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Can plasma-free DNA concentration be a diagnostic tool in critically ill septic patients?

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Aim: To investigate the diagnostic value of plasma-free DNA concentration (PF-DNA) in septic patients compared to nonseptic patients and to correlate it with clinical outcome.

Materials and methods: Forty-two mechanically ventilated consecutive patients (11 septic, 31 nonseptic) admitted to the intensive care unit (ICU) were recruited. On admission, besides PF-DNA concentration, APACHE II and SOFA scores and serum C-reactive protein (CRP), procalcitonin (PCT), and serum lipid concentrations, together with clinical outcome, were assessed. Plasma samples from 11 volunteers were also collected. Appropriate statistics were used for analysis.

Results: Median PF-DNA concentrations on admission were significantly higher in septic patients compared to nonseptic patients [14,285 (48–180,311) GE/mL versus 546.5 (0–7674) GE/mL, $P < 0.0001$]. For distinguishing septic and nonseptic patients on admission, the area under the curve obtained for PF-DNA concentration was 0.9 (sensitivity 84%, specificity 95%; cutoff 4083 GE/mL). There were no significant differences between PF-DNA concentrations of nonsurvivors and survivors. Additionally, DNA concentration demonstrated a significant correlation with CRP, PCT, and high-density lipoprotein concentrations.

Conclusion: Plasma DNA seems to be a potentially valuable tool to confirm sepsis diagnosis upon ICU admission.

Key words: Plasma-free DNA, critical illness, sepsis, diagnosis

1. Introduction

Despite increasing knowledge regarding their pathophysiology as well as new therapeutic strategies and new-generation antibiotics, sepsis and septic shock remain the leading causes of death in critically ill patients (1,2). The overall mortality rate associated with severe sepsis is still as high as 30% to 50% (3). Mortality rate and outcome are closely related to early diagnosis and treatment, along with meticulous follow-up. Thus, early and correct prediction of sepsis and its outcome are important factors in modern health management. Several biomarkers, such as interleukins, procalcitonin (PCT), C-reactive protein (CRP), fibronectin, and haptoglobin, and some scoring systems have been evaluated to predict prognosis in patients with sepsis and its sequel, but none have proved entirely useful (4).

Nevertheless, data retrieved on nucleic acids as a possible noninvasive diagnostic and prognostic biomarker have opened up a new research interest for several clinical conditions (5). Recently, increased concentration of plasma-free plasma DNA (PF-DNA) has been shown in various clinical situations including cancer, trauma, burns, myocardial infarction, acute stroke, and maternal blood (6–11). Accordingly, current studies have focused on PF-DNA concentration as a tool for diagnosis and follow-up of these situations, and as a prognostic marker for outcome as well as prenatal genetic diagnosis (6–9,11). However, its predictive value is arguable for these circumstances.

Theoretically, PF-DNA can be defined as acellular DNA fragments detectable in the extracellular fluid. Very low levels are present in normal, healthy populations. It has been suggested that DNA enters into the circulation following cell death, which can be as a result of cell necrosis or apoptosis, although the exact mechanism is yet not clear (8,12). Circulating DNA has a short half-life and is removed mainly by the liver. Accumulation of DNA in the circulation can result from either an excessive release of DNA caused by massive cell death or inefficient removal, or a combination of both.

Sepsis is associated with cell necrosis and apoptosis, and recently PF-DNA concentrations have also been shown to be increased in patients with sepsis (7,8,13).

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Furthermore, preliminary data from critically ill intensive care unit (ICU) patients have suggested that PF-DNA concentrations measured on admission were higher in nonsurvivors than in survivors (7,8).

The primary aim of this study was to evaluate the prognostic and diagnostic value of PF-DNA concentrations in mechanically ventilated patients with sepsis, severe sepsis, and septic shock. The secondary aim was to evaluate the correlation between PF-DNA and different markers (PCT, CRP, serum lipid levels, and scoring systems) already used to predict prognosis in patients with sepsis and its sequel.

2. Materials and methods

Forty-two consecutive patients with systemic inflammatory response syndrome (SIRS) or sepsis, admitted to the mixed ICU of our tertiary care hospital, were enrolled in the study after obtaining local ethics committee approval and informed consent from patients or their relatives. Patients who were younger than 18 years of age, pregnant, immunosuppressed, diagnosed with malignancy or genetic disease, or admitted because of acute drug overdose were excluded.

