Plasma cell-free DNA and qSOFA score predict 7-day mortality in 481 emergency department bacteraemia patients

Juha Rannikko[ab*] MD, Tapio Seiskari[c] MD PhD, Reetta Huttunen[a] MD PhD, Iina Tarkiainen[b] BM, Juulia Jylhävä[d] MD PhD, Mikko Hurme[b] MD PhD, Jaana Syrjänen[a] MD PhD and Janne Aittoniemi[c] MD PhD

*Corresponding author

[a]Department of Internal Medicine, Tampere University Hospital, Box 2000, FI-33521 Tampere, Finland

[b]Faculty of Medicine and Life Sciences, University of Tampere, Box 100, FI-33014 University of Tampere, Finland

[c]Department of Clinical Microbiology, Fimlab Laboratories, Box 66, FI-33101 Tampere, Finland

[d]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden

Running headline up to 30 characters: cfDNA predicts 7-day mortality

Running headline up to 60 characters: cfDNA and qSOFA predict 7-day mortality

Abstract

Background

A few studies have shown that both quick Sequential Organ Failure Assessment (qSOFA) score and cell-free DNA (cfDNA) have potential use as a prognostic marker in patients with infection. We studied these two markers alone and in combination to identify those emergency department (ED) patients with the highest risk of death.

Methods

Plasma cfDNA level was studied on days 0 to 4 after admittance to the ED from 481 culture-positive bloodstream infection cases. The qSOFA score was evaluated retrospectively according to Sepsis-3 definitions. The primary outcome was death by day 7.

Results

CfDNA on day 0 was significantly higher in non-survivors than in survivors (2.02 µg/ml vs. 1.35 µg/ml, $p<0.001$). CfDNA level was high (>1.69 µg/ml) in 134 (28%) out of 481 cases and the qSOFA score was ≥2 in 128 (28%) out of 458 cases. High cfDNA and qSOFA score ≥2 had 70% and 77% sensitivity and 76% and 76% specificity in predicting death by day 7, respectively. High cfDNA alone had odds ratio (OR) of 7.7 (95% CI 3.9-15.3) and qSOFA score ≥2 OR of 11.6 (5.5-24.3), but their combination had OR of 20.3 (10.0-41.4) in predicting death by day 7 when compared with those with low cfDNA and qSOFA score <2. Among the five cases with the highest cfDNA levels, there were three patients with severe disseminated intravascular coagulation.

Conclusion

CfDNA and qSOFA score can be used independently to identify those bacteraemia patients at high risk of death, and combining these two markers gives additional advantage.

Key words: biomarker; sepsis; bacteraemia

Introduction

Cell-free plasma DNA (cfDNA), also called extracellular DNA, comprises fragments of DNA that are released from apoptotic and necrotic cells [1]. Elevated levels of cfDNA in blood have been found in various clinical conditions, such as cancer, trauma, stroke and sepsis [2-5]. A few studies have indicated that cfDNA may have value in predicting the mortality of sepsis patients in intensive care units (ICUs) [3, 6-11] and in emergency department (ED) patients [12, 13]. In recent years, cfDNA has also been found to have a role as a link between innate immunity and coagulation [14], and increased levels of cfDNA are found in patients with deep vein thrombosis [15]. To the best of our knowledge, no studies have evaluated cfDNA as a possible marker of disseminated intravascular coagulation (DIC).

The criteria for sepsis were recently changed, and the quick Sequential Organ Failure Assessment (qSOFA) score has been introduced [16]. The qSOFA score was not invented to replace systemic inflammatory response syndrome (SIRS), but instead to help identify patients with suspected infection outside ICUs who are likely to develop complications [17, 18]. The qSOFA score has been shown to be better than SIRS as a prognostic marker of in-hospital mortality in an ED setting [19].

