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Prognostic value of circulating DNA levels in critically ill and trauma patients

Valor prognóstico dos níveis de DNA circulante em pacientes graves e em pacientes com trauma

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ABSTRACT

The number of studies investigating circulating nucleic acids as potential biomarkers has increased in recent years. The detection of such biomarkers is a minimally invasive alternative for the diagnosis and prognosis of various clinical conditions. The value of circulating DNA levels as a predictive biomarker has been demonstrated in patients suffering from numerous acute pathologies that have a high risk of intensive care needs and in-hospital deaths. The mechanism by which circulating DNA levels increase in patients with these conditions remains unclear. In this review, we focused on the potential use of this biomarker for prognosis prediction in critically ill and trauma patients. The literature review was performed by searching MedLine using PubMed in the English language.

Keywords: Critical illness; Critical care; Wounds and injuries; Biological markers; Prognosis; DNA/blood

INTRODUCTION

The number of studies investigating the use of circulating nucleic acids as potential biomarkers has grown significantly in recent years. The detection of such biomarkers is a minimally invasive alternative for the diagnosis and prognosis of various clinical conditions. The presence of extracellular nucleic acids in the bloodstream was first described by Mandel and Métais in 1948.[1] However, the analysis of circulating nucleic acids, particularly circulating DNA, in a number of chronic conditions, including neoplasms, has only been explored in the last 15 years.[2]

The potential of circulating DNA levels as a biomarker has been demonstrated in patients with numerous acute pathologies that have a high risk of intensive care needs and in-hospital deaths. The mechanism by which plasma DNA levels increases in patients with these conditions remains unclear. The rapidly elevated concentrations of circulating DNA levels that are observed in patients with several acute injuries suggest that extracellular DNA originates from tissue necrosis.[3] In contrast, apoptosis may contribute to persistent increases in circulating DNA levels. The reduced clearance of DNA caused by impaired organ function during systemic inflammation may also be a contributing factor.[4]

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METHODS

The literature was reviewed by searching MedLine using PubMed and the following Medical Subject Heading (MeSH) terms in the English language: "intensive care", "critically ill", "trauma", "brain traumatic injury", "free-serum DNA", and "plasma DNA". A focused search was performed using the references in the papers. The article selection criteria were clinical studies and adult patient population. For the purposes of this review, the article exclusion criteria were as follows: burns, strokes, and acute coronary syndromes. There was no restriction by the publication date.

RESULTS

The papers on nuclear DNA are organized according to pathological conditions. A special topic discusses the results of the papers regarding mitochondrial DNA. The relevant data, which are summarized in tables 1 and 2, are divided into trauma and other critical care conditions, respectively.

Circulating nuclear DNA levels in trauma patients

Trauma scores were introduced more than 30 years ago to describe injury-related anatomical and physiological alterations, as well as other consequences of injuries. Trauma scores are useful tools for facilitating triage and pre-hospital treatment, generating records with consistent terminology, describing the severity of injuries, assessing quality of care and clinical outcomes, evaluating and comparing the systems of care for trauma victims, and standardizing definitions for epidemiology, research and funding.[23]

A template is created when a trauma score is used to predict an outcome or to correlate with a prognostic measure. These models allow for predictions and comparisons between groups of patients and systems of assistance for trauma victims.[23] A simple score is suitable for decision-making with respect to screening. However, the complex consequences of trauma are best described using scores and prediction models that include anatomical and physiological changes, as well as age and co-morbidities.[23]

Numerous scoring systems have been described in the last 30 years. Several of these scores have been regionally applied with success. However, no scoring system or triage system is universally accepted. The rapid and widely available laboratory tests can be extremely useful in this context.[23]

Lo et al.[5] first evaluated circulating nuclear DNA levels in 84 trauma patients approximately 60 minutes after a closed trauma. The measured circulating nuclear DNA levels were higher in patients with higher Injury Severity Scores. Additionally, circulating nuclear DNA levels were 11.6 to 12 times higher in patients who experienced complications, such as acute lung injury, acute respiratory distress syndrome and death, than in patients who did not develop these complications.[5]

Rainer et al.[6] measured circulating nuclear DNA levels in 83 trauma patients. The samples were collected up to 210 minutes after the trauma, and a combination of circulating nuclear DNA levels and alanine transaminase (ALT) predicted post-traumatic organ failure and multiple organ dysfunction syndrome in 93% and 87% of patients, respectively.

