delta DB_7$ > 7.3 μmol/L were reliable predictors (DB7: 86.4% sensitivity, 88.6% specificity (area under the curve (AUC): 0.881, P < 0.001; ΔDB7: 84.4% sensitivity, 85.1% specificity, AUC: 0.865, P < 0.001) for mortality.The combination form ($DB_7$ > 12.8 μmol/L + $\Delta PCT_7$ < 5.3 ng/ml) had greatest predictive value (AUC: 0.894, P < 0.001). Their predictive values were confirmed in the validation cohort. Conclusions: Serum direct bilirubin was a reliable predictor for mortality in enteric fistula patients, which should be paid close attention in the critical care.

**Keywords:** Bilirubin, direct bilirubin, predictor, enteric fistula, abdominal sepsis

## Introduction

Despite advances in the supportive care, sepsis is still the 11th leading cause of death. It is demonstrated that the mortality of severe sepsis is nearly 50% [1]. Enteric fistula (EF)-related abdominal sepsis constitutes a major part of sepsis. EF is an abnormal communication between the enteric tract and surrounding tissues, involving leak of bowel contents. Contamination of the abdominal cavity with enterogenic microorganisms and toxins stimulates production of inflammatory cytokines, leading to multiple organ dysfunction syndrome and death [2]. Biomarkers which identify patients who are at high risk of poor outcomes could facilitate early interventions. Conventional biomarkers of sepsis, such as C-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 (IL-6) are insufficient in sensitivity and specificity [3, 4]. The disappointing results prompt clinicians to seek a more representative predictor.

During the sepsis, the liver is the second most commonly affected organ. Studies have shown that hepatocellular functions are depressed in the early sepsis [5]. Hepatocellular functions are commonly assessed by serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). These conventional markers are relatively unspecific, given their long half-life [6]. Recently, Patel JJ et al revealed an association between serum bilirubin levels and mortality during the sepsis [7]. It enlightened us that serum bilirubin could be a potential predictor of mortality in this population.

However, previous studies have the limitations of reporting bias with heterogeneous popula-

tions. Few studies focused exclusively on patients with EF-related abdominal sepsis. Moreover, the influence of hyperbilirubinemia acquisition time and alteration magnitude on outcomes has not been adequately investigated. Notably, total bilirubin (TB) consists of direct bilirubin (DB) and indirect bilirubin (IB). Previous studies were limited to TB. In several studies concerning cardiovascular disease, DB was reported to have better predictive value than TB [8, 9]. We prospectively monitored all subtypes of bilirubin during one week since admission, to evaluate their predictive capacity in EF patients complicated with sepsis.

## Methods

### *Study design and ethics*

This was a prospective observational study of patients admitted to the department of Surgery, Jinling Hospital in China. The treatment protocol was approved by the Jinling Hospital Ethical Subcommittee, with written informed consent obtained from patients or their relatives. All research work with humans was in compliance with the Helsinki Declaration.

### *Patient population*

We identified predictors with the derivation cohort and subsequently evaluated their performance in the validation cohort. Between January 1st, 2012 to January 13rd, 2013, 354 patients were enrolled in the derivation cohort. Between January 14th, 2013 to October 20th, 2013, 247 patients were enrolled in the validation cohort.

The enrollment criteria for two cohorts were the same: 1. ≥ 18 years old and ≤ 65 years old; 2. Enteric fistula confirmed by CT-scan or gastroenterography, 3. At least two of four criteria of systemic inflammatory response syndrome (SIRS) complicated with organ dysfunction or hypotension (systolic blood pressure (SBP) < 90 mmHg). SIRS was defined by two or more of the following conditions: temperature > 38 or < 36°C; heart rates > 90 beats/min; respiratory rate > 20 breaths/min or $PaCO_2$ < 4.26 kPa; white blood cell count > 12,000 or < 4,000 cells/μL (or > 10% immature forms). 4. Sepsis diagnosis based on the definition established in the 2012 International Sepsis Conference: the clinical manifestations of infection were shown by clinical symptoms, physical examination, laboratory tests such as elevation of plasma C-reactive protein and plasma procalcitonin more than 2 standard deviations above the normal value, and/or blood culture [6].

Exclusion criteria included: preexisting renal diseases or liver diseases (including hepatitis, cirrhosis, cholestatic liver disease, hemolytic disease, and liver cancer), lactation or gestational period, leucopenia, confirmed immunodeficiency or coagulation disorders and requirement for blood purification. Patients who died within 7 days since admission or withdrew due to personal reasons were excluded.

According to the exclusion criteria, 162 patients in the derivation cohort and 116 patients in the validation cohort were eligible and finally enrolled.

### *Management of abdominal sepsis*

The standard management includes: 1. Fluid resuscitation; 2. Norepinephrine (intravenously, 10 μg/min, 3 h) as primary vasopressor; 3. Early goal-directed therapy (MAP > 65 mmHg, and $SvO_2$ > 65%); 4. Source control by surgical or percutaneous drainage; 5. Antibiotics therapy. For fistula management, the four pivotal principles consisted of correction of intravascular volume deficit, drainage of abscess, control of fistula output, and protection of the skin. Mechanical ventilation and other assistant treatments were applied as needed. Hyperglycemia was controlled with short-acting insulin analogs [6].

### *Observing severity scores and outcomes*

Routine examinations, blood gas analysis, drug administrations (types of vasopressor, dosage, etc.) and culture results (blood, stool and urine) were monitored after admission. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Sequential Organ Failure Assessment (SOFA) score were calculated for all patients [10, 11]. The primary outcome was the 28-day overall mortality after admission. The secondary outcomes included duration of ICU stay.

### *Laboratory parameters*

The variables were prospectively collected from the patient's electronic health record.