In our earlier study, we evaluated 497 cases with blood culture-positive infection in the ED to establish how often death was related to sepsis and how often underlying diseases played a major role [20]. In the present study, we have measured plasma cfDNA in 481 of these blood culture-positive infection cases and evaluated the use of cfDNA as a prognostic marker in addition to the qSOFA score. We have also evaluated the possible association of high cfDNA to the clinical characteristics of bacteraemia. We used death by day 7 as a primary endpoint, since in our earlier study most of the sepsis-related deaths in patients with a prognosis of more than 6 months occurred early after admittance to the ED. Our secondary endpoint was death by day 28.

Methods

Tampere University Hospital is a tertiary hospital situated in the Pirkanmaa region of Finland with a catchment population of approximately 524 700 inhabitants. Blood cultures are routinely taken from patients with signs or symptoms of systemic infection. As described in our previous study [20], blood culture-positive cases in the specialised care ED of Tampere University Hospital were selected during the period March 1, 2012 to February 28, 2014. In the present study, all cases with an available plasma sample for cfDNA analysis were included. The clinical data of the patients were gathered retrospectively. The primary and secondary endpoints were death by day 7 and 28, respectively.

Blood cultures were collected in BacT/Alert Aerobic (FA Plus) and Anaerobic (FN Plus) blood culture bottles and placed in an automated microbial detection system BacT/Alert 3D (bioMérieux, Marcy l'Etoile, France). All culture-positive cases were selected by a clinical microbiologist. Cultures positive for coagulase-negative Staphylococcus, Propionibacterium, Bacillus, Micrococcus and Corynebacterium, with detection in a single blood culture bottle and without clinical relevance, were considered to be contaminants and were excluded. Also, positive samples were excluded if the first routine blood sample within the first 24 hours after the blood culture collection (day 0) was missed. This could be because the culture became positive later than 72 hours after admission.

The level of plasma cfDNA was analysed from blood samples from days 0 to 4. The day 0 sample was gathered mostly at the same time as the collection of the blood cultures, but samples gathered within 24 hours of the collection were included. Qubit dsDNA HS Assay Kit and Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA) were used for quantification of plasma cfDNA. The manufacturer's instructions and protocols were followed at each step. After plasma extraction, the turnaround time spent on the laboratory analysis of cfDNA level was approximately 10 minutes. The intra-assay coefficients of variation from control samples were between 3.55% and 5.73%. The final result was the average of the two samples.

Diagnoses of sepsis, severe sepsis and septic shock were made according to Sepsis-2 consensus definitions [21]. A criterion for qSOFA score was calculated based on Sepsis-3 definitions [16]. The

qSOFA score comprises the following criteria: altered mentation, respiratory rate ≥22 and systolic blood pressure ≤100 mmHg. In this study, altered mentation was classified based on the verbal section of the Glasgow Coma Scale: if the patient had less than 5 points in this section, the patient was considered to have altered mentation. The Pitt Bacteraemia Score was calculated as presented by Korvick et al. [22].

IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. A p-value of <0.05 was considered significant. Nonparametric data were analysed by Mann-Whitney U Test or Kruskall Wallis test when appropriate. The Youden index was used to select optimal cut-off analysis. Predictive performance (accuracy) of the cfDNA level and qSOFA score was assessed using the AUC/ROC statistic. A logistic regression model was used to study the independent effect of high cfDNA level on mortality models adjusted for potential confounders. The survival curve was assessed using the Kaplan-Meier method, and survival differences between groups were compared by log-rank test. The study was approved by the Ethics Committee of Tampere University Hospital and the National Supervisory Authority for Welfare and Health. The need for informed consent was waived as no additional blood sampling was needed and routine patient care was not modified.

Results

There were 800 consecutive positive blood cultures during the study period. Contaminants (n=136) were excluded and 167 blood cultures were excluded for missing the blood sample from day 0. The first sixteen of the gathered samples went missing later. A total of 481 cases among 469 patients were thus included. There were 253 (53%) male cases, 228 (47%) female cases, all were adults (>16 years), and the median age was 68 years. Table 1 provides information on patient characteristics, clinical presentation and microbiology of the infection.