Lam et al.[4] studied the kinetic profile of circulating nuclear DNA levels in 2 populations of trauma patients. In the first population, the samples were collected from 25 patients every 20 minutes for 3 hours after the trauma. In the second population, a daily sample was collected from 36 patients who were admitted to the intensive care unit (ICU). In the first population,

Table 1 - Characteristics of the included trauma studies

Source	Design	N	Condition	Technique/gene primer	DNA source	Results
Lam et al.[4]	Prospective observational	61	Trauma	qPCR/β-globin gene	Nuclear	Higher in patients with severe injuries and in those who developed organ failure
Lo et al.[5]	Prospective observational	84	Trauma	qPCR/β-globin gene	Nuclear	Higher in patients with elevated ISS and in patients who experienced complications
Rainer et al.[6]	Prospective observational	83	Trauma	qPCR/β-globin gene	Nuclear	Combined with alanine transaminase predicted post-traumatic organ failure and multiple organ dysfunction syndrome
Campello Yurgel et al.[7]	Prospective observational	41 males	TBI	qPCR/β-globin gene	Nuclear	Higher levels in patients who died in the ICU
Macher et al.[8]	Prospective observational	65	TBI	qPCR/β-globin gene	Nuclear	Higher levels in patients who died in the hospital

qPCR - quantitative polymerase chain reaction; ISS - injury severity score; TBI - traumatic brain injury; ICU - intensive care unit.

Table 2 - Characteristics of the included intensive care unit studies

Source	Design	N	Condition	Technique/gene primer	DNA source	Results
Rhodes et al.[9]	Prospective observational	19	Sepsis	qPCR/β-globin gene	Nuclear	Higher levels in patients who developed sepsis and in patients who died in the ICU or in the hospital
Saukkonen et al.[10]	Prospective observational	255	Sepsis and septic shock	qPCR/β-globin gene	Nuclear	Higher levels in patients who died in the hospital
Moreira et al.[11]		110	Febrile		Nuclear	Levels correlated with the severity of infection
Ha et al.[12]	Hospital-based case control	192	Hemorrhagic dengue fever	qPCR/GAPDH gene	Nuclear	Levels correlated with dengue severity and shock
Huttunen et al.[13]	Prospective observational	132	Bacteremia	Quant-iT™ dsDNA HS assay	Nuclear	High levels were an independent risk factor for death
Wijeratne et al.[14]	Prospective observational	94	Prognosis prediction	qPCR/β-globin gene	Nuclear	Higher levels in patients who required mechanical ventilation and non-survivors
Saukkonen et al.[15]	Prospective observational	228	Prognosis prediction	qPCR/β-globin gene	Nuclear	The maximum level was an independent predictor of hospital mortality
Okkonen et al.[16]	Prospective observational	580	Prognosis prediction	qPCR/β-globin gene	Nuclear	Circulating DNA level at baseline was an independent predictor of 90-day mortality
Arnalich et al.[17]	Prospective observational	85	Cardiac arrest	qPCR/β-globin gene	Nuclear	Higher levels at the time of admission in patients who died at 24 h or in the hospital
Huang et al.[18]	Prospective observational	42	Cardiac arrest	qPCR//β-globin gene	Nuclear	Independent predictor for in-hospital mortality and associated with higher 90-day mortality
Gornik et al.[19]	Prospective observational	67	Cardiac arrest	qPCR/human telomerase reverse transcriptase gene	Nuclear	Level at 24 h after ICU admission was independently associated with hospital mortality
Gornik et al.[20]	Prospective observational	30	Pancreatitis	qPCR/human telomerase reverse transcriptase gene	Nuclear	Higher levels on the first day correlated with extent of necrosis and were a good predictor of severity
Gornik et al.[21]	Prospective observational	104	Pancreatitis	qPCR/human telomerase reverse transcriptase gene	Nuclear	Higher levels on the first day correlated with severity
Nakahira et al.[22]	Prospective observational	443	Prognosis prediction	qPCR/human NADH dehydrogenase 1 gene	Mitochondrial	Higher levels associated with ICU mortality

qPCR - quantitative polymerase chain reaction; ICU - intensive care unit; GAPDH - glyceraldehyde-3-phosphate dehydrogenase; dsDNA - double stranded deoxyribonucleic acid; HS - high sensitivity; NADH - nicotinamide-adenine dinuleotide reduced.