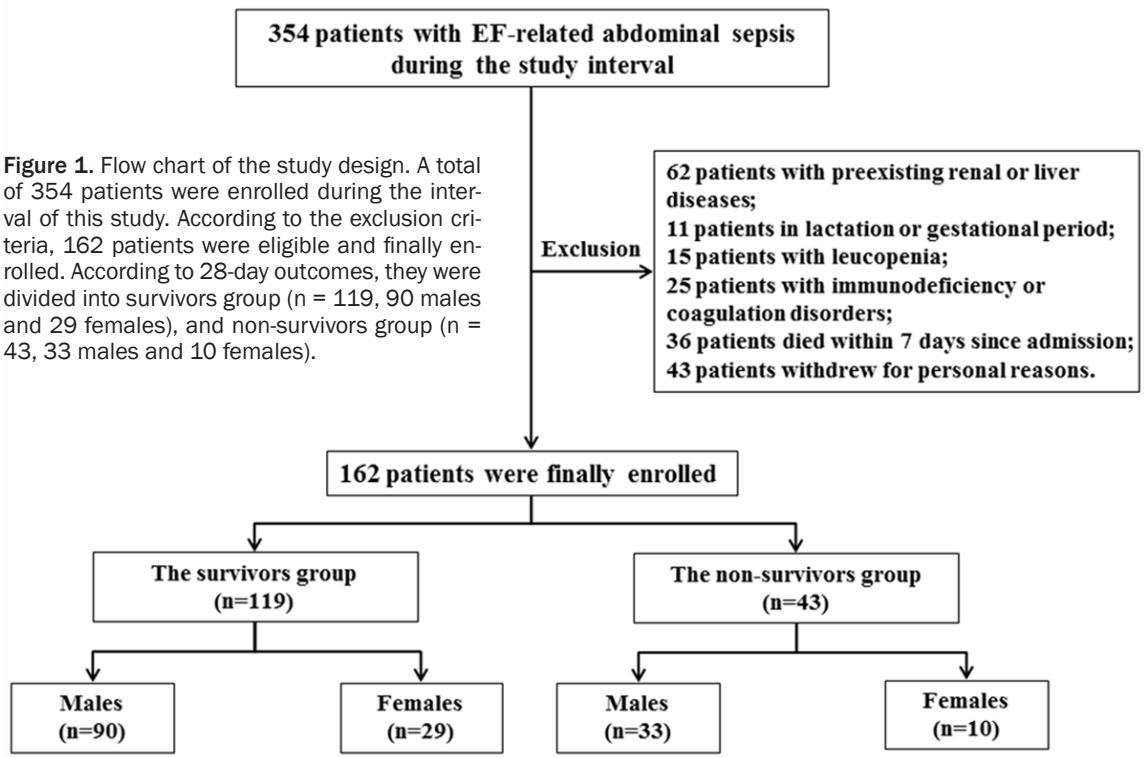


**Figure 1.** Flow chart of the study design. A total of 354 patients were enrolled during the interval of this study. According to the exclusion criteria, 162 patients were eligible and finally enrolled. According to 28-day outcomes, they were divided into survivors group (n = 119, 90 males and 29 females), and non-survivors group (n = 43, 33 males and 10 females).

For each enrolled patient, laboratory variables were collected on admission (day 0), day 3 and day 7. Venous blood in all laboratory tests was drawn between 5 am and 6 am in the morning. Blood Samples were collected into heparinized syringes (Sodium heparinate, Ratiopharm, China) and laboratory values were calculated within 2 hours.

Serum TB, DB, and IB levels were measured by the vanadate oxidation method using automatic biochemical analyzer Olympus AU5400 (Olympus America Inc., Melville, NY, USA). $\Delta TB_3 = TB_3\text{-}TB_0$; $\Delta TB_7 = TB_7\text{-}TB_0$ and $\Delta TB_{7\text{-}3} = TB_7\text{-}TB_3$. The definition applied to ΔDB and ΔIB.

Other hepatic function indexes [γ-glutamyltransferas (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), albumin (Alb)] and inflammatory parameters [white blood cells (WBC), CRP, PCT] were determined at each time point.

The standard values of laboratory tests in our hospital were as follows: TB, 0-19 μmol/L; DB, 0-6.8 μmol/L; IB, 0-12.2 μmol/L; GGT, < 50 U/L; ALP, 30-120 U/L; ALT, 2-50 U/L; WBC, 3.9-10 × $10^9$/L; CRP, < 8 mg/L; PCT < 0.5 ng/ml; Alb, 35-55 g/L. The imprecision of the assay was 5% or less of the total coefficient of variation, as reported by the manufacturer.

*Statistical analysis*

Normal distributed variables were compared using variance or Student's t test. Wilcoxon rank-sum test or Mann-Whitney U test were applied for non-normally distributed variables. Continuous variables were presented as means ± standard deviation (SD).

To identify the variables associated with mortality, univariate logistic regression analyses were performed. The variables with a *P* value of not more than 0.10 in univariate analysis were entered in the further multiple logistic regression analyses with the forward step-wise method. Given that differentΔTB, ΔDB and ΔIB were not independent variables, they were entered into the model separately. The Hosmer-Lemeshow test was used to check the goodness-of-fit of the logistic regression. All tests were assessed by OR (odds ratio) and their 95% CI (confidential intervals).

Receiver operating characteristic (ROC) curves were performed to determine the discriminating threshold of each parameter. The optimal

**Table 1.** Demographics and Clinical Characteristics of Patients in the Derivation Cohort

| Characteristics | Pooled (n = 162) | Survivors (n = 119) | Non-survivors (n = 43) | *P* value |
|---|---|---|---|---|
| Age (years), mean ± SD | 46.8 ± 9.6 | 44.7 ± 10.7 | 47.3 ± 13.6 | 0.279 |
| Male gender, n (%) | 123 (75.9) | 90 (75.6) | 33 (76.7) | 0.521 |
| BMI, mean ± SD | 19.8 ± 2.6 | 20.2 ± 1.5 | 19.1 ± 2.3 | 0.235 |
| Scores in admission | | | | |
| APACHE II (24 h) | 17.9 ± 3.6 | 17.6 ± 2.4 | 18.4 ± 4.1 | 0.076 |
| SOFA (24 h) | 10.3 ± 2.5 | 10.0 ± 2.1 | 10.9 ± 2.4 | 0.106 |
| Etiology, n (%) | | | | |
| Trauma | 37 (22.8) | 32 (26.9) | 5 (11.6) | 0.212 |
| Malignancy | 31 (19.1) | 18 (15.1) | 13 (30.2) | 0.036* |
| Operations | 62 (38.3) | 45 (37.8) | 17 (39.5) | 0.727 |
| Pancreatitis | 9 (5.6) | 7 (5.9) | 2 (4.7) | 0.210 |
| IBD | 20 (12.3) | 15 (12.6) | 5 (11.6) | 0.759 |
| Others[a] | 3 (1.9) | 2 (1.7) | 1 (2.3) | 0.086 |
| Fistula Location, n (%) | | | | |
| Stomach | 14 (8.6) | 10 (8.4) | 4 (9.3) | 0.805 |
| Duodenum | 40 (24.7) | 28 (23.5) | 12 (27.9) | 0.711 |
| Small bowel | 68 (42.0) | 49 (41.2) | 19 (44.2) | 0.748 |
| Colon | 20 (12.3) | 14 (11.8) | 6 (14.0) | 0.234 |
| Pancreas | 20 (12.3) | 18 (15.1) | 2 (4.7) | 0.021* |
| Underlying disease, n (%) | | | | |
| Cancer | 28 (17.3) | 18 (15.1) | 10 (23.3) | 0.032* |
| Cardiovascular disease | 11 (6.8) | 8 (6.7) | 3 (7.0) | 0.855 |
| Diabetes | 18 (11.1) | 13 (10.9) | 5 (11.6) | 0.703 |
| COPD | 13 (8.0) | 10 (8.4) | 3 (7.0) | 0.551 |
| None | 92 (56.8) | 70 (58.8) | 22 (51.2) | 0.322 |
| Fistula Output, n (%) | | | | |
| > 500 ml/day | 137 (84.6) | 98 (82.4) | 39 (90.7) | 0.264 |
| 200-500 ml/day | 18 (11.1) | 15 (12.6) | 3 (7.0) | 0.372 |
| < 200 ml/day | 7 (4.3) | 6 (5.0) | 1 (2.3) | 0.079 |
| ICU stay (days), Mean ± SD | 9.4 ± 3.0 | 5.9 ± 1.8 | 14.7 ± 2.5 | < 0.001* |

BMI, body mass index; APACHE II, acute physiology score and chronic health evaluation II; SOFA, sequential organ failure assessment score; IBD, inflammatory bowel disease; COPD, chronic obstructive pulmonary disease. Others [a]includes duodenum obstruction, acute suppurative cholangitis and acute gastric perforation. Data were presented as mean ± SD. *P* value < 0.05 was deemed significant. *indicates that *P* value < 0.05.

cutoff points were determined by maximizing the sum of sensitivity and specificity. We also determined the sensitivity, specificity, positive (PPV) and negative (NPV) predictive values. To study whether a combination of markers improved performance, a new ROC curve was compiled which included the relative contribution of each marker based on its concentration, as assessed by conditional logistic regression analysis with backward selection of all biomarkers. The predictors determined by the derivation cohort were evaluated in the validation cohort. All tests were assessed by their 95% confidence interval (CI). A *P* value < 0.05 was deemed significant. Statistical analyses were performed by SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA) and Stata software (version 8.0; Stata Corporation).