The minimum cfDNA level on day 0 to day 4 was 0.72 µg/ml, and the maximum was 66.95 µg/ml. The mean level on day 0 was 1.73 µg/ml, and the median was 1.38 µg/ml. Table 2 and Figure 1 show levels of cfDNA during days 0 to 4 in all cases and in relation to death by day 7. CfDNA day 0 levels in the non-survivors were significantly higher compared with survivors. Furthermore, levels remained at a significantly higher level on day 1 to day 4.

The optimal cut-off value for the plasma cfDNA values on day 0 in predicting death by day 7 was estimated using ROC curve, as illustrated in Figure 2. CfDNA had an AUC of 0.77 (95%CI 0.69 to 0.85) in predicting death by day 7. A cut-off value of 1.69 µg/ml had sensitivity of 70.4% and specificity of 76.4% in predicting death by day 7 with an AUC of 0.73 (95% Confidence Interval (CI) 0.65 to 0.82, $p<0.001$). Plasma cfDNA levels above this cut-off value are hereafter referred to as high and levels below this value are referred to as low. CfDNA level was high in 134 (28%) out of 481 cases, and qSOFA score was ≥2 in 128 (28%) out of 458 cases. A comparison of high cfDNA and qSOFA score ≥2 in relation to death by day 7 is given in Table 3. QSOFA score ≥2 together with high cfDNA level on day 0 resulted in a 20-fold risk of death by day 7, when compared with those with low cfDNA level and qSOFA score <2. Odds ratios of death by day 7 were 7.7 (95% CI 3.9 to 15.3) and 11.6 (95% CI 5.5 to 24.3) for high vs. low cfDNA and qSOFA score ≥2 vs. qSOFA score <2, respectively (Table 3). Figure 3 presents the Kaplan-Meier survival curves by day 28 for high cfDNA, qSOFA score ≥2 and both.

Death by day 28 was a secondary endpoint. CfDNA on day 0 was significantly higher in non-survivors than in survivors (1.95 µg/ml vs. 1.33 µg/ml, p<0.001). CfDNA had an AUC of 0.76 (95%CI 0.69 to 0.82) in predicting death by day 28. The optimal cut-off value of cfDNA in predicting death by day 28 was the same as in death by day 7 (1.69 µg/ml). The results for the secondary endpoint (day 28) are shown in Table 3.

The maximum level of cfDNA at day 0 was 38.85 µg/ml and in this patient it remained between 29.50 µg/ml and 66.95 µg/ml on days 1 to 4. This patient suffered from a severe pneumococcal infection with DIC resulting in multiple amputations. Among the five highest cfDNA levels at day 0, there were two other patients with DIC caused by *Capnocytophaga canimorsus* sepsis that resulted in skin necrosis or amputations. Their cfDNA levels were 9.65 µg/ml and 8.92 µg/ml.

Median level of cfDNA at day 0 in cases with qSOFA score <2 was 1.34 µg/ml (Inter-Quartile Range (IQR) 1.16 µg/ml to 1.64 µg/ml). Ten patients with qSOFA score <2 died by day 7 with the median day 0 cfDNA level of 1.45 µg/ml (IQR 1.38 µg/ml to 1.90 µg/ml). The difference of day 0 cfDNA level between survivors and non-survivors in patients with qSOFA score <2 was statistically insignificant (p=0.10). C-reactive protein (CRP) level on day 0 did not have a significant predictive value for death by day 7 (AUC 0.52 (95% CI 0.43 to 0.60), p=0.677, data on 478 cases). In predicting transfer to ICU within 24 hours of admission, cfDNA level had an AUC of 0.70 (95% CI 0.61 to 0.80, p<0.001) and qSOFA score ≥2 0.72 (0.63 to 0.80, p<0.001).