All patients were mechanically ventilated. The diagnoses of SIRS, sepsis, severe sepsis, and septic shock were established according to the definition of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference and evidence-based management was performed (14).

Determination of PF-DNA concentrations, PCT, CRP, and other laboratory parameters were performed from peripheral venous blood. For that, venous blood samples were taken on admission as soon as possible. In addition to this, blood samples were collected from 11 healthy subjects (control group) to determine the PF-DNA concentrations. Sequential Organ Failure Assessment (SOFA), Simplified Acute Physiology Score (SAPS), and Acute Physiology and Chronic Health Evaluation (APACHE II) scores were also calculated simultaneously with blood sampling.

Furthermore, age, sex, body weight, reason for admission, clinical diagnosis, and the length of ICU and hospital stay, as well as ICU, hospital, and 28-day ICU mortality data, were recorded.

Peripheral venous blood samples (4 mL) were collected into tubes containing ethylenediamine tetraacetic acid for PF-DNA evaluation. All blood samples were separated within 3 h of venesection. Plasma samples were stored at –20 °C until further processing. Cell-free plasma was prepared as described by Rhodes et al. (8).

DNA isolation from plasma samples was performed by phenol/chloroform extractions. PF-DNA was measured with a real-time PCR assay for the β-goblin gene, which is present in all nucleated cells of the body. For quantitative results, human genomic DNA was used to perform a plasma DNA curve. The unit "genome-equivalents/mL" was used. One genome-equivalent was defined as the amount of a particular target sequence contained in a single diploid human cell (6).

The nephelometric method (Dade-Behring) for plasma CRP and Kryptor assay (B.R.A.H.M.S. Diagnostica, Berlin, Germany) for PCT measurements were used.

Data were expressed as mean ± standard deviation (SD), median and interquartile range, and number of patients. Differences in continuous variables were compared with the nonparametric Mann–Whitney U test and Kruskal–Wallis tests. Spearman's rho correlation tests were used for correlation analysis and receiver operating characteristic (ROC) analysis was used for plasma-free DNA concentration, PCT, CRP, and SOFA score as a predictor of sepsis in patients admitted to the ICU. A univariate analysis was performed to compare various parameters (age; PF-DNA concentration; SOFA, SAPS, and APACHE II scores; PCT, CRP, and HDL levels) as predictors of mortality on admission. Logistic regression analysis was performed to identify factors that had independent predictive value for ICU mortality. Statistical analysis was performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered significant.

3. Results

Forty-two mechanically ventilated ICU patients were recruited into the study. From these 42 patients, 31 were nonseptic and 11 were septic. Demographic data are presented in Table 1. Additionally, primary infectious sources and sites and causative organisms of the 11 septic patients are presented in Table 2. On admission to the ICU, the mean APACHE II score was 14.6 ± 6.4 and the mean SOFA score was 4.9 ± 3.2. The mean length of stay of patients in the ICU was 25.4 ± 20 days and the mean length of stay in the hospital was 33.3 ± 27 days. ICU and hospital mortalities were 26.2% and 33.3%, respectively.

The PF-DNA concentration on admission was significantly higher in patients admitted to the ICU compared to the control group [1476 (0–180,311) GE/mL versus 371 (33–757) GE/mL, $P < 0.05$] and in the septic compared to nonseptic ICU patients [14,285 (48–180,311) GE/mL versus 546.5 (0–7674) GE/mL, $P < 0.0001$].

There were no statistically significant differences between PF-DNA concentrations of survivors compared to nonsurvivors (when comparing 28-day, ICU, and hospital mortalities). Additionally, there was no significant correlation between PF-DNA concentration and hospital and ICU stay.

PF-DNA levels at admission were correlated with CRP ($r = 0.365$, $P = 0.037$), PCT ($r = 0.457$, $P = 0.007$), and high-density lipoprotein (HDL) ($r = –0.415$, $P = 0.015$) concentrations.

Table 1. Demographic and medical characteristics of patients on admission and sample distribution according to the diagnosis.