## Results

### *General characteristics of patients*

As for the derivation cohort, 354 patients were enrolled during the interval of this study. According to the exclusion criteria, 162 patients were eligible and finally enrolled (**Figure 1**). The

**Table 2.** Characteristics of Patients in the Derivation and Validation Cohorts

| Characteristics | Derivation cohort (n = 162) | Validation cohort (n = 116) | *P* value |
|---|---|---|---|
| Age (years), mean ± SD | 46.8 ± 9.6 | 49.4 ± 11.2 | 0.382 |
| Male gender, n (%) | 123 (75.9) | 91 (78.4) | 0.283 |
| BMI, mean ± SD | 19.8 ± 2.6 | 19.1 ± 3.8 | 0.327 |
| Scores on admission | | | |
| APACHE II (24 h) | 17.9 ± 3.6 | 18.3 ± 5.2 | 0.129 |
| SOFA (24 h) | 10.3 ± 2.5 | 10.7 ± 3.1 | 0.205 |
| Etiology, n (%) | | | |
| Trauma | 37 (22.8) | 24 (20.7) | 0.883 |
| Malignancy | 31 (19.1) | 23 (19.8) | 0.926 |
| Operations | 62 (38.3) | 36 (31.0) | 0.264 |
| Pancreatitis | 9 (5.6) | 5 (4.3) | 0.195 |
| IBD | 20 (12.3) | 26 (22.4) | 0.042* |
| Others[a] | 3 (1.9) | 2 (1.7) | 0.405 |
| Fistula Location, n (%) | | | |
| Stomach | 14 (8.6) | 8 (6.9) | 0.432 |
| Duodenum | 40 (24.7) | 21 (18.1) | 0.563 |
| Small bowel | 68 (42.0) | 30 (25.9) | 0.036* |
| Colon | 20 (12.3) | 24 (20.7) | 0.067 |
| Pancreas | 20 (12.3) | 33 (28.4) | 0.007* |
| Underlying disease, n (%) | | | |
| Cancer | 28 (17.3) | 25 (21.6) | 0.718 |
| Cardiovascular disease | 11 (6.8) | 10 (8.6) | 0.301 |
| Diabetes | 18 (11.1) | 9 (7.8) | 0.334 |
| COPD | 13 (8.0) | 5 (4.3) | 0.038* |
| None | 92 (56.8) | 67 (57.8) | 0.887 |
| Fistula Output, n (%) | | | |
| > 500 ml/day | 137 (84.6) | 88 (75.9) | 0.217 |
| 200-500 ml/day | 18 (11.1) | 16 (13.8) | 0.426 |
| < 200 ml/day | 7 (4.3) | 12 (10.3) | 0.082 |
| ICU stay (days), Mean ± SD | 9.4 ± 3.0 | 10.1 ± 4.2 | 0.126 |

BMI, body mass index; APACHE II, acute physiology score and chronic health evaluation II; SOFA, sequential organ failure assessment score; IBD, inflammatory bowel disease; COPD, chronic obstructive pulmonary disease. Others [a]includes duodenum obstruction, acute suppurative cholangitis and acute gastric perforation. Data were presented as mean ± SD. *P* value < 0.05 was deemed significant. *indicates that *P* value < 0.05.

derivation cohort's demographics are summarized in **Table 1**. According to 28-day outcomes, 162 patients were divided into survivors group (n = 119, 90 males and 29 females), and non-survivors group (n = 43, 33 males and 10 females). There were no significant differences with respect to age, gender, BMI and severity scores between the two groups. By contrast, ICU stay (14.7 ± 2.5 in the non-survivors vs. 5.9 ± 1.8 in the survivors, $P < 0.001$, the same order hereinafter), and malignancy prevalence (23.3% vs. 15.1%, $P = 0.032$) were significantly higher in non-survivors compared with survivors. Operations were listed as the top etiology for both groups (37.8% for survivors and 39.5% for non-survivors), followed by trauma in survivors group (26.9%) and malignancy in non-survivors group (30.2%). The demographics of the derivation cohort and validation cohort were generally similar (**Table 2**), which method was consistent with previous studies [12].

*Comparison of variables between survivors and non-survivors in the derivation cohort*

The dynamic changes of laboratory variables in the derivation cohort are summarized in **Table 3**. The measured variables were not significantly different between survivors and non-survi-

**Table 3.** Dynamic Changes of variables in the Derivation Cohort

| Variables | Groups | Day 0 | Day 3 | Day 7 |
|---|---|---|---|---|
| TB (μmol/L) | Survivors | 22.1 ± 7.9 | 19.6 ± 5.7 | 25.9 ± 6.5 |
| | Non-survivors | 26.4 ± 10.4 | 30.1 ± 13.5 | 42.3 ± 17.9 |
| | *P* | 0.281 | 0.027* | 0.011* |
| DB (μmol/L) | Survivors | 7.1 ± 2.6 | 9.6 ± 1.1 | 11.2 ± 1.1 |
| | Non-survivors | 9.8 ± 3.7 | 14.5 ± 6.8 | 23.1 ± 10.6 |
| | *P* | 0.331 | 0.204 | < 0.001* |
| IB (μmol/L) | Survivors | 14.3 ± 2.6 | 10.6 ± 2.9 | 14.5 ± 2.7 |
| | Non-survivors | 16.2 ± 5.5 | 15.4 ± 4.4 | 18.9 ± 3.8 |
| | *P* | 0.562 | 0.312 | 0.327 |
| CRP (mg/L) | Survivors | 165.7 ± 14.8 | 217.1 ± 17.8 | 64.6 ± 13.8 |
| | Non-survivors | 184.8 ± 38.7 | 171.9 ± 34.9 | 107.1 ± 35.7 |
| | *P* | 0.667 | 0.162 | 0.263 |
| PCT (ng/ml) | Survivors | 9.5 ± 0.2 | 2.5 ± 0.3 | 1.7 ± 0.3 |
| | Non-survivors | 8.6 ± 0.7 | 6.8 ± 1.2 | 5.2 ± 2.8 |
| | *P* | 0.724 | 0.253 | 0.006* |

TB, total bilirubin; DB, direct bilirubin; IB, indirect bilirubin; CRP, C-reactive protein; PCT, procalcitonin. Data were presented as mean ± SD. *P* value < 0.05 was deemed significant. *indicates that *P* value < 0.05.

vors initially. Compared with survivors, non-survivors had markedly higher $TB_3$ and $TB_7$ (30.1 ± 13.5 vs. 19.6 ± 5.7, *P* = 0.027, 42.3 ± 17.9 vs. 25.9 ± 6.5, *P* = 0.011, respectively). Non-survivors tended to exhibit higher $DB_7$ (23.1 ± 10.6 vs. 11.2 ± 1.1, *P* < 0.001). There were no significant differences with respect to other liver function indicators, including ALT, ALP, GGT and Alb levels (data not shown).

The PCT levels decreased progressively in survivors and non-survivors, with significant differences on day 7 (1.7 ± 0.3 in survivors vs. 5.2 ± 2.8 in non-survivors, *P* = 0.006). The CRP levels also exhibited a descendent trend for both groups, but no significant differences were detected throughout the observation period. The corresponding data were in a similar profile in the validation cohort (data not shown).

