

# Long Pentraxin 3 as a Predictive Marker of Mortality in Severe Septic Patients Who Received Successful Early Goal-Directed Therapy

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**Purpose:** Pentraxin 3 (PTX3) has been suggested to be a prognostic marker of mortality in severe sepsis. Currently, there are limited data on biomarkers including PTX3 that can be used to predict mortality in severe sepsis patients who have undergone successful initial resuscitation through early goal-directed therapy (EGDT).

**Materials and Methods:** A prospective cohort study was conducted among 83 severe sepsis patients with fulfillment of all EGDT components and the achievement of final goal. Plasma PTX3 levels were measured by sandwich ELISA on hospital day (HD) 0, 3, and 7. The data for procalcitonin, C-reactive protein and delta neutrophil index were collected by electric medical record. The primary outcome was 28-day all-cause mortality.

**Results:** 28-day all-cause mortality was 19.3% and the median (interquartile range) APACHE II score of total patients was 16 (13–19). The non-survivors (n=16) had significantly higher PTX3 level at HD 0 [201.4 (56.9–268.6) ng/mL vs. 36.5 (13.7–145.3) ng/mL,  $p=0.008$ ]. PTX3 had largest AUC<sup>ROC</sup> value for the prediction of mortality among PTX3, procalcitonin, delta neutrophil index, CRP and APACHE II/SOFA score at HD 0 [0.819, 95% confidence interval (CI) 0.677–0.961,  $p=0.008$ ]. The most valid cut-off level of PTX3 at HD 0 was 140.28 ng/mL (sensitivity 66.7%, specificity 73.8%). The PTX3 and procalcitonin at HD 0 showed strong correlation ( $r=0.675$ ,  $p<0.001$ ). However, PTX3 at HD 0 was the only independent predictive marker in Cox's proportional hazards model ( $\geq 140$  ng/mL; hazard rate 7.16, 95% CI 2.46–15.85,  $p=0.001$ ).

**Conclusion:** PTX3 at HD 0 could be a powerful predictive biomarker of 28-day all-cause mortality in severe septic patients who have undergone successful EGDT.

**Key Words:** Pentraxin 3, severe sepsis, early-goal directed therapy, mortality, predictive biomarker

## INTRODUCTION

Pentraxins are a superfamily of soluble multi-functional pattern recognition receptors.<sup>1</sup> Pentraxin 3 (PTX3) is the prototyp-

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•The authors have no financial conflicts of interest.

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ic long pentraxin, and the classic short pentraxin of C-reactive protein (CRP) and serum amyloid P component (SAP) are other key members of this family.<sup>1</sup> PTX3 plays a major role in innate immunity and inflammation.<sup>1</sup> It is rapidly produced and released by various inflammatory cells in response to pro-inflammatory stimuli,<sup>2–4</sup> and acts as an acute phase protein, similar to short pentraxins, because the plasma level of PTX3 increases rapidly and drastically during inflammation.<sup>1</sup> However, the normal human population without any inflammatory conditions has a low plasma PTX3 level of  $<2$  ng/mL.<sup>1</sup>

Despite the introduction of updated early intensive resuscitating strategies and new antibiotics, sepsis-related morbidities and mortality remain considerably high.<sup>5–7</sup> Therefore, new treatment strategies with immunomodulatory drugs such as

the targeted therapy according to the status of inflammatory response in each patient need to be developed to dramatically improve outcomes, especially in septic patients with high severity and risk of poor outcome. To achieve improvement, we must identify new prognostic biomarkers that can predict the mortality caused by severe sepsis, in spite of successful initial intensive resuscitation.<sup>6-9</sup>

Plasma PTX3 has been reported to be higher in patients with severe sepsis of high severity compared with healthy controls and patients with low severity sepsis.<sup>10-13</sup> It has been suggested to be a good predictive marker of mortality in sepsis, because it may reflect tissue involvement by inflammatory processes more directly.<sup>1,14</sup> However, there have been few studies to evaluate novel prognostic biomarkers including PTX3 to predict mortality in patients with severe sepsis receiving initial intensive resuscitation. The aim of this study, therefore, was to evaluate the relationship between PTX3 and poor outcome, and also to explore the role of PTX3 as a predictive biomarker of 28-day all-cause mortality in severe sepsis patients who received successful early goal-directed therapy (EGDT) and initial resuscitation according to Surviving Sepsis Campaign.<sup>6,15</sup>