Table 4 presents the day 0 plasma cfDNA levels stratified by various demographic features, underlying conditions and severity of sepsis. The level of cfDNA was statistically significantly higher in the following conditions: liver disease, alcohol abuse, septic shock, admitted from ED to ICU, Pitt Bacteraemia Score ≥4 and death by day 7 or day 28. The level of cfDNA was statistically significantly lower in patients with hematologic cancer.

Statistically significant variables associated with death by day 7 in univariate analysis were cardiovascular disease, liver disease, alcohol abuse, MRSA carriage, Pitt Bacteraemia Score ≥4, high cfDNA, qSOFA score ≥2 and both qSOFA score ≥2 and high cfDNA. The following variables were

not associated with death by day 7: sex, age over 60, age over 80, neurological disease, pulmonary disease, rheumatological disease, kidney disease, substance abuse (other than alcohol), metastatic tumour, hematological malignancy, ESBL carriage, gram stain result, E.coli urosepsis or CRP over 100 mg/l on the first day. The odds ratios of death by day 7 for high cfDNA, qSOFA score ≥2 and both adjusted for these variables were 7.7 (95% CI 3.9 to 15.3), 11.6 (95% CI 5.5 to 24.3) and 21.3(95% CI 10.5 to 43.5), respectively. In multivariable analysis, we included all statistically significantly associated variables in univariate analysis. QSOFA score ≥2, high cfDNA, qSOFA score ≥2 combined with high cfDNA, cardiovascular disease and Pitt Bacteraemia Score ≥4 remained significant in this model.

Discussion

The present study shows that qSOFA score and cfDNA identify patients with a high risk of death due to bacteraemia infection. In this study, 34 out of 128 (27%) patients with qSOFA score ≥2 in the ED had died by day 7. If the patients had both qSOFA score ≥2 and cfDNA of more than 1.69 µg/ml on day 0, 47% of them died. Thus, the combination of high cfDNA level and qSOFA score ≥2 elevated the risk of death by day 7 from 12-fold to 20-fold.

QSOFA has recently been shown to predict organ failure and in-hospital mortality in 1009 patients with suspected infection [23]. Yet, the authors recommended the use of further confirmatory test. Given the present results, the combination of qSOFA score ≥2 and high cfDNA does not improve sensitivity in predicting death by day 7, but it could be an option to further improve specificity. As an illustration, we suggest a two-step approach where qSOFA score ≥2 is followed by cfDNA analysis: in the present material comprising 458 sepsis cases, qSOFA score ≥2 results in 128 cases at higher risk of death due to bacteraemia infection during the first week. Analysing the cfDNA of these 128 exposes 57 cases with even higher 7-day mortality of 47%. Moreover, the turnaround time spent on laboratory analysis of cfDNA level was approximately 10 minutes (after plasma extraction). This does not make turnaround time a significant obstacle for clinical use, but due to the need for plasma extraction, no point-of-care test is yet available

The Sepsis-3 task force did not find relevant change in predictive validity when lactate was added to qSOFA [24]. Clinicians will continue measuring lactate as the result is a numerical value rather than just the value of positive or negative. Especially high levels of lactate are seen in patients with severe infections, and the kinetics of lactate have been shown to have value in the prognosis of sepsis patients [25]. In this study, especially high levels of cfDNA were seen in patients with DIC that resulted in amputations. This is not surprising since a growing body of evidence suggests a role for cfDNA and DNA-binding proteins in the pathogenesis of DIC [14]. Because there were only few patients with DIC in our material, further studies are needed to establish cfDNA as an early marker for DIC.

Nevertheless, this finding also suggests that cfDNA could be an option to identify those patients with severe consequences of sepsis.

The AUC of cfDNA in predicting death by day 7 and day 28 were 0.77 and 0.76, respectively. These are somewhat lower than in two studies done in ED with soluble urokinase-type plasminogen activator receptor (suPAR) [26, 27]. These studies had an AUC of 0.79 and 0.84 in predicting death by day 30. When using their cut-off values, suPAR seems to be more sensitive in predicting death (76% and 83% vs. 65%) and cfDNA has a slightly better specificity (69% and 76% vs. 78%). In another cfDNA study done by our group, these predictors are more or less the same as in the suPAR studies (AUC 0.84, sensitivity 83% and specificity 76%) [12]. As the biochemical mechanism of these two markers differs greatly, it is possible that these markers perform differently depending on the subgroups within septic patients. Cases with suspected DIC might be one subgroup where cfDNA could be of help.