*Univariate and multivariate logistic regression analysis*

We further performed univariate and multivariate logistic regression analysis to find out potential risk factors associated with mortality, as shown in Tables S1 and S2.

In univariable analysis, numerous factors were associated with mortality, including age, $TB_7$, $DB_7$, $\Delta DB_7$, $IB_7$, $ALP_7$, $GGT_7$, $WBC_7$ and $PCT_7$. Other variables were unrelated to outcomes. The variables which were strongly associated with mortality in univariate analysis were entered in the further multiple logistic regression analyses. In multivariable logistic regression analysis, age (> 46, OR: 2.44, 95% CI: 1.03-7.76, *P* = 0.046), $TB_7$ > 30.7 μmol/L, OR: 2.57, 95% CI: 1.15-15.30, *P* = 0.035), $DB_7$ (> 12.8 μmol/L, OR: 4.26, 95% CI: 2.54-16.11, *P* < 0.001), $\Delta DB_7$ (> 7.3 μmol/L, OR: 3.42, 95% CI: 2.16-8.50, *P* = 0.002) and $PCT_7$ (> 3.3 ng/ml, OR: 2.35, 95% CI: 1.55-11.86, *P* = 0.037) still remained significantly associated with mortality.

Taken together, these results indicated that hyperbilirubinemia and serum bilirubin fluctuations were strongly associated with mortality in abdominal septic patients.

*Predictive value of individual and combined parameters in the derivation cohort*

To investigate the predictive values of variables, ROC curve analysis was constructed (**Table 4**). The AUC of $DB_7$ (AUC: 0.881, 95% CI: 0.784-0.927, *P* < 0.001) was much greater than other biomarkers. The optimal cutoff value of $DB_7$ was > 12.8 μmol/L, with 86.4% sensitivity, 88.6% specificity, 90.6% PPV and 80.5% NPV. Besides, a $\Delta DB_7$ value of > 7.3 μmol/L allowed discrimination between survivors and non-survivors, with a sensitivity of 84.4% and a specificity of 85.1% (AUC: 0.865, 95% CI: 0.701-0.914, PPV: 76.2%, NPV: 87.3%, *P* < 0.001). The AUC of $\Delta PCT_7$ was 0.834 (95% CI: 0.759-0.906, *P* < 0.001), with 80.5% sensitivity, 81.6% specificity, 76.6% PPV and 88.2% NPV at the best threshold value of < 5.3 ng/ml. The predictive value of $TB_7$ and $PCT_7$ were less accurate with a sensitivity or specificity less than 80% [13]. None of other variables was useful to predict mortality, with AUC ranging from 0.518 to 0.647 (data not shown).

We selected the predictors with significant AUC to evaluate whether their combination form

**Table 4.** Predictive value of variables for 28-day mortality in the Derivation Cohort

| Variables | Cutoff | Sensitivity | Specificity | AUC | 95% CI | PPV | NPV | *P* value |
|---|---|---|---|---|---|---|---|---|
| $TB_7$ (μmol/L) | > 30.7 | 64.5% | 85.1% | 0.782 | 0.721-0.883 | 43.5% | 91.8% | 0.025* |
| $DB_7$ (μmol/L) | > 12.8 | 86.4% | 88.6% | 0.881 | 0.784-0.927 | 90.6% | 80.5% | < 0.001* |
| $\Delta DB_7$ (μmol/L) | > 7.3 | 84.4% | 85.1% | 0.865 | 0.701-0.914 | 76.2% | 87.3% | < 0.001* |
| $PCT_7$ (ng/ml) | > 3.3 | 70.5% | 84.6% | 0.766 | 0.683-0.850 | 52.3% | 93.2% | 0.036* |
| $\Delta PCT_7$ (ng/ml) | < 5.3 | 80.5% | 81.6% | 0.834 | 0.759-0.906 | 76.6% | 88.2% | < 0.001* |

To investigate the role of variables in prediction of mortality, ROC curve analysis for individual and combined parameters were constructed. AUC: area under the curve, CI, confidential intervals; PPV, positive predictive values; NPV, negative predictive values. TB, total bilirubin; DB, direct bilirubin; PCT, procalcitonin. *P* value < 0.05 was deemed significant. *indicates that *P* value < 0.05.

could promote the predictive accuracy further (**Table 5**). Notably, the combination form of ($DB_7$ > 12.8 μmol/L + $\Delta PCT_7$ < 5.3 ng/ml) resulted in the greatest AUC (AUC: 0.894, *P* < 0.001) than other variables, either alone or in combination.

ROC curve analysis showed that $DB_7$, $\Delta DB_7$, and $\Delta PCT_7$ were reliable predictors of mortality in abdominal sepsis. The predictive value of $DB_7$ was superior to conventional biomarkers such as CRP or PCT. Prominently, the conjunctive form ($DB_7$ + $\Delta PCT_7$) showed the best discriminatory power.

*Evaluation of predictors in the validation cohort*

To evaluate the reproducibility of these predictors, we tested them in an independent validation cohort. We confirmed that the AUCs of $DB_7$, $\Delta DB_7$, and $\Delta PCT_7$ were statistically identical in the derivation cohort and the validation cohort (0.881 vs. 0.864; 0.865 vs. 0.856; 0.834 vs. 0.825; respectively), as shown in **Figures 2** and **3**. When we applied the cutoff points determined in the derivation cohort to the validation cohort, we found 82.9% sensitivity (95% CI, 76.4%-90.1%) and 84.4% specificity (95% CI, 78.6%-89.5%) for $DB_7$; 81.3% sensitivity (95% CI, 74.9%-88.4%) and 82.4% specificity (95% CI, 76.9%-89.1%) for $\Delta DB_7$; 76.7% sensitivity (95% CI, 72.8%-81.1%) and 77.5% specificity (95% CI, 70.9%-84.6%) for $\Delta PCT_7$ (**Figure 3**). They all met the requirements that a good area under the ROC curve is at least 0.75, and the best threshold needs to be identified with a sensitivity and specificity of at least 75% [13].

## Discussion

In this study, we monitored laboratory parameters to predict mortality of abdominal septic patients. $DB_7$ and $\Delta DB_7$ were reliable predictors. The combined biomarkers ($DB_7$ + $\Delta PCT_7$) provided greater predictive accuracy than individual markers. Their performance was confirmed in an independent validation cohort. To the best of our knowledge, it is the first study focusing exclusively on EF patients to sequentially analyze bilirubin subtypes and to establish the concept of DB as a biomarker.

Hyperbilirubinemia has been reported to occur in 31% of critically ill patients. A retrospective study suggested hyperbilirubinemia as an independent risk factor for mortality [14]. The authors suggested that prospective studies should be taken to confirm their findings. Our study was such a study. Bilirubin is the breakdown product of hemoglobin. Unconjugated bilirubin (namely IB) reversibly bounds to plasma albumin. Within hepatocytes, IB becomes conjugated bilirubin (namely DB) and flows into bile across the hepatocyte canalicular membranes. Hyperbilirubinemia may be a consequence of hemolysis, hepatic dysfunction or post-hepatic occlusion. Infections, multiple drugs, hypersplenism and massive blood transfusions all contribute to hemolysis [15]. However, hemolysis is not the principal etiology, since 70% of serum bilirubin consists of conjugated bilirubin during the sepsis. It is speculated that hepatocyte excretory dysfunction plays a central role in the sepsis-induced hyperbilirubinemia. Gonnert et al demonstrated that transporters at the hepatocyte canalicular pole are particularly susceptible to sepsis. The energy-dependent transporters required for bile secretion are impaired by inflammatory mediators [16]. Taken together, abdominal septic patients are at high-risk of hyperbilirubinemia. We determined the predictive value of this routinely measured variable, in comparison with conventional biomarkers (CRP and PCT).