## MATERIALS AND METHODS

### Study subjects and design

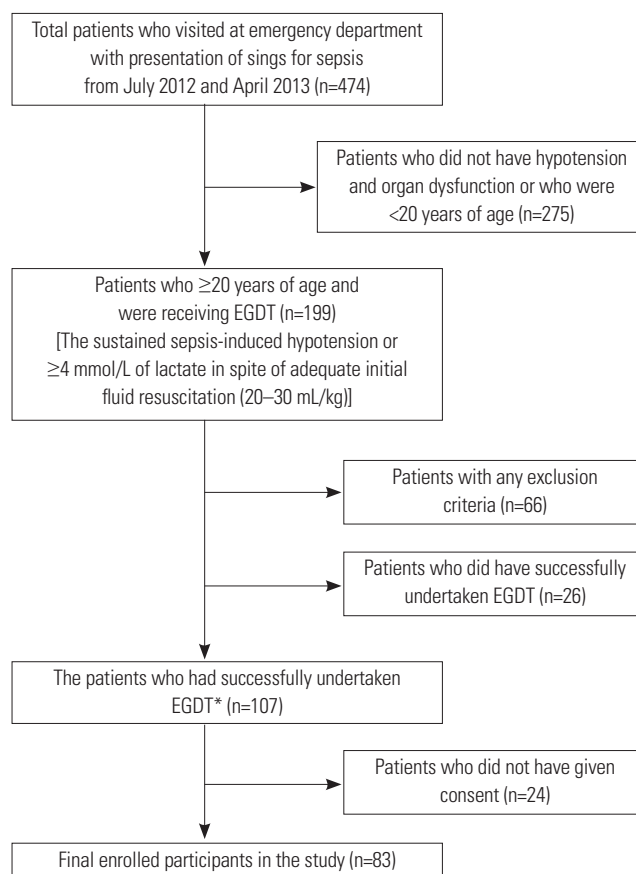
A prospective cohort study was conducted at Severance Hospital, a 2000-bed, tertiary-care, University-affiliated referral center located in Seoul, Republic of Korea. Total 474 patients visited at emergency department (ED) with presentation of the defined criteria for systemic inflammatory response syndrome (SIRS) by infection from July 2012 and April 2013. The 199 patients were eligible if they were  $\geq 20$  years of age and had the sustained sepsis-induced hypotension or  $\geq 4$  mmol/L of lactate in spite of adequate initial fluid resuscitation (20–30 mL/kg) with crystalloid<sup>6,15,16</sup> as well as were receiving EGDT. We excluded 66 patients based on the following criteria: 1) pregnancy, 2) acute cerebrovascular accident, 3) acute coronary syndrome, 4) contraindication to central venous catheterization, 5) active gastrointestinal bleeding, 6) trauma, 7) drug overdose, 8) requirement of immediate surgery, and 9) do-not-resuscitate status. We prospectively enrolled 107 patients who had successfully undertaken EGDT, which was defined as the fulfillment of all EGDT components and achievement of the final goal of superior vena cava oxygen saturation ( $S_{cv}O_2$ )  $\geq 70\%$  within 6 hrs after arrival at the ED, as well as faithfully implemented EGDT and initial resuscitation of Surviving Sepsis Campaign international guidelines for management of severe sepsis and septic shock according to the recent update version at enrollment.<sup>6,15,17</sup> We finally included total 83 participants in this prospective longitudinal study (Fig. 1). All participants were fulfilled with the definition for severe sepsis and septic shock.<sup>6,15,16</sup>

The primary outcome of this study was the 28-day all-cause

mortality. Written informed consents were obtained from all patients before study enrollment. This study was approved by our local Ethic Committee of Institutional Review Board.

### Data collection

The clinical characteristics and laboratory data of study participants were collected from electronic medical records. The clinical characteristics included age, gender, body mass index (BMI), primary infectious origin, underlying comorbidities, Charlson index,<sup>18</sup> Sepsis-related Organ Failure Assessment (SOFA) score<sup>19</sup> assessed upon arrival at the ED, acute renal / liver failure, bacteremia, methods for treatment of severe sepsis and appropriate initial empirical antibiotic use within 2 hrs from the arrival at the ED and the alive status on hospital day (HD) 0, 3, 7, 14, and 28. Acute Physiology and Chronic Health Evaluation II (APACHE II) score was assessed based on the worst values over the first 24 hrs of intensive care unit (ICU) care.<sup>20</sup> Laboratory data included plasma procalcitonin (PCT; ng/mL), serum CRP (mg/L) and delta neutrophil index (DNI;



**Fig. 1.** The flow chart on the enrollment processes for study participants. \*This was defined as the fulfillment of all EGDT components and achievement of the final goal of superior vena cava oxygen saturation  $\geq 70\%$  within 6 hrs after arrival at the emergency department, as well as faithfully implemented EGDT and initial resuscitation of Surviving Sepsis Campaign international guidelines for management of severe sepsis and septic shock according to the recent update version at enrollment. EGDT, early goal-directed therapy.

%) measured at HD 0 (n=83), 3 (n=78), 7 (n=76), and 14 (n=70) from all alive patients at each HD. As the components of EGDT, the central venous pressure (CVP), mean arterial blood pressure (MAP) and  $S_{cv}O_2$  values were measured and collected upon arrival at the ED (0 hr) and 2, 4, and 6 hrs upon arrival at the ED.

### Definitions

HD 0 was defined as the time within 24 hrs after arrival at the ED. The other HDs were exactly calculated from HD 0 and consistently applied at all data collections and measurements of plasma PTX3. The survivors were defined as patients who were alive at HD 28 and the non-survivors were defined as patients who were dead by HD 28, irrespective of direct cause of death. The patients with any of the following criteria due to the infection were defined as severe sepsis; 1) sepsis-induced hypotension of systolic blood pressure (SBP) <90 mm Hg or MAP <70 mm Hg or SBP decrease >40 mm Hg, 2) lactate >4 mmol/L, 3) urine output <0.5 mL/kg/hr for  $\geq 2$  hrs despite adequate fluid resuscitation, 4) acute lung injury, 5) creatinine (Cr) increase >0.5 mg/dL, 6) bilirubin >4 mg/dL, 7) platelet <100000/ $\mu$ L, and 8) international normalized ratio (INR) >1.5.<sup>6,15,16</sup> Septic shock was defined as sepsis-induced hypotension persisting despite adequate fluid resuscitation.<sup>6,15,16</sup> We used the definitions of SIRS and sepsis by 2001 International Sepsis Definitions Conference.<sup>16</sup> Bacteremia was confined into the clinically significant bacteremia, which was defined as  $\geq 1$  positive blood culture performed before the first administration of antimicrobial agents and clinical features compatible with sepsis.<sup>21</sup> Acute renal failure was diagnosed as an absolute increase in serum Cr of 0.3 mg/dL over 48 hrs or a 50% increase in Cr over 7 days according to Kidney Disease Improving Global Outcomes.<sup>22</sup> Acute liver failure referred to the development of severe acute liver failure with encephalopathy and coagulopathy (INR  $\geq 1.5$ ) in a patient without prior known liver disease within 26 wks of the onset of any hepatic symptoms.<sup>23</sup>