circulating nuclear DNA levels were increased 20 minutes after the trauma and were significantly higher in patients with more severe trauma and in patients who developed multi-organ failure. In the second population, circulating nuclear DNA levels were higher in patients who developed multi-organ failure between the 2nd day and the 4th day after admission to the ICU. In patients with persistent multi-organ failure who remained in the ICU, circulating nuclear DNA levels remained elevated for 28 days after the trauma. The majority of survivors with multi-organ dysfunction exhibited an extremely high initial peak in circulating nuclear DNA levels, followed by a smaller sustained increase.

Circulating nuclear DNA levels in traumatic brain injured patients

Globally, trauma is a leading cause of death for individuals under 45 years of age. Up to half of the deaths in this group are caused by brain trauma.[24,25] Establishing prognostic criteria is important for the effective treatment of patients with severe traumatic brain injury (TBI) because the accurate identification of patients at increased risk requires an effective predictor. The Glasgow coma scale (GCS) is one of the most important short-term prognostic predictors after TBI.[26] Although the GCS is useful in the acute phase, the Glasgow outcome scale (GOS) is the most widely used scale for assessing medium- and long-term prognosis after TBI and other non-traumatic brain injuries.[27] However, associated conditions and the low predictive value of clinical assessment in patients with severe TBI complicate the identification of patients at increased risk for the development of secondary brain damage and death.[28] A practical and sensitive biomarker is required to identify such patients as early as possible and provide earlier interventions.[29]

Consequently, an increasing amount of effort has been devoted to the development of new biomarkers. Potential biomarkers for brain damage include S100-B protein, C-tau, neuron-specific enolase and Hsp70.[30] However, none of these proteins has been established as a broadly applicable marker for predicting brain damage.

Campello Yurgel et al.[7] investigated circulating nuclear DNA levels in 41 male victims with isolated severe TBI or isolated severe TBI with associated extracerebral injuries. The results indicated a significant correlation between higher circulating nuclear DNA levels in the first 24 hours after severe TBI and death. Furthermore, persistent, elevated, circulating nuclear DNA levels appeared to correlate with higher mortality.

Recently, Macher et al.[8] investigated circulating nuclear DNA levels in 65 patients with severe TBI during the first 96 hours after admission to the ICU. After an initial peak in circulating nuclear DNA levels, a larger reduction in circulating nuclear DNA levels was detected within the first 24 hours among survivors than among non-survivors.

Circulating nuclear DNA levels in sepsis patients

Severe sepsis and septic shock are the leading causes of death in the ICU, with a mortality rate ranging between 30% and 60%.[31,32] Several biomarkers have been evaluated for predicting mortality and morbidity in septic patients; however, none of these markers has proven to be useful in clinical practice.[33]

Martins et al.[34] were the first authors to describe the presence of circulating DNA in a small group of septic patients. Subsequently, Rhodes et al.[9] assessed circulating nuclear DNA levels in 52 ICU patients, and 19 of these patients developed severe sepsis or septic shock. High levels of circulating nuclear DNA measurements were observed in patients who developed sepsis and in patients who died in the ICU or in the hospital. Circulating nuclear DNA levels were an independent predictor of mortality. The sensitivity and the specificity of circulating nuclear DNA levels for predicting death in ICU patients were 92% and 80%, respectively, when concentrations greater than 127ng/mL were used.

Saukkonen et al.[10] assessed circulating nuclear DNA levels in 255 patients with severe sepsis and septic shock at the time of ICU admission and 72 hours after ICU admission. At both time points, the circulating nuclear DNA levels were significantly higher in non-survivors than in survivors. Additionally, the circulating nuclear DNA levels were higher in patients who did not survive hospitalization than in survivors. Notably, the circulating nuclear DNA levels on the first day in the ICU constituted an independent predictor of mortality.