**Table 5.** Predictive value of combined variables for 28-day mortality in the Derivation Cohort

| Multivariable model | AUC | 95% CI | *P* value |
|---|---|---|---|
| $DB_7$ + $\Delta DB_7$ | 0.862 | 0.828-0.938 | < 0.001* |
| $DB_7$ + $\Delta PCT_7$ | 0.894 | 0.832-0.910 | < 0.001* |
| $\Delta DB_7$ + $\Delta PCT_7$ | 0.840 | 0.777-0.908 | < 0.001* |
| $DB_7$ + $\Delta DB_7$ + $\Delta PCT_7$ | 0.868 | 0.822-0.933 | < 0.001* |

To study whether a combined biomarkers improved performance, a new ROC curve was compiled which included the relative contribution of each biomarker. AUC: area under the curve, CI, confidential intervals; PCT, procalcitonin. *P* value < 0.05 was deemed significant. *indicates that *P* value < 0.05.

We observed that compared with survivors, TB and DB on day 7 were dramatically higher in non-survivors. There are several explanations for this phenomenon. Firstly, bilirubin has an antioxidant effect greater than vitamin C, vitamin E and β-carotene. A physiological concentration of bilirubin could scavenge oxygen free radicals efficiently [17]. However, this antioxidant property impairs the reactive oxygen species-dependent bactericidal activity of neutrophils. Secondly, bilirubin inhibited vascular cell adhesion molecule-1-mediated leukocyte migration. Bilirubin could suppress protein kinase C activity and p38 protein phosphorylation [18]. Thirdly, bilirubin showed an inhibitory effect on complement-dependent reactions [19]. They all contribute to diminished immune function, leading to increased infection severity. Previous studies indicated that patients with hyperbilirubinemia had a 3-fold increased risk of infection compared with healthy controls [20].

It is pivotal that the alteration trend of variables and assessment time should be taken into account. In the current study, we revealed that $DB_7$ and $\Delta DB_7$ had better prediction capacity than CRP and PCT. Sepsis is a rapidly-changing syndrome which is too complex to be evaluated by a single measurement. The alteration trend reflects disease evolution more accurately, particularly when absolute values are high initially. We emphasized the importance of analyzing dynamic alterations of variables, rather than just absolute values. The assessment time is another crucial factor. The predictive value of $DB_3$ was not sensitive as $DB_7$. We presumed that during the early sepsis, most patients were still capable to maintain reasonable serum bilirubin levels by compensation mechanisms. Besides, the different predictive accuracy was probably due to the wider 95% CI of $DB_3$ in comparison with $DB_7$. It was also found that $\Delta DB_{7\text{-}3}$ was not as sensitive as $\Delta DB_7$ in predicting mortality. A possible explanation is that $\Delta DB_7$ was calculated from the values on day 7 to baseline, but $\Delta DB_{7\text{-}3}$ was calculated from the values on day 7 to day 3, meaning that $\Delta DB_{7\text{-}3}$ might be affected by more confounding factors.

It is noteworthy that the prognosis was associated particularly with DB rather than TB. Since DB is reversibly bound to albumin, it is easily separated from albumin and be in active form compared with other bilirubin subtypes. We speculated that DB might act on the target organs and molecules directly. Secondly, we observed that the time-dependent alterations and the predictive value of IB were not statistically significant. The interference factor of IB might account for the superior predictive value of DB to TB. Current scoring systems incorporate serum TB to evaluate disease severity [6]. We showed that DB had more accurate predictive value than TB in patients with EF-related abdominal sepsis. DB and DB dynamics should be incorporated in assessing severity of this population.

Since we tested the predictors again in an independent validation cohort, the predictive values of these biomarkers were confirmed.

Although CRP was suggested as a predictor of outcomes in sepsis, Lee et al found no associations between CRP levels and mortality [4]. We proved that CRP levels were not different between survivors and non-survivors throughout the observation period. Moreover, the predictive value of ΔCRP was not as high as ΔPCT, which was in accordance with previous studies showing that PCT reflects the severity of sepsis better than CRP [21].

PCT has a moderate to strong correlation with sepsis outcomes [22]. However, the PCT value in predicting survival is still controversial, because the serum concentrations and peak time may be influenced by clinical conditions [3]. Therefore, we focused on a limited population to reduce heterogeneity. Previous studies demonstrated that there was a considerable overlap of PCT baseline values in survivors and non-survivors. PCT levels became significantly lower in survivors than in non-survivors during the second week of sepsis [23]. We revealed that PCT levels of non-survivors were initially