DNI is evaluated by subtracting the fraction of mature polymorphonuclear leukocytes from the sum of myeloperoxidase-reactive cells.<sup>24</sup> Administration of low-dose steroid was defined as the use of intravenous hydrocortisone at a dose of 200 mg per day.<sup>25</sup> The successful achievement of EGDT goals was defined as attaining all of the following hemodynamic endpoints within 6 hrs upon arrival at the ED; 1) CVP  $\geq 8$ –12 mm Hg (12–15 mm Hg in ventilated patients), 2) MAP  $\geq 65$  mm Hg, 3)  $S_{cv}O_2 \geq 70\%$ , and 4) urine output  $\geq 0.5$  mL/kg/hr.<sup>26</sup>

### The measurement of plasma pentraxin 3 levels

Samples for PTX3 measurement were obtained from all living patients on HD 0, 3, 7, and 14. The plasma was stored at  $-70^\circ\text{C}$  until analysis. PTX3 levels were measured by sandwiched ELISA, using a commercial kit of Human PTX3/TSG-14 Quantikine<sup>®</sup> Immunoassay (R&D Systems Inc., Minneapolis, MN, USA) with the detection limit of 0.1 ng/mL, according to the

manufacturer's instruction. We made the seven standard solutions (from 20 ng/mL to 0.313 ng/mL) by serial 1:2 dilution with zero standard (0 ng/mL) for standard curve. The optical densities were measured at 450 nm using the Spectramax<sup>®</sup> 190 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Each sample including standard solution was tested in triplicate and the mean values were used in analyses. Both intra and interassay coefficients of variation were <10%. The PTX3 does not cross-react with short pentraxin of CRP and SAP.

### Statistical analyses

Statistical analyses were performed using SPSS software, version 20 (SPSS Inc., Chicago, IL, USA) and a two-sided *p* value less than 0.05 was considered as statistically significant. All data for continuous variables were presented as median (interquartile range). Categorical variables were analyzed by  $\chi^2$  test or Fisher's exact test, and continuous variables were analyzed by Mann-Whitney U test. The correlation between two continuous variables was evaluated by Pearson's correlation coefficient (*r*). The differences in 28-day all-cause mortality by survival curve were compared using the log-rank test. The Cox's proportional hazard model was computed to evaluate the independent predictive effect of initial PTX3 at HD 0 on 28-day all-cause mortality and to find the independent predictive variables of 28-day all-cause mortality with the variables with *p* values less than 0.05 in univariate analyses. The accuracy and cut-off levels of initial PTX3, PCT, and DNI for predicting 28-day all-cause mortality were investigated using receiver operating characteristic (ROC) curves.<sup>27</sup> Area under the ROC curve (AUC)<sup>ROC</sup> was expressed with 95% confidence interval (CI) and *p* value in each ROC curve.

## RESULTS

### The characteristics of study subjects

For the 83 enrolled subjects, the 28-day all-cause mortality was 19.3%. The number of patients who died on HD 3, 7, 14, and 28 was 5, 7, 13, and 16, respectively. The survivors (n=67) and non-survivors (n=16) were similar in age, gender, BMI and Charlson index including the frequency of various underlying comorbidities.

The APACHE II score was significantly higher in the non-survivors than in the survivors [20 (15–29) vs. 15 (12–19), *p*=0.011]. The frequency of acute liver failure in the non-survivors was significantly higher than that in the survivors (68.8% vs. 7.5%, *p*<0.001), but acute renal failure occurred at a similar frequency in the two groups. The most common primary infectious origin of severe sepsis was the urinary tract and lung in the survivors (38.8%) and non-survivors (50%), respectively. The frequency of initial bacteremia concomitance was also not significantly different between the two groups (56.3% vs. 47.8%, *p*=0.588). Among the treatment modalities of severe sepsis according to

the Surviving Sepsis Campaign updated in 2008 and 2012,<sup>6,15</sup> mechanical ventilation and vasopressin use were significantly more frequent in the non-survivors than with the survivors

(37.5% vs. 10.4%,  $p=0.016$  and 31.3% vs. 4.5%,  $p=0.006$ ). However, the frequencies of continuous renal replacement therapy, low-dose steroid treatment, norepinephrine use, and adminis-