Age (years) (mean ± SD)		51.1 ± 18.7
Weight (kg) (mean ± SD)		74.2 ± 10.1
Height (cm) (mean ± SD)		167.8 ± 9.2
Female/male ratio (n = 42)		20/22
Chronic disease (COPD, CAD, CKF, HT, DM, CLF) (n = 42): (+)/(-)		23/19
Admission diagnosis (n): medical/emergency surgery/elective surgery		21/17/4
APACHE II score	On admission (mean ± SD)	14.6 ± 6.4
	On admission, related to the diagnosis: NS/S (mean ± SD)	13.19 ± 4.3/17.4 ± 8.2
SOFA score	On admission (mean ± SD)	4.9 ± 3.2
	On admission, related to the diagnosis: NS/S (mean ± SD)	4 ± 2.2/5.9 ± 4.5
SAPS score	On admission (mean ± SD)	29.9 ± 12.2
	On admission, related to the diagnosis: NS/S (mean ± SD)	27.4 ± 8.3/34.8 ± 15.8
Diagnosis on admission (n = 42): NS/S/SS/Ssh		31/4/6/1

NS: nonseptic; S: septic; SS: severe sepsis; Ssh: septic shock; COPD: chronic obstructive pulmonary disease; CAD: coronary artery disease; CKF: chronic kidney failure; HT: hypertension; DM: diabetes mellitus; CLF: chronic liver failure.

Table 2. Primary infectious sources and sites, and causative organisms of septic patients (n = 11).

	Patient distribution
Primary source of infections:	
Nosocomial	10
Community-acquired	1
Primary site of infections*:	
Lung	6
Urinary tract	2
Blood	3
Intraabdominal	3
CNS**	1
Soft tissue	1
Causative organisms ***:	
Acinetobacter baumannii	8
Streptococcus pneumoniae	1
MRSA****	1
Staphylococcus aureus	1
Staphylococcus hominis	1
Enterococcus	1

*Five patients had multiple infections in different sites.

**CNS: central nervous system.

***Two patients had polymicrobial infections.

****Methicillin-resistant *Staphylococcus aureus.*

ROC curves were constructed to define admission PF-DNA concentration as a predictor of sepsis in critically ill ICU patients. The cut-off value for distinguishing between septic and nonseptic patients on admission was 4083 GE/mL (95% CI: 0.789–1.021) with 84% sensitivity, 95% specificity, and area under the curve (AUC) of 0.9 (Figure 1). ROC curve analysis of CRP, PCT, HDL, and SOFA score on admission for the prediction of sepsis are presented in Figure 2. The AUC for PCT was 0.9 (95% CI:

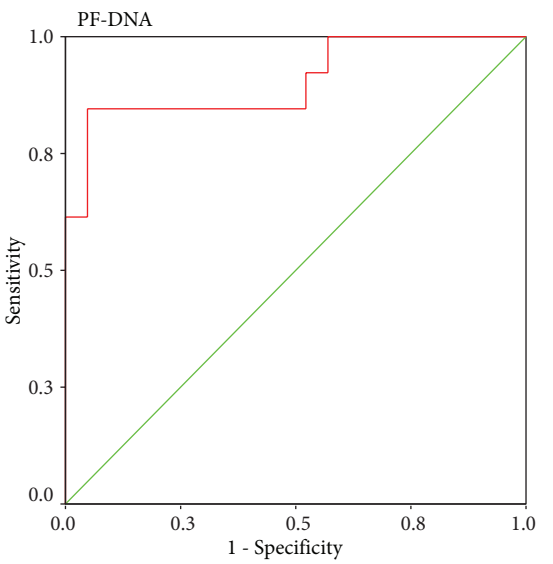

Figure 1. ROC curves for PF-DNA concentration on admission to the ICU to predict sepsis diagnosis. The area under the curve for PF-DNA concentration is 0.9 (95% CI: 0.78–1.02).