Moreira et al.[11] measured the levels of circulating nuclear DNA, procalcitonin and C-reactive protein in 110 febrile patients with a diagnosis of fever of unknown origin, localized infection, sepsis or septic shock. The circulating nuclear DNA levels correlated with the severity of infection. The best thresholds for predicting infection and sepsis were 2,800 GE/mL and 14000 GE/mL, respectively. Importantly, higher concentrations of circulating nuclear DNA levels were found in non-survivors than in survivors. Additionally, the diagnostic efficiency of the circulating nuclear DNA levels was similar to the diagnostic efficiency of procalcitonin and C-reactive protein levels. Thus, the results indicated that normal circulating nuclear DNA levels might exclude the presence of infection in a febrile patient, whereas high concentrations indicate a severe infection and exhibit a high prognostic value for predicting mortality in the absence of other causes that justify the values.

Ha et al.[12] assessed the levels of circulating nuclear DNA levels in 192 patients with hemorrhagic dengue fever and reported that the higher circulating nuclear DNA levels correlated with the severity and the presence of shock.

Huttunen et al.[13] evaluated the circulating nuclear DNA levels in 132 patients with bacteremia caused by *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus β-hemolyticus* and *Escherichia coli*. The maximum values obtained between 1 day and 4 days after the first positive blood cultures were significantly higher in non-survivors than in survivors. These values exhibited a discriminatory power of 0.81 for predicting death. High levels (1.52mg/mL) of circulating nuclear DNA levels were an independent risk factor for death. Thus, the majority of deaths in septic bacteremia patients and some deaths in patients with serious viral infections appear to be the result of an inadequate immune response that is caused by extensive immune cell death. This process is also called immunoparalysis of sepsis.[35]

Circulating nuclear DNA levels for predicting prognosis in critically ill patients

During the last three decades, several prognostic scores have been used to predict severity in critically ill patients. The principal scores used for predicting prognosis in critically ill adult patients are the Acute Physiology and Chronic Health Evaluation (APACHE) Score, the Simplified Acute Physiology Change (SAPS) and the Probability of Death Model (MPM).[36] The predictor variables that are commonly used in these scores are age, co-morbidities, physiological changes, acute diagnoses, and bias as a function of time (e.g., treatment and measurement at different times). However, these scores require constant updating[37] because they deteriorate with time and lose the capacity for discrimination and calibration. Moreover, these prognostic scores usually assume that unavailable data values are normal, which can negatively affect their performance.[38] Factors, such as patient preferences for life support, individual responses to the disease, environment and treatment effects, also influence outcomes and may not be included in the prediction models. The majority of models do not include a measure of bias as a function of time. In theory, many limitations of these traditional prognostic models could be overcome by the early collection of samples for laboratory tests, such as the free nucleic acid test.

Wijeratne et al.[14] first investigated 94 patients and the prognostic value of circulating nuclear DNA levels measured at the time of their admission to the ICU. The circulating nuclear DNA levels correlated with C-reactive protein levels and the Sequential Organ Failure Assessment (SOFA) scores. Additionally, the patients who required mechanical ventilation and the non-survivors had significantly higher circulating nuclear DNA levels. The best threshold for predicting death was 6109 GE/mL.

Saukkonen et al.[15] studied 228 patients admitted to one clinical ICU and two clinical-surgical ICUs. The samples were collected at the time of admission, the following morning and 48 hours after the second sample. The peak circulating nuclear DNA levels correlated significantly with the APACHE II scores and with the maximum SOFA scores. Additionally, the circulating nuclear DNA levels at the time of hospital admission were higher in non-survivor patients than in survivors, and the maximum circulating nuclear DNA level was an independent predictor of hospital mortality.

Okkonen et al.[16] studied 580 patients who required invasive or noninvasive mechanical ventilation for more than 6 hours in 25 Finnish ICUs. The samples for circulating nuclear DNA determination were collected at the time of admission and 2 days later. The circulating nuclear DNA levels at the time of admission were significantly higher in patients who did not survive the 90th day, and the circulating nuclear DNA levels higher than 16,000 GE/mL were independent predictors of death.

Circulating nuclear DNA levels in cardiac arrest patients

The survival rates for patients who experience out-of-hospital cardiac arrest have not improved despite advances in cardiopulmonary resuscitation.[39] Clinical variables in the patient's history, variables related to cardiopulmonary resuscitation and post resuscitation, and several biomarkers have been examined for prognostic purposes. However, none of these factors has proven to be effective.