**Table 1.** Comparison of Clinical Characteristics and Sepsis Treatment between Survivors and Non-Survivors

Characteristics	Total (n=83)	Survivors (n=67)	Non-survivors (n=16)	p value
Age	71 (64–77)	71 (64–77)	70 (63–76)	0.599
Male (%)	47 (56.6)	37 (55.2)	10 (62.5)	0.598
BMI (kg/m <sup>2</sup> )	23 (20–26)	23 (20–25)	23 (21–26)	0.710
Charlson index	3 (2–4)	3 (2–4)	4 (3–5)	0.106
Underlying comorbidities				
Diabetes mellitus	28 (33.7)	23 (34.3)	5 (31.3)	0.815
Chronic kidney disease	12 (14.5)	8 (11.9)	4 (25.0)	0.233
Cardiovascular disease*	18 (21.7)	14 (20.9)	4 (25.0)	0.741
Cerebrovascular disease	19 (22.9)	17 (25.4)	2 (12.5)	0.340
Solid cancer	27 (32.5)	20 (29.9)	7 (43.8)	0.286
SOFA score	8 (6–9)	8 (6–9)	8 (6–10)	0.490
APACHE II score	16 (13–19)	15 (12–19)	20 (15–29)	0.011
Acute kidney failure	42 (50.6)	34 (50.7)	8 (50.0)	0.100
Acute liver failure	16 (19.3)	5 (7.5)	11 (68.8)	<0.001
Primary infectious origin <sup>†</sup>				
Urinary tract	30 (36.1)	26 (38.8)	4 (25.0)	0.392
Respiratory	29 (34.9)	21 (31.3)	8 (50.0)	0.242
Intra-abdomen	27 (32.5)	23 (34.3)	4 (25.0)	0.564
Bacteremia	41 (49.4)	32 (47.8)	9 (56.3)	0.588
Treatment of severe sepsis				
CRRT	13 (15.7)	8 (11.9)	5 (31.3)	0.117
Mechanical ventilation	13 (15.7)	7 (10.4)	6 (37.5)	0.016
Low-dose steroid	42 (50.6)	31 (46.3)	11 (68.8)	0.164
Norepinephrine	69 (83.1)	56 (83.6)	13 (81.3)	1.000
Vasopressin	8 (9.6)	3 (4.5)	5 (31.3)	0.006
Appropriate empirical antibiotics use within 2 hr	73 (88.0)	58 (86.6)	15 (93.8)	0.678
Components of EGDT				
CVP (mm Hg)				
0 hr	6 (4–10)	6 (3–9)	6 (4–11)	0.491
2 hr	7 (5–10)	7 (5–9)	7 (4–11)	0.771
4 hr	8 (5–11)	9 (5–11)	8 (5–11)	0.364
6 hr	9 (6–12)	10 (6–12)	9 (5–11)	0.694
MAP (mm Hg)				
0 hr	59 (50–69)	57 (50–68)	60 (56–71)	0.339
2 hr	71 (62–81)	71 (62–79)	68 (61–85)	0.798
4 hr	82 (75–91)	83 (75–92)	78 (67–85)	0.095
6 hr	85 (78–93)	87 (79–98)	77 (64–87)	0.009
S <sub>cv</sub> O <sub>2</sub> (%)				
0 hr	66 (58–72)	65 (59–71)	69 (54–76)	0.244
2 hr	68 (61–73)	67 (62–71)	71 (61–74)	0.371
4 hr	73 (61–78)	72 (60–77)	77 (64–86)	0.289
6 hr	79 (71–87)	77 (69–84)	83 (75–89)	0.105
Total hospital stay (days)	16 (8–24)	17 (11–29)	8 (3–13)	<0.001

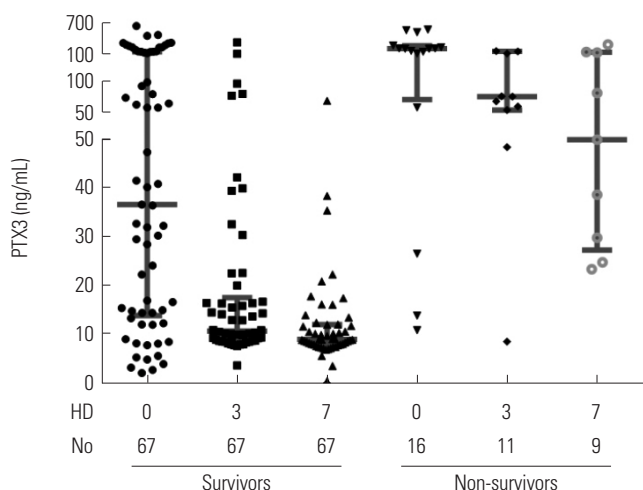
BMI, body mass index; SOFA, sequential organ failure assessment; APACHE, acute physiology and chronic health evaluation; CRRT, continuous renal replacement therapy; EGDT, early goal-directed therapy; CVP, central venous pressure; MAP, mean arterial pressure; S<sub>cv</sub>O<sub>2</sub>, superior vena cava oxygenation saturation. Values are presented as the median (interquartile range) or number (percent). SOFA score was assessed upon arrival at the emergency department and APACHE II score was assessed based on the worst values over the first 24 hrs of intensive care unit care.

\*Except essential hypertension, <sup>†</sup>Multiple selection was possible in one patient.

tration of the appropriate initial empirical antibiotics within 2 hrs upon arrival at the ED were not different between the two groups. The detailed components of EGDT at 0, 2, 4, and 6 hrs were similar between the two groups, except for the MAP level at 6 hrs (Table 1).

**Change of inflammatory markers at various hospital days**

The levels of PTX3, PCT, CRP, and DNI continually decreased with time for all study subjects. In particular, only PTX3 presented with a consistent and significant difference for HD 0, 3,



**Fig. 2.** Changing pattern of PTX3 levels at HD 0, 3, and 7 in survivors and non-survivors. The middle and upper/lower error bars mean the median and interquartile ranges, respectively. PTX3, pentraxin 3; HD, hospital day.

and 7 between the survivors and non-survivors. The non-survivors had a significantly higher PTX3 at HD 0, 3, 7, and 14 than the levels in the survivors [HD 0: 201.4 (56.9–268.6) ng/mL vs. 36.5 (13.7–145.3) ng/mL,  $p=0.008$ ; HD 3: 66.4 (49.3–146.1) ng/mL vs. 10.5 (8.5–17.3) ng/mL,  $p=0.002$ ; HD 7: 49.7 (25.8–163.6) ng/mL vs. 8.8 (7.7–11.8) ng/mL,  $p=0.003$ ] (Fig. 2, Table 2). Comparing with the 28-day all-cause mortality by quartile of PTX3 at HD 0, the mortality rates were higher in the groups with greater levels of PTX3 (Q1: 9.5%, Q2: 5.0%, Q3: 19.0%, Q4: 38.1%,  $p=0.024$ ). The PCT levels did not reveal any significant difference at all on HD 0, 3, and 7. The CRP levels showed significantly higher values in the non-survivors at only HD 7. The DNI values showed significantly higher levels in the non-survivors only at HD 0 (Table 2).