This study has some limitations. Our study was a single-centre tertiary hospital study comprising mostly community-onset infections. Furthermore, our material comprises only culture-positive cases, so one has to be cautious when extrapolating these results to culture-negative cases with infection. In addition, 167 of the culture-positive cases were excluded as the routine blood sample from day 0 was missed. However, the microbiological data of these missed cases were analysed and we did not find any differences that would have altered the findings of this study [20].

In line with previous studies [28-30], the results of the present study show that CRP-level has no value in predicting fatality. A high level of CRP (or leucocytes) is still an important sign for clinicians; at least they have to think could the patient have an infection or sepsis. When sepsis is suspected, a clinician in the ED has to decide, for example, whether the patient needs care in the ICU. A recent study suggested that only clinical impression of condition might be used to make this decision [31], but occasionally clinicians might need some extra help in making these decisions. Therefore, counting qSOFA score and measuring the level of cfDNA might help the decision-making process.

In conclusion, the present study shows that the combined information on qSOFA score and cfDNA can identify patients with the highest risk of death due to bacteraemia infection. These markers may find those patients admitted to ED with the highest need of care.

Potential conflicts of interest:

Juha Rannikko: No conflict

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Corresponding author: Juha Rannikko, MD, Department of Internal Medicine, Tampere University Hospital, Box 2000, FI-33521 Tampere, Finland. Tell: +358-50-5832249, Fax: +358-3-31164333, e-mail: juha.rannikko@gmail.com

Table 1. Patient characteristics, clinical presentation and microbiological data of the study population

		Data
Demographic		
	Cases/Patients	481/469
	Patients admitted twice/three times	10/1
	Gender (female/male))	228/253
	Median age, y (range)	68 (16-95)
Chronic medical condition		**n (%)**
	Coronary artery disease, chronic vascular disease or chronic heart failure	155 (32)
	Diabetes mellitus	137 (29)
	Social or medical problems of alcohol abuse	49 (10)
	Solid tumour with metastasis	55 (11)
	Hematological malignancy	45 (9)
Severity of the sepsis		**n (%)**
	Sepsis	481 (100)
	Severe sepsis (≥1 organ failure)	145 (30)
	Septic shock	36 (8)
	qSOFA score ≥2[1]	128 (28)
	Case transferred from ED to ICU	44 (9)
Site of infection		**n (%)**
	Urinary	134 (28)
	Intra-abdominal	83 (17)
	Skin, soft tissue and bones	71 (15)
	Pulmonary	48 (10)
	Unknown	116 (24)
Causative organism		**n (%)**
Gram-positive		213 (44)
	Staphylococcus aureus	71 (15)
	Streptococcus pneumonia	46 (11)
Gram-negative		219 (46)
	E. coli	156 (32)

[1] Data available on 458 cases

Others		46 (10)
	Anaerobes	15 (3)
	Fungi	1 (0.2)
	Polymicrobial	30 (6)
Day of case fatality		**n (%)**
	Days 0 to 7	44 (9)
	Days 0 to 28	69 (14)
	Days 0 to 90	97 (20)

qSOFA, quick Sepsis-related Organ Failure Assessment; ED, Emergency Department; ICU, Intensive Care Unit

Table 2. Plasma cell free DNA (cfDNA) levels during days 0 to 4 after admission to emergency department in all cases and in relation to death by day 7