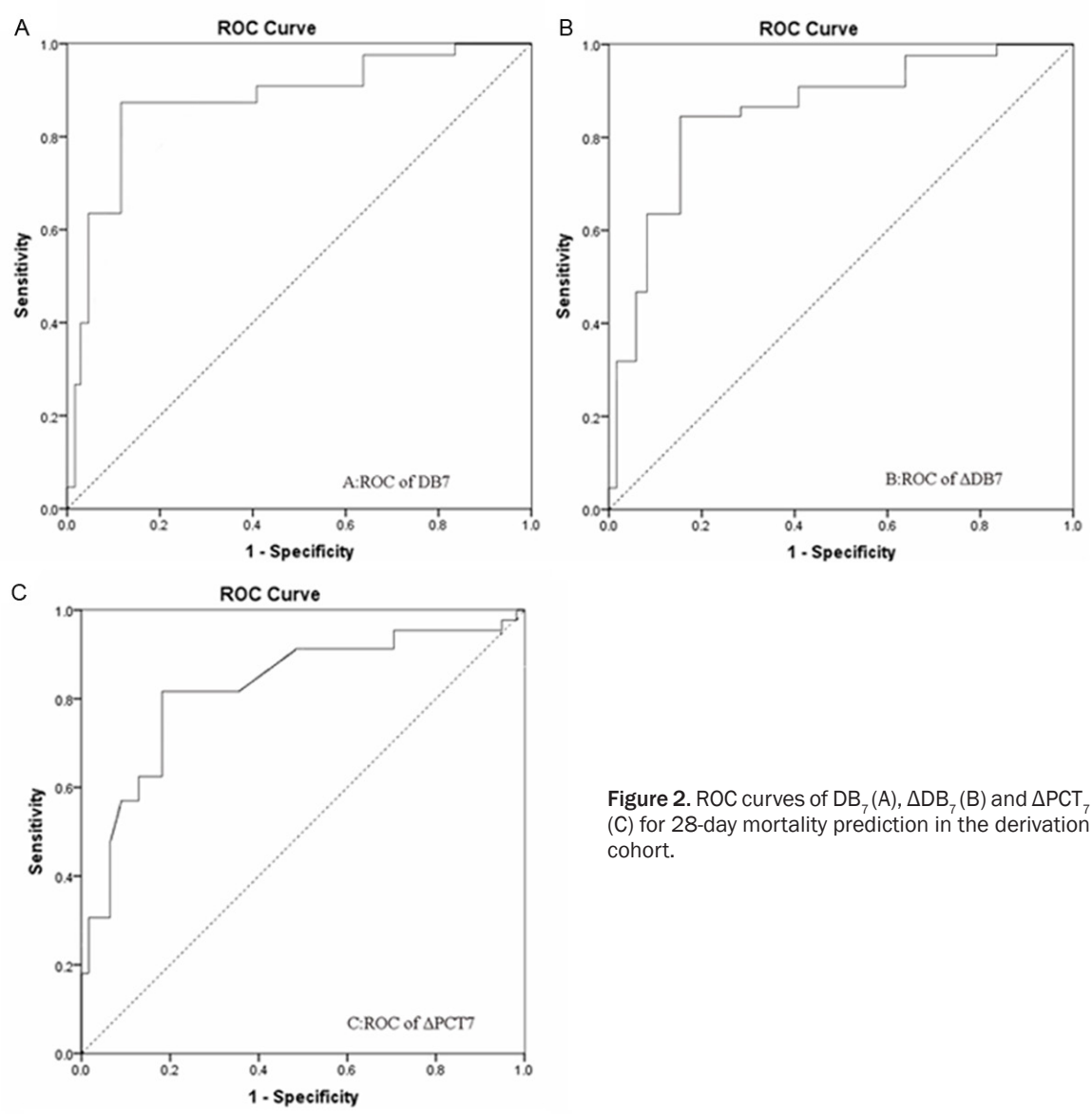


**Figure 2.** ROC curves of $DB_7$ (A), $\Delta DB_7$ (B) and $\Delta PCT_7$ (C) for 28-day mortality prediction in the derivation cohort.

similar with survivors but increased significantly on day 7. We determined that $\Delta PCT_7$ had significant predictive value. The slow decrease in serum PCT levels was consistently associated with poor outcomes. Povoa P et al also observed that the PCT predictive value seems to be more related to alteration trend rather than a single measurement [24]. We also found that the combination of $DB_7$ and $\Delta PCT_7$ improved the predictive accuracy. Given the complex pathophysiology of sepsis, it is unlikely that a single biomarker could provide adequate information.

The serial bilirubin determinations have an impact on the management of EF patients. Firstly, $DB_7$ and $\Delta DB_7$ provide early and sensitive alert signals. Even mild bilirubin deviations from normal levels (particularly $DB_7$ and $\Delta DB_7$) were associated with mortality. But they are usually ignored in clinics. With the cut-off values, we could identify patients who would otherwise miss the curative window and initiate targeted intervention quickly. Secondly, biomarker-based protocols guide therapeutic adjustments, preventing under- or over-treatments. Thirdly, compared with PCT and CRP, bilirubin measurements are cost-effective (per test: $24.9 for PCT, $5.7 for CRP and for $1.9 for bilirubin).

## Conclusion

By focusing on abdominal septic patients, we confirmed a predictive role of serum bilirubin

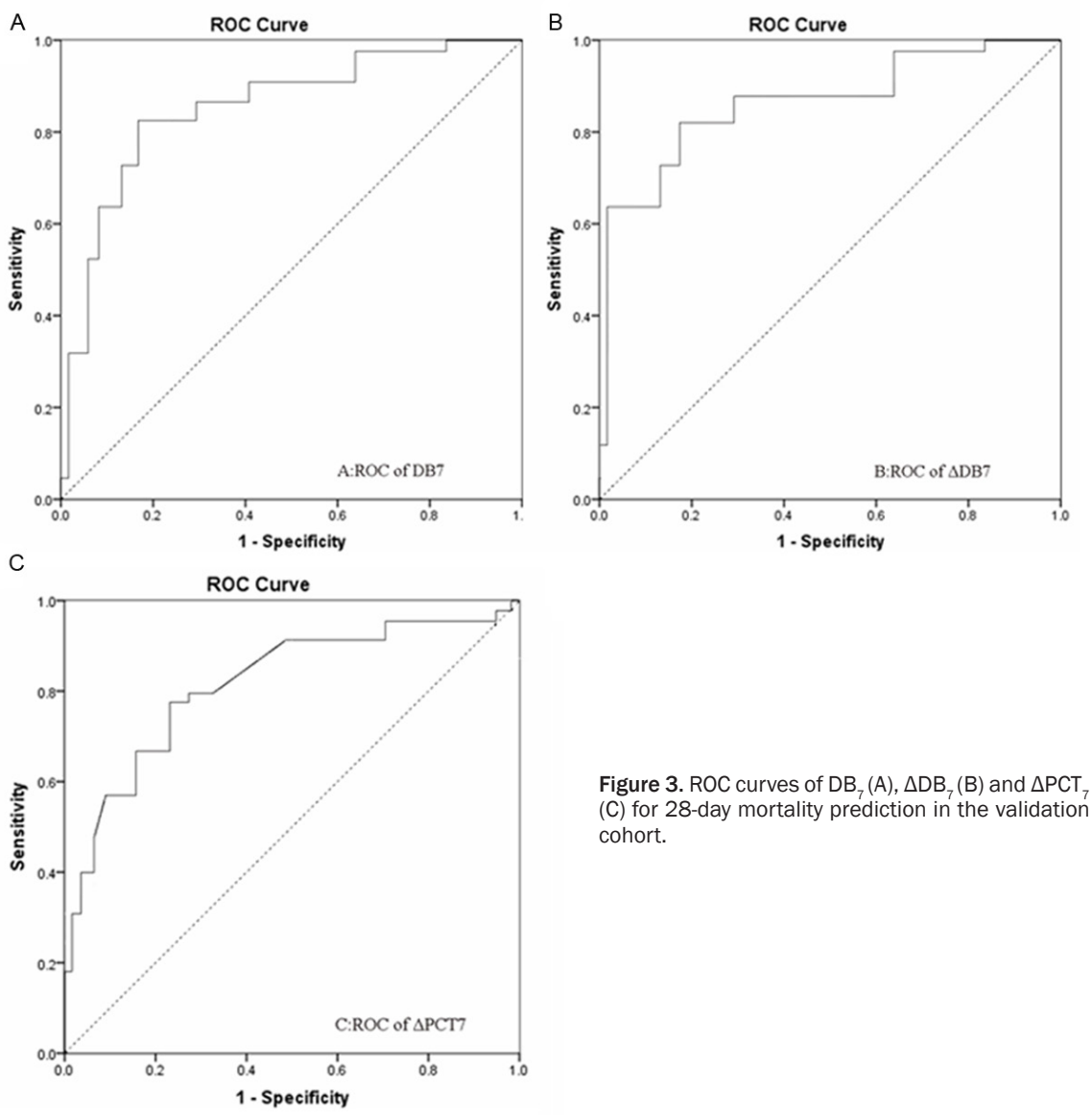


**Figure 3.** ROC curves of $DB_7$ (A), $\Delta DB_7$ (B) and $\Delta PCT_7$ (C) for 28-day mortality prediction in the validation cohort.

subtypes, which was underestimated previously. We demonstrated that $DB_7$ and $\Delta DB_7$ were reliable predictors of mortality in patients with EF-related abdominal sepsis, better than CRP or PCT. The combined biomarkers could improve the predictive accuracy. Strict monitoring of bilirubin kinetics potentiates early warning.

## Acknowledgements

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## Disclosure of conflict of interest

None.

## Abbreviations

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APACHE II, Acute Physiology and Chronic Health Evaluation II; AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; DB, direct bilirubin; EF, enteric fistula; GGT, γ-glutamyltransferas; IB, indirect bilirubin; NPV, negative predictive values; OR, odds ratio; PCT, procalcitonin; PLT, platelet; PPV, positive predictive values; RBC, red blood cells; ROC, Receiver operating characteristic; SOFA, Sequential Organ Failure Assessment; TB, total bilirubin; TP, total protein; WBC, white blood cells.