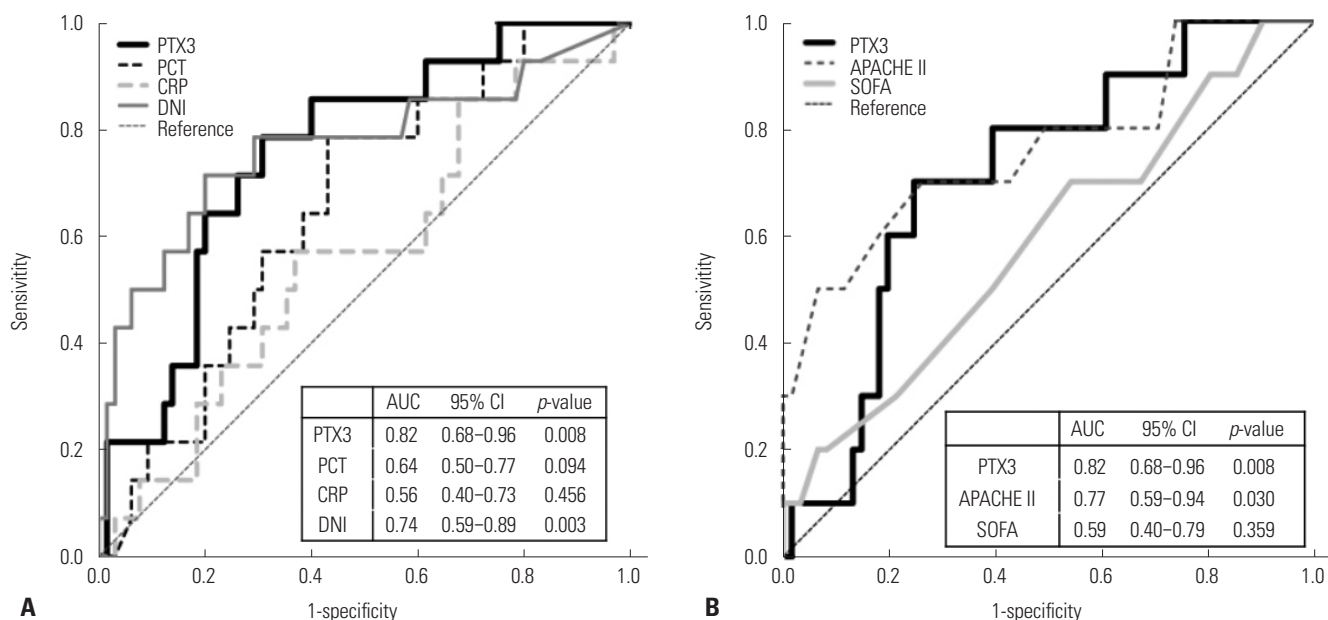
**The cut-off levels of various markers measured at HD 0 for the prediction of 28-day all-cause mortality**

For PTX3, PCT, DNI, and CRP at HD 0, the largest and smallest AUC<sup>ROC</sup> values for the prediction of 28-day all-cause mortality were for PTX3 and CRP, respectively (0.819, 95% CI 0.677–0.961,  $p=0.008$  and 0.564, 95% CI 0.400–0.728,  $p=0.456$ , respectively). The most valid cut-off level to predict the 28-day mortality of PTX3 at HD 0 was 140.28 ng/mL (sensitivity 66.7% and specificity 73.8%). In addition, the most appropriate cut-off points of DNI were 10.6% with the AUC<sup>ROC</sup> values of 0.739 (95% CI 0.591–0.887,  $p=0.003$ ) (sensitivity 68.8% and specificity 77.6%) (Fig. 3A). The AUC<sup>ROC</sup> values of APACHE II and SOFA score for the prediction of 28-day all-cause mortality (0.765 and 0.591, respectively) were smaller than PTX3 (Fig. 3B).

**Table 2.** Comparison of Inflammatory Markers Measured on Hospital day 0, 3, and 7 between Survivors and Non-Survivors

HD	Total	Survivors	Non-survivors	p value
<b>PTX3 (ng/mL)</b>				
0	51.6 (14.3–197.6)	36.5 (13.7–145.3)	201.4 (56.9–268.6)	0.008
3	12.7 (8.7–34.1)	10.5 (8.5–17.3)	66.4 (49.3–146.1)	0.002
7	8.9 (7.8–13.5)	8.8 (7.7–11.8)	49.7 (25.8–163.6)	0.003
<b>Procalcitonin (ng/mL)</b>				
0	9.9 (0.6–30.6)	7.8 (0.3–30.6)	17.4 (9.7–33.4)	0.114
3	8.2 (0.2–12.7)	5.2 (0.1–12.9)	4.5 (0.3–21.7)	0.909
7	0.6 (0.1–2.1)	0.6 (0.1–1.8)	1.6 (0.6–19.1)	0.113
<b>CRP (mg/L)</b>				
0	106.1 (42.3–174.2)	106.1 (35.4–169.2)	117.0 (55.6–201.5)	0.723
3	64.1 (29.6–156.7)	64.1 (27.7–156.7)	58.8 (45.9–236.1)	0.634
7	36.3 (12.7–76.9)	35.3 (12.3–70.7)	102.5 (47.0–172.6)	0.004
<b>DNI (%)</b>				
0	6.1 (1.9–13.6)	5.1 (1.3–10.0)	14.3 (5.8–50.1)	0.003
3	0.8 (0.0–2.9)	0.8 (0.0–2.1)	2.3 (0.4–4.1)	0.224
7	1.6 (0.1–3.0)	1.2 (0.0–3.0)	1.8 (1.7–4.7)	0.145

PTX3, pentraxin 3; CRP, C-reactive protein; DNI, delta neutrophil index; HD, hospital day. Values are presented as the median (interquartile range). The number of total participants who received the measurement of above four inflammatory markers were 83, 78, and 76 at HD 0, 3, and 7, respectively. In survivors, the inflammatory markers were measured in 67 patients at all HD 0, 3, and 7. In non-survivors, the number of patient who received the measurement of the inflammatory markers were 16, 11, and 9 at HD 0, 3, and 7, respectively.