Thus, Arnalich et al.[17] evaluated circulating nuclear DNA levels in 85 victims of out-of-hospital cardiac arrest who experienced a sustained return of spontaneous circulation after approximately 27 minutes. Of 85 patients studied, 30 died within 24 hours and 58 patients died during hospitalization. The circulating nuclear DNA levels were significantly higher in non-survivors at 24 hours and in patients who died during hospitalization than in survivors. The best cut-off for predicting mortality at 24 hours was 4340 GE/mL, and the best cut-off for predicting hospital mortality was 3485 GE/mL. The risk of death within 24 hours and during hospitalization increased by 1.75 times and 1.36 times, respectively, for every increase of 500 GE/mL in circulating nuclear DNA levels.

Huang et al.[18] assessed 42 victims of out-of-hospital cardiac arrest. The circulating nuclear DNA levels measured within 2 hours of the event were higher in non-survivor patients compared to survivors, and a value higher than 1170 GE/mL was an independent predictor of in-hospital mortality after 90 days.

Gornik et al.[19] recently assessed 67 victims of non-traumatic out-of-hospital cardiac arrest in three

university-associated intensive care units. The circulating nuclear DNA levels were measured at the time of admission and at 24±1 hours after admission. The multivariate analysis showed that "low-flow" time and circulating nuclear DNA levels at 24 hours after ICU admission were independently associated with hospital mortality. The ROC curve for the circulating nuclear DNA levels 24 hours post admission showed 81.0% sensitivity and 78.3% specificity for the cut-off value of 0.115ng/μL.

Circulating nuclear DNA levels in pancreatitis patients

Acute pancreatitis is typically a mild illness; however, approximately 20% of patients have severe disease with local and systemic complications and high mortality. Early identification of this subgroup of patients is critical because early treatment can reduce morbidity and mortality. The methods used for stratifying risk include Ranson and APACHE II scores and C-reactive protein levels. Abdominal computed tomography is also useful for assessing pancreatic necrosis,[40] which is an important determinant of severity.

Gornik et al.[20] first measured the circulating nuclear DNA levels in 30 patients with pancreatitis, using samples collected at the time of admission and after 1 day, 4 days, and 7 days. The patients who developed severe pancreatitis had significantly higher circulating nuclear DNA levels on the first day compared to the patients with mild disease. The circulating nuclear DNA levels also correlated with the extent of necrosis and were a good predictor of severity (ROC AUC, 0.97).

Gornik et al.[21] extended their initial studies by determining the circulating nuclear DNA levels in 104 patients suffering from pancreatitis. A total of 33 patients met the criteria for severe disease, and 71 patients had mild disease. Of the patients with severe pancreatitis, 28 patients were hospitalized in the ICU. The samples were collected on the first day. The circulating nuclear DNA levels were lower in the patients with mild disease than in the patients with severe disease. Additionally, circulating nuclear DNA levels differentiated the patients with severe disease from the patients with mild disease according to high sensitivity and specificity (90.9% and 88.7%, respectively) using a cut-off value of >0.304ng/μL. This level of circulating nuclear DNA was more sensitive and more specific than C-reactive protein levels, APACHE II scores and Ranson scores.

Circulating mitochondrial DNA levels

Additional cell-free DNA species, such as cell-free mitochondrial DNA (mtDNA), are under evaluation for clinical relevance.[41] Thus far, mtDNA has been shown to be more sensitive to oxidative damage than nuclear DNA.[41]

This observation has made available exciting opportunities for non-invasive diagnostic evaluation that can be performed with high accuracy and at a reasonable cost.

Earlier studies suggested that circulating mitochondrial DNA (mtDNA) levels are increased in response to stimuli, such as trauma[42] and sepsis.[43]

Nakahira et al.[22] recently assessed blood samples obtained from two prospective, observational, cohort studies of ICU patients (n=443). The medical ICU patients with elevated circulating mtDNA levels (≥3200 copies/ml plasma) had increased odds of dying within 28 days of ICU admission in both cohorts (odds ration - OR, 7.5 and 8.4). Nakahira et al. demonstrated that elevated circulating mtDNA levels may improve risk prediction in the medical ICU patients.