Days after admission	**Plasma cfDNA (µg/ml), median (quartiles)**			**p-values**
	All	**Non-survivors**	**Survivors**	
Day 0	1.38 (1.20-1.77)	2.02 (1.51-2.92)	1.35 (1.19-1.66)	<0.001
Day 1	1.36 (1.18-1.73)	2.20 (1.57-3.63)	1.33 (1.17-1.64)	<0.001
Day 2	1.35 (1.16-1.65)	1.87 (1.59-2.92)	1.33 (1.16-1.61)	<0.001
Day 3	1.33 (1.16-1.61)	1.81 (1.48-4.34)	1.32 (1.14-1.58)	<0.001
Day 4	1.35 (1.17-1.71)	1.66 (1.49-2.38)	1.33 (1.15-1.68)	0.017
Maximum value	1.49 (1.28-1.91)	2.24 (1.56-4.55)	1.45 (1.27-1.81)	<0.001

cfDNA values available on 481 patients on day 0, 446 patients on day 1, 389 patients on day 2, 300 patients on day 3, 137 patients on day 4 and 481 patients on days 0 to 4 (maximum value).

Table 3. Diagnostic values of day 0 cfDNA with cut-off value of 1.69 µg/ml, qSOFA score ≥2 and both qSOFA score ≥2 and cfDNA>1.69 µg/ml in predicting death by day 7 and day 28.

	n (deceased/ survivors)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Odds Ratio with 95%CI	AUC with 95%CI	p-values
Death by day 7								
cfDNA >1.69 µg/ml	134 (31/103)	70.4	76.4	23.1	96.2	7.7 (3.9-15.3)	0.73[1] (0.65-0.82)	<0.001
qSOFA score ≥2[2]	128 (34/94)	77.3	77.3	26.6	97.0	11.6 (5.5-24.3)	0.77 (0.70-0.85)	<0.001
cfDNA >1.69 µg/ml and qSOFA score ≥2[2]	57 (27/30)	61.4	92.8	47.4	95.8	20.3 (10.0-41.4)	0.77 (0.68-0.86)	<0.001
Death by day 28								
cfDNA >1.69 µg/ml	134 (45/89)	65.2	78.4	33.6	93.1	6.8 (3.9-11.8)	0.72[1] (0.65-0.79)	<0.001
qSOFA score ≥2[2]	128 (45/83)	66.2	78.7	35.2	93.0	7.2 (4.1-12.6)	0.72 (0.66-0.79)	<0.001
cfDNA >1.69 µg/ml and qSOFA score ≥2[2]	57 (35/22)	51.5	94.3	61.4	91.8	17.7 (9.3-33.7)	0.73 (0.65-0.81)	<0.001

[1] See results for the AUC of cfDNA as a continuous variable
[2] Data available on 458 cases

Table 4. Day of admission plasma cell free DNA (cfDNA) values stratified by various demographic features, underlying conditions and severity of sepsis.

	Plasma cfDNA (ug/ml) on day of admission to emergency department		**p-value**
	factor present, median (quartiles)	**factor absent, median (quartiles)**	
Characteristics and underlying conditions			
Male	1.42 (1.24-1.85)	1.30 (1.16-1.64)	0.002
Age over 60 years	1.39 (1.22-1.80)	1.32 (1.16-1.60)	0.035
Age over 80 years	1.46 (1.25-1.89)	1.35 (1.18-1.69)	0.020
Cardiovascular disease	1.40 (1.22-1.75)	1.36 (1.19-1.82)	0.822
Diabetes any type	1.38 (1.18-1.70)	1.36 (1.20-1.77)	0.894
Chronic kidney disease[1]	1.38 (1.26-1.73)	1.36 (1.19-1.77)	0.561
Liver disease	1.91 (1.41-2.15)	1.35 (1.19-1.67)	<0.001
Alcohol abuse[2]	1.95 (1.47-2.38)	1.34 (1.18-1.64)	<0.001
Solid tumour with metastasis	1.51 (1.20-1.97)	1.36 (1.19-1.71)	0.142
Hematologic cancer	1.27 (1.12-1.68)	1.37 (1.22-1.76)	0.041
Gram-positive bacteraemia[3]	1.35 (1.17-1.67)	1.38 (1.23-1.80)	0.180
Polymicrobial infection	1.58 (1.25-2.13)	1.36 (1.19-1.75)	0.058
Severity of sepsis			
qSOFA score ≥2[4]	1.56 (1.26-2.20)	1.34 (1.16-1.64)	<0.001
Severe sepsis	1.61 (1.31-2.23)	1.31 (1.17-1.60)	<0.001
Septic shock	1.96 (1.32-2.83)	1.36 (1.19-1.70)	<0.001