**Fig. 3.** Comparison of ROC curves for inflammatory markers (A) and APACHE II/SOFA score (B) measured at hospital day 0 for the prediction of 28-day all-cause mortality. PTX3, delta neutrophil index, CRP and procalcitonin were measured at hospital day 0 within 24 hrs of arrival at the emergency department. SOFA score was assessed upon arrival at the emergency department and APACHE II score was assessed based on the worst values over the first 24 hrs of intensive care unit care. ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidential interval; PTX3, pentraxin 3; PCT, procalcitonin; CRP, C-reactive protein; DNI, delta neutrophil index; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment.

### Correlation of pentraxin 3 with various predictive markers according to hospital day

The PTX3 and PCT levels measured at HD 0 and 14 had a significant correlation ( $r=0.675$ ,  $p<0.001$  and  $r=0.757$ ,  $p<0.001$ ). However, there was no significant correlation between the PTX3 and PCT levels measured at HD 3 ( $r=-0.261$ ,  $p=0.437$ ). The significant correlation between PTX3 and CRP levels was observed for HD 0, 3, 7, and 14 with an increase in correlation coefficient with time (HD 14:  $r=0.496$ ,  $p=0.003$ ). There was a significantly strong correlation between PTX3 and DNI only on HD 0 and 3 (HD 0:  $r=0.507$ ,  $p<0.001$  and HD 3:  $r=0.911$ ,  $p<0.001$ ). The PTX3 measured at HD 0 and SOFA/APACHE II score assessed upon arrival at the ED/ICU had significant correlations with a low correlation coefficient (Table 3).

### Independent marker for the prediction of 28-day all-cause mortality

In Cox's proportional hazards model, the plasma PTX3 level measured at HD 0 was the only independent predictive marker for 28-day all-cause mortality in patients with severe sepsis who were receiving EGDT. The level of more than 140 ng/mL of PTX3 at HD 0 was correlated with a 7-fold increase in 28-day all-cause mortality [hazard rate (HR) 7.16, 95% CI 2.46–15.85,  $p=0.001$ ] (Table 4). In addition, the 28-day cumulative survival rate in patients with a less than 140 ng/mL level of PTX3 at HD 0 was significantly higher than in the patients with a level of PTX3 at HD 0 more than 140 ng/mL in Kaplan-Meier survival analysis ( $p=0.003$ ) (Fig. 4A). Among the 27 patients with a PTX3 level greater than 140 ng/mL at HD 0, 10 (37.0%) patients had

died by HD 28, but only 6 (10.7%) patients among the 56 patients with PTX3 level lower than 140 ng/mL at HD 0 were dead by HD 28. The percentage of subjects with PTX3 level greater than 140 ng/mL at HD 0 was significantly higher in the non-survivors than in the survivors [68.8% (11 of 16) vs. 23.9% (16 of 67),  $p=0.003$ ].

### Short-term change in plasma PTX3 level

We subtracted the PTX3 level at HD 3 from the PTX3 level at HD 0 to identify the effect of the short-term change in PTX3 level on mortality. These short-term changes in values were significantly lower in the survivors than in the non-survivors [-33.0 (-154.0–3.8) ng/mL vs. 84.9 (-5.4–259.3) ng/mL,  $p<0.001$ ] (Fig. 4B). The plasma PTX3 levels at HD 3 showed decreasing values compared to those at HD 0 in 55 of 67 (82.1%) patients in the survivors. On the other hand, 8 of 11 (72.7%) patients in the non-survivors had a short-term increase in PTX3 level. The 28-day cumulative survival rate was 80% (12 of 15) in patients with the short-term decrease in PTX3 level at HD 3 in spite of greater than 140 ng/mL level of PTX3 at HD 0. In addition, 11 of 12 (91.7%) patients with PTX3 less than 140 ng/mL at HD 0 were alive at HD 28 in spite of the short-term increase in PTX3 value at HD 3.

## DISCUSSION

Our present results suggest that the plasma PTX3 level measured within 24 hrs upon arrival at the ED could be a powerful

predictive biomarker for 28-day all-cause mortality in severe septic patients who have undertaken successful EGDT and initial resuscitation. The PTX3 level at HD 0 was the only independent marker associated with 28-day all-cause mortality in Cox's proportional hazards model. The patients with a PTX3 level greater than 140 ng/mL at HD 0 had a 7-fold HR, and the mortality of these patients was as high as 68.8% in spite of the achievement at final goal of EGDT.