FINAL CONSIDERATIONS

The studies presented in this review demonstrate that circulating DNA levels could be a valuable tool in diagnosis and prognosis prediction for a number of critical diseases. Circulating DNA levels may facilitate the stratification of patients, particularly when one or more measurements are performed early in the clinical time frame. However, different genomic sequences used for amplification and small samples obtained limit the extrapolation of these results. Therefore, future studies with larger samples of patients are necessary to standardize the technique and to define more accurate cut-off points.

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RESUMO

Nos últimos anos, o número de estudos que investigam os ácidos nucleicos circulantes como potenciais biomarcadores tem aumentado. A detecção desses biomarcadores é uma alternativa minimamente invasiva para o diagnóstico e o prognóstico de diversas condições clínicas. O valor dos níveis de DNA circulante como biomarcador preditivo foi demonstrado em pacientes com numerosas patologias agudas que apresentam riscos elevados de necessitar cuidados intensivos e de sofrer mortalidade hospitalar. Os mecanismos pelos quais os níveis de DNA circulante aumentam em pacientes com essas condições ainda são obscuros. Nesta revisão, focalizamos o potencial uso deste biomarcador para predição prognóstica em pacientes graves e pacientes com trauma. A revisão da literatura foi feita por meio de busca no MedLine utilizando o PubMed em inglês.

Descritores: Doença crítica; Cuidados críticos; Ferimentos e lesões; Marcadores biológicos; Prognóstico; DNA/sangue