[1] History of creatinine more than 120 µmol/l
[2] Social or medical problems of alcohol abuse in the past 12 months
[3] Excluding polymicrobial (n=30) and anaerobical (n=15) bacteraemia
[4] Data available on 458 cases

Admitted from ED to ICU	1.95 (1.40-2.89)	1.35 (1.19-1.69)	<0.001
Pitt Bacteraemia Score ≥4[5]	1.83 (1.34-2.73)	1.35 (1.19-1.68)	<0.001
Death by day 7	2.02 (1.51-2.92)	1.35 (1.19-1.66)	<0.001
Death by day 28	1.95 (1.50-2.58)	1.33 (1.18-1.64)	<0.001

Abbreviations: ED, emergency department; ICU, intensive care unit

[5] Data available on 474 cases

Figure 1. A line plot diagram showing cell free DNA (cfDNA) levels during days 0 to 4 after admission to emergency department in 44 patients who died by day 7 (black plots) and 437 survivors (open plots). One case with all cfDNA levels over 30 µg/ml excluded from the figure. *Cutoff, cfDNA value of 1.69 µg/ml had an optimal sensitivity of 70.4% and specificity of 76.4% in predicting day 7 mortality from the day 0 sample based on the AUC-ROC-analysis.

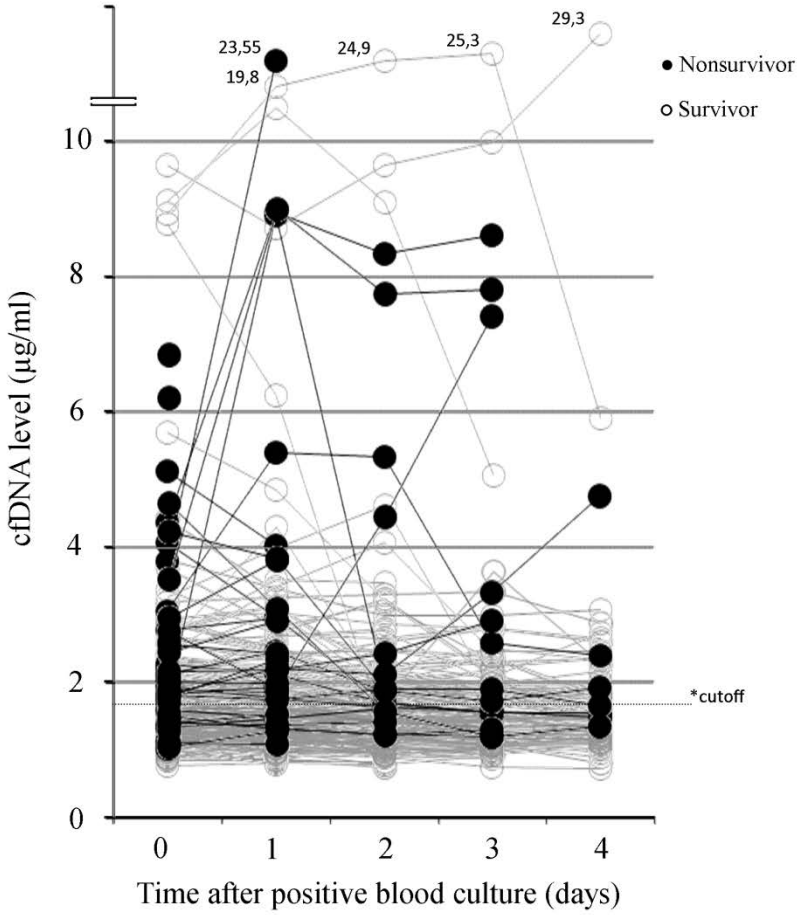

Figure 2. Receiver operating characteristic (ROC) curve on plasma cell-free DNA level, qSOFA score ≥2 and both cfDNA>1.69 µg/ml and qSOFA score ≥2 measured on day of admission to the Emergency Department in relation to case fatality by day 7.