The plasma PTX3 level was previously evaluated to identify the association with the severity and mortality or the predic-

**Table 3.** The Correlation of PTX3 with Other Inflammatory Markers Measured in All Participants Who were Alive at Each HD

HD	r	p value
PTX3 and procalcitonin		
0	0.675	<0.001
3	-0.261	0.437
7	0.343	0.010
14	0.757	<0.001
PTX3 and CRP		
0	0.253	0.024
3	0.268	0.044
7	0.308	0.022
14	0.496	0.003
PTX3 and DNI		
0	0.507	<0.001
3	0.911	<0.001
7	0.085	0.533
14	0.268	0.132
PTX3 and APACHE II score at HD 0	0.242	0.042
PTX3 at HD 0 and SOFA score	0.390	0.001

PTX3, pentraxin 3; CRP, C-reactive protein; DNI, delta neutrophil index; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; HD, hospital day.

r refers to the Pearson's correlation coefficient. SOFA score was assessed upon arrival at the emergency department and APACHE II score was assessed based on the worst values over the first 24 hrs of intensive care unit care. The number of patients at HD 0, 3, 7, and 14 were 83, 78, 76, and 70, respectively.

**Table 4.** Independent Predictive Factors for 28-Day All-Cause Mortality in Severe Sepsis Patients Receiving Successful Early-Goal Directed Therapy

Variables	HR	95% CIs	p value
Age ≥ 71 yrs	3.27	0.57–10.69	0.377
Male	1.19	0.29–2.31	0.958
APACHE II score ≥ 16 point	2.18	0.51–9.25	0.685
Mechanical ventilation	1.92	0.64–5.81	0.089
Acute liver injury	1.28	0.43–3.79	0.888
Vasopressin use	2.89	0.92–9.18	0.546
PTX3 at HD 0 ≥ 140 ng/mL	7.16	2.46–15.85	0.001
DNI at HD 0 ≥ 10.6%	1.23	0.13–9.03	0.217

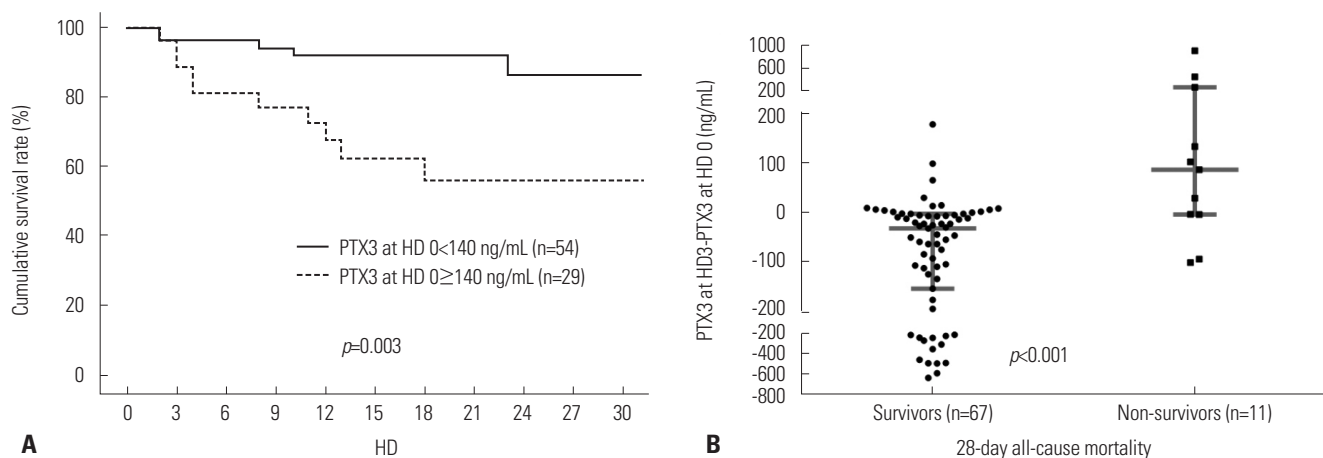
HR, hazard rate; CI, confidence interval; APACHE, acute physiology and chronic health evaluation; PTX3, pentraxin 3; DNI, delta neutrophil index; HD, hospital day.

tion of development of bacteremia or septic shock, mainly in heterogeneous populations, including SIRS and/or severe sepsis and/or critically ill or febrile neutropenic hematologic patients.<sup>11-13,28,29</sup> On the other hand, only a few studies on homogeneous infectious patients were performed to assess the role of PTX3 in severity or as a prognostic marker in patients with ventilator-associated pneumonia, community-acquired pneumonia, bacteremia, severe leptospirosis or severe meningococcal disease.<sup>30-34</sup> In spite of various infectious and/or inflammatory conditions, almost all the studies have shown that a higher level of PTX3 was related to severity or mortality, as indicated by our data. However, our study is unique because of our approach in assessing the role of serial PTX3 for the prediction of the mortality in the severe sepsis patients who have undertaken successful initial resuscitation.

Physicians worldwide fight desperately against severe sepsis through implementation of the Surviving Sepsis Campaign guidelines to improve survival rate.<sup>6</sup> The surviving sepsis campaign guidelines are currently a very important strategy for treating severe sepsis because many clinical trials using new therapeutic drugs have not resulted in an improvement in mortality.<sup>6</sup> The critical aspects of the Surviving Sepsis Campaign are initial resuscitation, which prevents the occurrence of multi-organ failure within 6 hrs of arrival at the ED and is mainly performed through the process of EGDT.<sup>6</sup> Therefore, the discovery of new prognostic markers and targeted therapy using new therapeutic drugs may be extremely important, especially in severe septic patients who are likely to die in spite of successful initial resuscitation. Considering such clinical importance, we explored whether PTX3 is an independent predictive marker of 28-day mortality in the severe septic patients who have undertaken successful EGDT.