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## References


[1] Sakr Y, Elia C, Mascia L, Barberis B, Cardellino S, Livigni S, Fiore G, Filippini C, Ranieri VM. The influence of gender on the epidemiology of and outcome from severe sepsis. Crit Care 2013; 17: R50.

[2] Martinez JL, Luque-de-Leon E, Ballinas-Oseguera G, Mendez JD, Juarez-Oropeza MA, Roman-Ramos R. Factors predictive of recurrence and mortality after surgical repair of enterocutaneous fistula. J Gastrointest Surg 2012; 16: 156-163; discussion 163-154.

[3] Karlsson S, Heikkinen M, Pettila V, Alila S, Vaisanen S, Pulkki K, Kolho E, Ruokonen E, Finnsepsis Study G. Predictive value of procalcitonin decrease in patients with severe sepsis: a prospective observational study. Crit Care 2010; 14: R205.

[4] Lee CC, Chen SY, Tsai CL, Wu SC, Chiang WC, Wang JL, Sun HY, Chen SC, Chen WJ, Hsueh PR. Prognostic value of mortality in emergency department sepsis score, procalcitonin, and C-reactive protein in patients with sepsis at the emergency department. Shock 2008; 29: 322-327.

[5] Baue AE, Durham R, Faist E. Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): are we winning the battle? Shock 1998; 10: 79-89.

[6] Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb SA, Beale RJ, Vincent JL, Moreno R; Surviving Sepsis Campaign Guidelines Committee including the Pediatric Subgroup. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med 2013; 41: 580-637.

[7] Patel JJ, Taneja A, Niccum D, Kumar G, Jacobs E, Nanchal R. The Association of Serum Bilirubin Levels on the Outcomes of Severe Sepsis. J Intensive Care Med 2013; [Epub ahead of print].

[8] Pineda S, Bang OY, Saver JL, Starkman S, Yun SW, Liebeskind DS, Kim D, Ali LK, Shah SH, Ovbiagele B. Association of serum bilirubin with ischemic stroke outcomes. J Stroke Cerebrovasc Dis 2008; 17: 147-152.

[9] Hwang HJ, Kim SH. Inverse relationship between fasting direct bilirubin and metabolic syndrome in Korean adults. Clin Chim Acta 2010; 411: 1496-1501.

[10] Jones AE, Trzeciak S, Kline JA. The Sequential Organ Failure Assessment score for predicting outcome in patients with severe sepsis and evidence of hypoperfusion at the time of emergency department presentation. Crit Care Med 2009; 37: 1649-1654.

[11] Delibegovic S, Markovic D, Hodzic S. APACHE II scoring system is superior in the prediction of the outcome in critically ill patients with perforative peritonitis. Med Arh 2011; 65: 82-85.

[12] Hu MY, Katchar K, Kyne L, Maroo S, Tummala S, Dreisbach V, Xu H, Leffler DA, Kelly CP. Prospective derivation and validation of a clinical prediction rule for recurrent Clostridium difficile infection. Gastroenterology 2009; 136: 1206-1214.

[13] Yin J, Tian L. Joint confidence region estimation for area under ROC curve and Youden index. Stat Med 2014; 33: 985-1000.

[14] Kramer L, Jordan B, Druml W, Bauer P, Metnitz PG; Austrian Epidemiologic Study on Intensive Care ASDI Study Group. Incidence and prognosis of early hepatic dysfunction in critically ill patients--a prospective multicenter study. Crit Care Med 2007; 35: 1099-1104.

[15] Scharte M, Fink MP. Red blood cell physiology in critical illness. Crit Care Med 2003; 31: S651-657.

[16] Bolder U, Ton-Nu HT, Schteingart CD, Frick E, Hofmann AF. Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. Gastroenterology 1997; 112: 214-225.

[17] Schwertner HA, Vitek L. Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. Atherosclerosis 2008; 198: 1-11.

[18] Paine A, Eiz-Vesper B, Blasczyk R, Immenschuh S. Signaling to heme oxygenase-1 and its anti-inflammatory therapeutic potential. Biochem Pharmacol 2010; 80: 1895-1903.

[19] Shiomi S, Habu D, Kuroki T, Ishida S, Tatsumi N. Clinical usefulness of conjugated bilirubin levels in patients with acute liver diseases. J Gastroenterol 1999; 34: 88-93.

[20] Field E, Horst HM, Rubinfeld IS, Copeland CF, Waheed U, Jordan J, Barry A, Brandt MM. Hyperbilirubinemia: a risk factor for infection in the surgical intensive care unit. Am J Surg 2008; 195: 304-306; discussion 306-307.

[21] Claeys R, Vinken S, Spapen H, ver Elst K, Decochez K, Huyghens L, Gorus FK. Plasma procalcitonin and C-reactive protein in acute septic shock: clinical and biological correlates. Crit Care Med 2002; 30: 757-762.

[22] Kibe S, Adams K, Barlow G. Diagnostic and prognostic biomarkers of sepsis in critical care. J Antimicrob Chemother 2011; 66 Suppl 2: ii33-40.

[23] Herrmann W, Ecker D, Quast S, Klieden M, Rose S, Marzi I. Comparison of procalcitonin, sCD14 and interleukin-6 values in septic patients. Clin Chem Lab Med 2000; 38: 41-46.
[24] Povoa P, Teixeira-Pinto AM, Carneiro AH; Portuguese Community-Acquired Sepsis Study Group SACIUCL. C-reactive protein, an early marker of community-acquired sepsis resolution: a multi-center prospective observational study. Crit Care 2011; 15: R169.