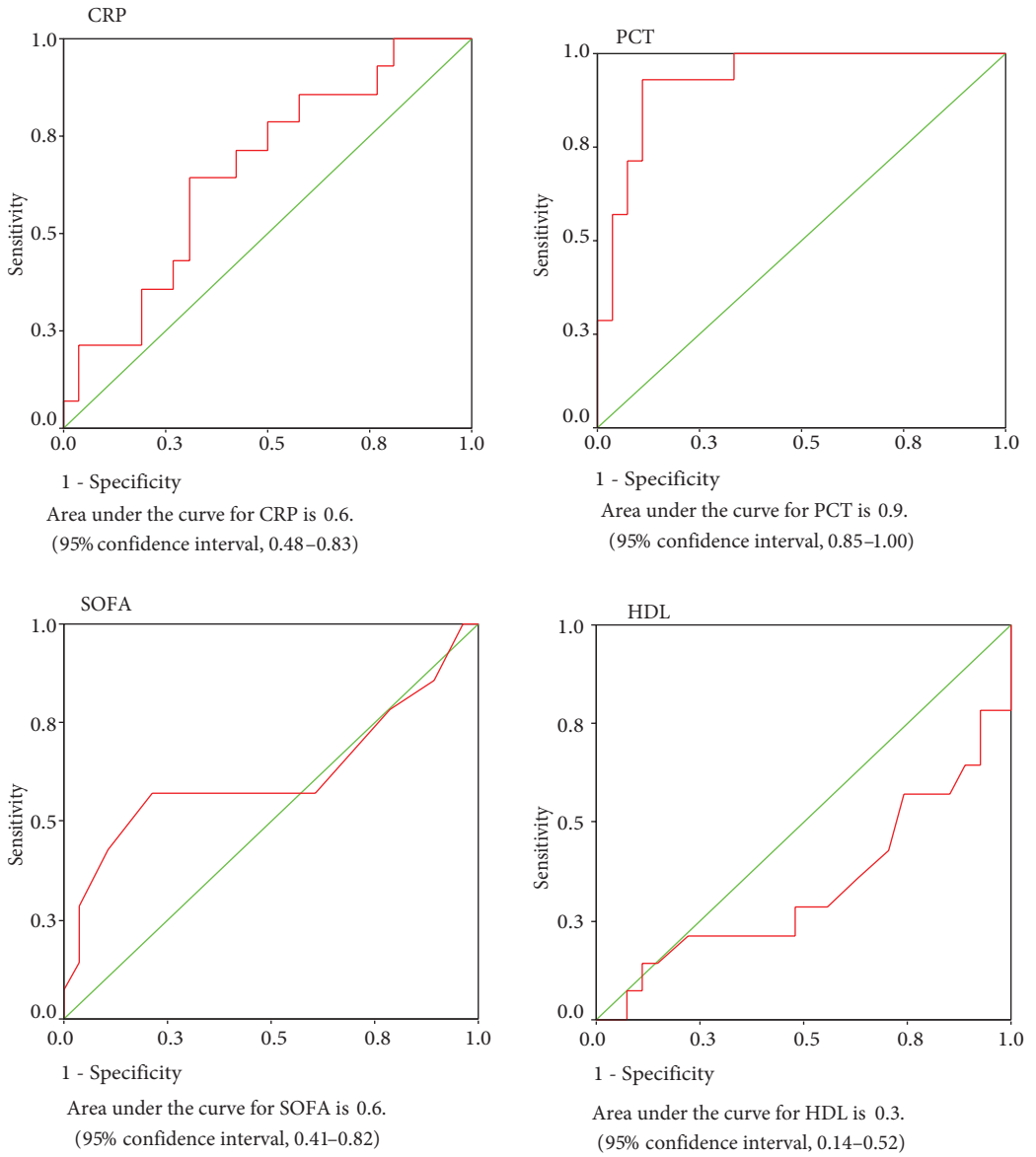

Figure 2. ROC curve analysis of CRP, PCT, HDL, and SOFA score for the prediction of sepsis on admission.

0.86–1.00), which was the most similar value to that for PF-DNA concentration.

Univariate analyses were performed to compare several parameters as predictors of mortality on admission. Age; SOFA, SAPS, and APACHE II scores; and PCT and HDL levels were significantly different between survivors and nonsurvivors (Table 3).