The short-term change after HD 3 as well as initial PTX3 at HD 0 could be useful for predicting the 28-day mortality in specific conditions. These values were significantly higher in the non-survivors than in the survivors. Non-survivors had a high rate of short-term increase in PTX3, but almost all of the survivors had a decreasing pattern. The predictive role of initial PTX3 level at HD 0 for 28-day all-cause mortality was affected by short-term change after HD 3. The 28-day mortality was as low as 20% in patients with a decreased PTX3 after HD 3 in spite of PTX3 greater than 140 ng/mL at HD 0, which was the only independent predictive factor in Cox's regression model. Therefore, if we assess both the PTX3 level at HD 0 and short-term PTX3 change after HD 3, we could most likely obtain useful information to predict 28-day all-cause mortality in severe septic patients who have undertaken successful EGDT.

Our data showed a strong correlation between PTX3 and PCT at HD 0 and 14, which is a well-known diagnostic and predictive biomarker for severity and mortality in sepsis.<sup>35</sup> However, initial PCT level at HD 0 was not an independent predictive marker for 28-day mortality, contrary to common expectations. The AUC value of PCT at HD 0 was lowest among



**Fig. 4.** Kaplan-Meier survival curve according to PTX3 level on hospital day 0 with a cut-off point of 140 ng/mL (A) and comparison of short-term change in PTX3 level between hospital day 0 and 3 in both survivors and non-survivors (B). PTX3, pentraxin 3; HD, hospital day.

PTX3 and DNI. Furthermore, we observed in the detailed patient description that there were obviously patients for whom PTX3 were helpful for predicting mortality, while PCT was not predictive: the patients who died due to severe sepsis from pneumonia had a high initial PTX3 level and increasing PTX3 level at HD 7, irrespective of very low continuous PCT levels. In addition, the short-term change in PCT after HD 3 or 7 did not have predictive value for 28-day all-cause mortality. To our best knowledge, there have been no published study to assess the usefulness of PCT under EGDT or initial resuscitation according to surviving sepsis campaign guidelines. Thus, further studies are warranted to identify any specific sepsis characteristics, such as the primary source of sepsis or specific clinical infectious circumstances, to establish the clinical utility of PTX3 as a biomarker for severe sepsis.

In the present study, we also evaluated the predictive role of DNI for 28-day mortality in severe septic patients who had undertaken successful EGDT and compared these values with PTX3. DNI, which are easily obtained as part of complete blood cell analysis and differential count can be determined without additional tests or additional cost, are new biomarkers for the prediction of severity and prognosis in sepsis.<sup>36-39</sup> Nevertheless, there have been no published studies to evaluate these biomarkers in severe septic patients who have undertaken successful EGDT.

In addition to important characteristics and homogeneity of our study population, our present study has several strengths and is unique in its approach to evaluate the prognostic role of PTX3 for mortality in severe sepsis. It has consistently been reported that initially high plasma PTX3 levels are associated with mortality or fatality in patients with inflammation by infection, including severe sepsis.<sup>12,13,29,31</sup> In the present study, we found much higher overall mean or median PTX3 at HD 0 than in previously reported studies (122.0 ng/mL in our study, 7.8 ng/mL in bacteremia patients by Huttunen, et al.,<sup>31</sup> and 16.7 ng/mL in 49 severe sepsis patients by Uusitalo-Seppälä, et al.,<sup>12</sup>

71.3 ng/mL in patients admitted to an ICU with SIRS by Bastrup-Birk, et al.<sup>13</sup>). Our high overall initial PTX3 level was similar to 118 ng/mL in the subgroup analysis carried out by Bastrup-Birk, et al.<sup>13</sup> These findings, along with 19.3% of total 28-day all-cause mortality and 16.2 of total APACHE II score in our study, suggest that the severity of sepsis in our study population was as high as those in severe septic patients encountered during routine clinical practice, although a direct comparison was impossible because of the absence of an APACHE II score in other studies.

The cut-off point for the prediction of mortality was higher in our study than in previously reported studies (140 ng/mL in our study, 15 ng/mL by Huttunen, et al.,<sup>31</sup> 7.7 ng/mL by Uusitalo-Seppälä, et al.,<sup>12</sup> and 32.6 ng/mL by Bastrup-Birk, et al.<sup>13</sup>) The AUC<sup>ROC</sup> of PTX3 for the prediction of mortality in our study was as high as those in previously reported studies (0.819 in our study, 0.82 by Huttunen, et al.,<sup>31</sup> and 0.69 by Uusitalo-Seppälä, et al.<sup>12</sup>). Mauri, et al.<sup>29</sup> reported that the high PTX3 HD 5: HD 0 ratio may be associated with mortality in the 90 severe septic patients who were most similar to our study population among the studies for PTX3 and infection. In contrast to our study, however, the initial PTX3 levels in the study of Mauri, et al.<sup>29</sup> were not different between non-survivors and survivors.

The main weakness of our study is relatively small number of enrolled study participants. We prospectively enrolled patients with an accurate definition of severe sepsis and successful achievement of EGDT, which is the core strategy of initial intensive resuscitation in the surviving sepsis campaign guidelines, and we obtained the results to suggest the powerful predictive role of initial PTX3. Therefore, our results have significant clinical relevance, and further studies for PTX3 are warranted, especially in septic patients with high severity and mortality irrespective of initial intensive resuscitation. Most recently, Cunha, et al.<sup>40</sup> analyzed the association between the single-nucleotide polymorphisms in the PTX3 gene and the risk of invasive aspergillosis, and suggested an important role of PTX3 in critical



infectious diseases.

In conclusion, we found that the initial plasma PTX3 at HD 0 is a strong predictive biomarker for 28-day all-cause mortality of severe sepsis patients who have undergone successful EGDT. The PCT level at HD 0 has a strong correlation with PTX3, however, it can not independently predict mortality. These findings suggest that PTX3 could play an important prognostic role in severe septic patients who received successful implantation of initial resuscitation.

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