**Table S1.** Logistic regression analysis of liver function indicators to differentiate survivors and non-survivors

| | | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|---|
| Dependent Variable | | OR | 95% CI | *P* value | OR | 95% CI | *P* value |
| Gender | Male | 1.00 | | | | | |
| | Female | 1.15 | 1.02-3.09 | 0.272 | | | |
| Age, years | ≤ 46 | 1.00 | | | | | |
| | > 46 | 3.04 | 1.90-7.34 | 0.025* | 2.44 | 1.03-7.76 | 0.046* |
| APACH II score | ≤ 18 | 1.00 | | | | | |
| | > 18 | 1.38 | 1.13-4.54 | 0.241 | | | |
| SOFA score | ≤ 7 | 1.00 | | | | | |
| | > 7 | 1.56 | 1.15-5.62 | 0.197 | | | |
| $TB_0$ (μmol/L) | ≤ 23.9 | 1.00 | | | | | |
| | > 23.9 | 3.07 | 2.17-10.20 | 0.056 | | | |
| $TB_3$ (μmol/L) | ≤ 24.9 | 1.00 | | | | | |
| | > 24.9 | 2.60 | 1.42-7.69 | 0.116 | | | |
| $TB_7$ (μmol/L) | ≤ 30.7 | 1.00 | | | | | |
| | > 30.7 | 3.86 | 2.38-9.14 | < 0.001* | 2.57 | 1.15-15.30 | 0.035* |
| $\Delta TB_3$ (μmol/L) | ≤ 3.7 | 1.00 | | | | | |
| | > 3.7 | 1.37 | 1.18-16.73 | 0.358 | | | |
| $\Delta TB_7$ (μmol/L) | ≤ 5.2 | 1.00 | | | | | |
| | > 5.2 | 1.52 | 1.07-2.01 | 0.311 | | | |
| $\Delta TB_{7-3}$ (μmol/L) | ≤ 4.6 | 1.00 | | | | | |
| | > 4.6 | 1.48 | 1.12-14.87 | 0.182 | | | |
| $DB_0$ (μmol/L) | ≤ 8.2 | 1.00 | | | | | |
| | > 8.2 | 1.41 | 1.22-12.59 | 0.223 | | | |
| $DB_3$ (μmol/L) | ≤ 11.3 | 1.00 | | | | | |
| | > 11.3 | 1.92 | 1.13-9.74 | 0.159 | | | |
| $DB_7$ (μmol/L) | ≤ 12.8 | 1.00 | | | | | |
| | > 12.8 | 5.23 | 3.82-13.46 | < 0.001* | 4.26 | 2.54-16.11 | < 0.001* |
| $\Delta DB_3$ (μmol/L) | ≤ 3.7 | 1.00 | | | | | |
| | > 3.7 | 1.12 | 0.74-10.31 | 0.417 | | | |
| $\Delta DB_7$ (μmol/L) | ≤ 7.3 | 1.00 | | | | | |
| | > 7.3 | 4.54 | 1.32-8.42 | 0.003* | 3.42 | 2.16-8.50 | 0.002* |
| $\Delta DB_{7-3}$ (μmol/L) | ≤ 3.6 | 1.00 | | | | | |
| | > 3.6 | 1.28 | 1.10-7.56 | 0.216 | | | |
| $IB_0$ (μmol/L) | ≤ 14.7 | 1.00 | | | | | |
| | > 14.7 | 2.89 | 1.83-22.07 | 0.107 | | | |
| $IB_3$ (μmol/L) | ≤ 12.5 | 1.00 | | | | | |
| | > 12.5 | 2.58 | 1.68-20.41 | 0.132 | | | |
| $IB_7$ (μmol/L) | ≤ 15.4 | 1.00 | | | | | |
| | > 15.4 | 4.19 | 2.89-20.01 | < 0.001* | 1.02 | 1.00-7.89 | 0.475 |
| $\Delta IB_3$ (μmol/L) | ≤ 4.8 | 1.00 | | | | | |
| | > 4.8 | 2.02 | 1.94-12.15 | 0.168 | | | |
| $\Delta IB_7$ (μmol/L) | ≤ 7.2 | 1.00 | | | | | |
| | > 7.2 | 1.63 | 1.42-17.03 | 0.185 | | | |
| $\Delta IB_{7-3}$ (μmol/L) | ≤ 6.7 | 1.00 | | | | | |
| | > 6.7 | 1.17 | 0.75-6.56 | 0.405 | | | |

OR, odds ratio; CI, confidential intervals; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; TB, total bilirubin; DB, direct bilirubin; IB, indirect bilirubin.

**Table S2.** Logistic regression analysis of laboratory parameters to differentiate survivors and non-survivors

| | | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|---|
| Dependent Variable | | OR | 95% CI | *P* value | OR | 95% CI | *P* value |
| $ALP_0$ (U/L) | ≤ 144.5 | 1.00 | | | | | |
| | > 144.5 | 1.34 | 1.06-5.59 | 0.205 | | | |
| $ALP_3$ (U/L) | ≤ 112.4 | 1.00 | | | | | |
| | > 112.4 | 1.08 | 1.02-9.14 | 0.341 | | | |
| $ALP_7$ (U/L) | ≤ 183.5 | 1.00 | | | | | |
| | > 183.5 | 3.88 | 2.52-10.79 | < 0.001* | 1.68 | 1.30-8.74 | 0.085 |
| $GGT_0$ (U/L) | ≤ 95.0 | 1.00 | | | | | |
| | > 95.0 | 1.02 | 1.01-4.96 | 0.424 | | | |
| $GGT_3$ (U/L) | ≤ 82.5 | 1.00 | | | | | |
| | > 82.5 | 1.16 | 1.07-5.71 | 0.375 | | | |
| $GGT_7$ (U/L) | ≤ 127.0 | 1.00 | | | | | |
| | > 127.0 | 2.91 | 1.45-9.38 | 0.045* | 1.49 | 1.14-6.23 | 0.175 |
| $TP_0$ (g/L) | ≤ 57.6 | 1.00 | | | | | |
| | > 57.6 | 0.36 | 0.04-0.62 | 0.294 | | | |
| $TP_3$ (g/L) | ≤ 57.9 | 1.00 | | | | | |
| | > 57.9 | 0.41 | 0.12-0.67 | 0.378 | | | |
| $TP_7$ (g/L) | ≤ 58.4 | 1.00 | | | | | |
| | > 58.4 | 0.78 | 0.56-4.12 | 0.156 | | | |
| $Alb_0$ (g/L) | ≤ 32.5 | 1.00 | | | | | |
| | > 32.5 | 0.05 | 0.02-3.46 | 0.921 | | | |
| $Alb_3$ (g/L) | ≤ 34.3 | 1.00 | | | | | |
| | > 34.3 | 0.35 | 0.15-4.67 | 0.245 | | | |
| $Alb_7$ (g/L) | ≤ 33.9 | 1.00 | | | | | |
| | > 33.9 | 0.77 | 0.05-4.89 | 0.278 | | | |
| $WBC_0$ ($10^9$/L) | ≤ 11.9 | 1.00 | | | | | |
| | > 11.9 | 2.92 | 2.45-8.34 | 0.068 | | | |
| $WBC_3$ ($10^9$/L) | ≤ 12.1 | 1.00 | | | | | |
| | > 12.1 | 1.52 | 1.05-8.33 | 0.203 | | | |
| $WBC_7$ ($10^9$/L) | ≤ 13.5 | 1.00 | | | | | |
| | > 13.5 | 3.71 | 2.23-10.77 | 0.031* | 1.32 | 1.09-9.15 | 0.159 |
| $CRP_0$ (mg/L) | ≤ 181.0 | 1.00 | | | | | |
| | > 181.0 | 1.49 | 1.05-10.78 | 0.177 | | | |
| $CRP_3$ (mg/L) | ≤ 208.3 | 1.00 | | | | | |
| | > 208.3 | 1.92 | 1.37-10.88 | 0.230 | | | |
| $CRP_7$ (mg/L) | ≤ 90.1 | 1.00 | | | | | |
| | > 90.1 | 1.92 | 1.13-14.38 | 0.028 | | | |
| $PCT_0$ (ng/ml) | > 8.8 | 1.00 | | | | | |
| | > 8.8 | 1.44 | 1.04-7.91 | 0.258 | | | |
| $PCT_3$ (ng/ml) | ≤ 3.6 | 1.00 | | | | | |
| | > 3.6 | 1.21 | 1.05-6.58 | 0.240 | | | |
| $PCT_7$ (ng/ml) | ≤ 3.3 | 1.00 | | | | | |
| | > 3.3 | 4.07 | 2.61-10.40 | < 0.001* | 2.35 | 1.55-11.86 | 0.037* |
| N $ratio_0$ (%) | ≤ 0.8 | 1.00 | | | | | |
| | > 0.8 | 2.35 | 1.25-10.95 | 0.162 | | | |
| N $ratio_3$ (%) | ≤ 0.8 | 1.00 | | | | | |
| | > 0.8 | 1.62 | 1.14-9.44 | 0.214 | | | |
| N $ratio_7$ (%) | ≤ 0.8 | 1.00 | | | | | |
| | > 0.8 | 2.05 | 1.45-12.00 | 0.146 | | | |

OR, odds ratio; CI, confidential intervals; ALP, alkaline phosphatase; GGT, γ-glutamyltransferas; TP, total protein; ALB, albumin; WBC, white blood cells; CRP, C-reactive protein; PCT, procalcitonin; N ratio, neutrophil ratio.