4. Discussion

The results obtained in this study show that PF-DNA concentration was significantly higher in critically ill patients compared to the healthy control group and in septic compared to nonseptic patients. On admission, PF-DNA concentration was positively correlated with CRP and PCT, while negatively correlated with HDL.

The diagnostic cutoff value for PF-DNA predicting sepsis was 4083 GE/mL, which was lower than in other studies. Furthermore, the PF-DNA concentrations of our patient population were also lower than in other studies. This discrepancy could be due to the impacts of organ dysfunctions as well as therapeutic modalities such as hemodialysis or plasmapheresis during the sampling time. These factors that were not evaluated in this study could have affected the PF-DNA clearances.

Recent studies measured PF-DNA concentration of various critically ill patients on admission to the ICU and correlated these values with the diagnosis and with the outcomes (7–9,15–18). Wijeratne et al., Rhodes et al., Saukkonen et al., and Huttunen et al. demonstrated that high PF-DNA levels on admission were related to poor

Table 3. Follow-up parameters on admission for survivors and nonsurvivors in the ICU.

	Survivors (n = 31)	Nonsurvivors (n = 11)	P-value
Age (years)	47 (16–83)	73 (25–81)	0.020*
SOFA	3 (0–10)	7 (2–17)	0.008*
SAPS	26 (12–42)	36 (20–73)	0.001*
APACHE II	13 (5–25)	17 (11–38)	0.006*
PCT (ng/mL)	0.425 (0.06–40.5)	2.39 (0.15–196.3)	0.019*
CRP (mg/L)	96.4 (9.77–344)	104 (45.1–358)	0.575
HDL (mg/dL)	24.5 (13–68)	19 (10–50)	0.045*
PF-DNA on admission (GE/mL)	1475.7 (0–180,311)	1567 (0–67,660)	0.596

PCT: procalcitonin, CRP: C-reactive protein, HDL: high-density lipoprotein.
Data presented as median with interquartile range. *P < 0.005.

prognosis and organ dysfunction and may be used as a predictor of mortality and sepsis in critically ill patients (7–9,19). Our results suggest that sepsis is associated with higher PF-DNA concentrations than those in nonseptic patients on admission and in the control group. These results are in accordance with the results of previous studies. However, in our study, admission PF-DNA concentrations were similar among survivors and nonsurvivors in the ICU, in the hospital and at 28 days. This discrepancy may be due to our relatively small sample size.

It has been suggested that cell apoptosis may be an important source of PF-DNA (19–21). Therefore, PF-DNA may be used as a marker of apoptosis. Furthermore, several studies comparing status of septic and nonseptic critically ill patients related to the apoptosis levels have shown that the quantity of apoptosis was correlated with sepsis prognosis (22,23). Brinkmann et al. also demonstrated that neutrophils activated by bacterial endotoxins release molecular complexes consisting in part of DNA (24).

Consequently, the level of PF-DNA may be correlated with the diagnosis and prognosis of sepsis in critically ill patients.

In recent studies, several biomarkers and tools were discussed to define the most appropriated ones for sepsis diagnosis and follow-up (25–28). We compared different biomarkers with PF-DNA concentration values and we have seen that PF-DNA concentration was positively correlated with CRP and PCT and negatively correlated with HDL. Previous studies have also shown correlations of PF-DNA with several biomarkers (7,16). However, some studies described no correlation between these markers (8).

All of the findings of this study point toward the importance and possible future use of PF-DNA concentrations as a reliable noninvasive prognostic factor in the modern management of septic patients in ICUs. Nevertheless, this study could be criticized for the small number of patients and an inconsistent patient population. Therefore, further studies should be designed with larger numbers and well-defined patient populations. Furthermore, multiple sampling from the same patients can be performed during the ICU stay for the follow-up of PF-DNA trends and to establish the value of PF-DNA even more precisely as a new follow-up tool in the critically ill patient population.

In conclusion, infectious processes and especially sepsis increase PF-DNA concentrations. PF-DNA concentration is a diagnostic marker for sepsis in critically ill patients during the overall ICU and hospital stay. Additionally, it is correlated with PCT, CRP, and HDL levels. Therefore, the use of PF-DNA concentration is a relatively simple and noninvasive rapid marker, which could be correlated within the current diagnosis and management of critically ill patients.

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