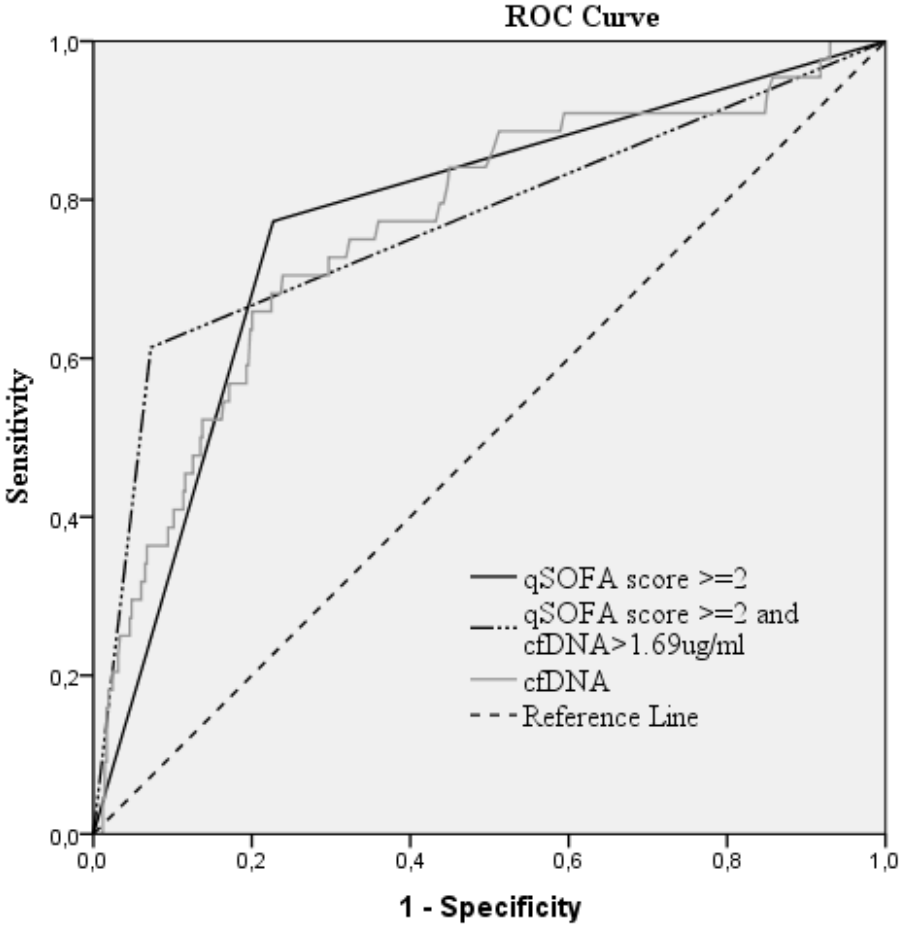

Figure 3. Cumulative day 28 survival in culture-positive cases with **A)** maximum plasma cell free DNA (cfDNA) > 1.69 µg/ml compared with those with cfDNA ≤ 1.69 µg/ml, **B)** qSOFA score ≥2 compared with those with qSOFA score <2 and **C)** cfDNA >1.69 and qSOFA score ≥2 compared with those with cfDNA≤ 1.69 µg/ml and qSOFA<2. The survival curve was calculated using the Kaplan-Meier method, and survival differences between groups were compared by log-rank test.