REFERENCES

1. Mandel P, Métais P. Les acides nucléiques du plasma sanguin chez l'homme. C R Acad Sci Paris. 1948;142:241-3.
2. van der Vaart M, Pretorius PJ. Is the role of circulating DNA as a biomarker of cancer being prematurely overrated? Clin Biochem. 2010;43(1-2):26-36.
3. Ziegler A, Zangemeister-Wittke U, Stahel RA. Circulating DNA: a new diagnostic gold mine? Cancer Treat Rev. 2002;28(5):255-71.
4. Lam NY, Rainer TH, Chan LY, Joynt GM, Lo YM. Time course of early and late changes in plasma DNA in trauma patients. Clin Chem. 2003; 49(8):1286-91.
5. Lo YM, Rainer TH, Chan LY, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. Clin Chem. 2000;46(3):319-23.
6. Rainer TH, Lo YM, Chan LY, Lam NY, Lit LC, Cocks RA. Derivation of a prediction rule for posttraumatic organ failure using plasma DNA and other variables. Ann N Y Acad Sci. 2001;945:211-20.
7. Campello Yurgel V, Ikuta N, Brondani da Rocha A, Lunge VR, Fett Schneider R, Kazantzi Fonseca AS, et al. Role of plasma DNA as a predictive marker of fatal outcome following severe head injury in males. J Neurotrauma. 2007;24(7):1172-81.
8. Macher H, Egea-Guerrero JJ, Revuelto-Rey J, Gordillo-Escobar E, Enamorado-Enamorado J, Boza A, et al. Role of early cell-free DNA levels decreased as a predictive marker of fatal outcome after severe traumatic brain injury. Clin Chem Acta. 2012;414:12-7.
9. Rhodes A, Wort SJ, Thomas H, Collinson P, Benett ED. Plasma DNA concentration as a predictor of mortality and sepsis in critically ill patients. Crit Care. 2006;10(2):R60.
10. Saukkonen K, Lakkisto P, Pettilä V, Varpula M, Karlsson S, Ruokonen E, Pulkki K; Finnsepsis Study Group. Cell-free plasma DNA as a predictor of outcome in severe sepsis and septic shock. Clin Chem. 2008;54(6):1000-7.
11. Moreira VG, Prieto B, Rodríguez JS, Alvarez FV. Usefulness of cell-free plasma DNA, procalcitonin and C-reactive protein as markers of infection in febrile patients. Ann Clin Biochem. 2010;47(Pt 3):253-8.
12. Ha TT, Huy NT, Murao LA, Lan NT, Thuy TT, Tuan HM, et al. Elevated levels of cell-free circulating DNA in patients with acute dengue virus infection. PLoS One. 2011;6(10):e25969.
13. Huttunen R, Kuparinen T, Jylhävä J, Aittoniemi J, Vuento R, Huhtala H, et al. Fatal outcome in bacteremia is characterized by high plasma cell free DNA concentration and apoptotic DNA fragmentation: a prospective cohort study. PLoS One. 2011; 6(7):e21700.
14. Wijeratne S, Butt A, Burns S, Sherwood K, Boyd O, Swaminathan R. Cell-free plasma DNA as a prognostic marker in intensive treatment unit patients. Ann N Y Acad Sci. 2004;1022:232-8.
15. Saukkonen K, Lakkisto P, Varpula M, Varpula T, Voipio-Pulkki LM, Pettilä V, et al. Association of cell-free plasma DNA with hospital mortality and organ dysfunction in intensive care unit patients. Intensive Care Med. 2007;33(9):1624-7.
16. Okkonen M, Lakkisto P, Korhonen AM, Parviai-nen I, Reinikainen M, Varpula T, Pettilä V; FINNALI Study Group. Plasma cell-free DNA in patients needing mechanical ventilation. Crit Care. 2011;15(4):R196.
17. Arnalich F, Menéndez M, Lagos V, Ciria E, Quesada A, Codoceo R, et al. Prognostic value of cell-free plasma DNA in patients with cardiac arrest outside the hospital: an observational cohort study. Crit Care. 2010;14(2):R47.
18. Huang CH, Tsai MS, Hsu CY, Chen HW, Wang TD, Chang WT, et al. Circulating cell-free DNA levels correlate with postresuscitation survival rates in out-of-hospital cardiac arrest patients. Resuscitation. 2012;83(2):213-8.
19. GorniK I, Wagner J, Gasparović V, Milicic D, Degoricija V, Skoric B, et al. Prognostic value of cell-free DNA in plasma of out-of-hospital cardiac arrest survivors at ICU admission and 24h post-admission. Resuscitation. 2014;85(2):233-7.
20. Gornik I, Wagner J, Gasparović V, Lauc G, Gornik O. Free serum DNA is an early predictor of severity in acute pancreatitis. Clin Biochem. 2009; 42(1-2):38-43.
21. Gornik O, Gornik I, Wagner J, Radić D, Lauc G. Evaluation of cell-free DNA in plasma and serum as early predictors of severity in acute pancreatitis. Pancreas. 2011;40(5):787-8.
22. Nakahira K, Kyung SY, Rogers AJ, Gazourian L, Youn S, Massaro AF, et al. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. PLoS Med. 2013;10(12):e1001577; discussion e1001577.
23. Champion HR. Trauma scoring. Scand J Surg. 2002;91(1):12-22. Review.
24. Gennarelli TA. Mechanisms of brain injury. J Emerg Med. 1993;11 Suppl 1:5-11. Review.
25. Jennett B. Epidemiology of head injury. Arch Dis Child. 1998;78(5):403-6.
26. Balestreri M, Czosnyka M, Chatfield DA, Steiner LA, Schmidt EA, Smielewski P, et al. Predictive value of Glasgow Coma Scale after brain trauma: change in trend over the past ten years. J Neurol Neurosurg Psychiatry. 2004;75(1):161-2.
27. Wilson JT, Pettigrew LE, Teasdale GM. Structured interviews for the Glasgow Outcome Scale and the extended Glasgow Outcome Scale: guidelines for their use. J Neurotrauma. 1998;15(8):573-85.
28. Ghajar J. Traumatic brain injury. Lancet. 2000;356(9233):923-9.
29. Townend WJ, Guy MJ, Pani MA, Martin B, Yates DW. Head injury outcome prediction in the emergency Department: a role for protein S-100B? J Neurol Neurosurg Psychiatry. 2002;73(5):542-6.
30. Oliveira CO, Ikuta N, Regner A. Outcome biomarkers following severe traumatic brain injury. Rev Bras Ter Intensiva. 2008;20(4):411-21.
31. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29(7):1303-10.
32. Alberti C, Brun-Buisson C, Burchardi H, Martin C, Goodman S, Artigas A, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Med. 2002;28(2):108-21. Erratum in: Intensive Care Med. 2002;28(4):525-6.

33. Kibe S, Adams K, Barlow G. Diagnostic and prognostic biomarkers of sepsis in critical care. J Antimicrob Chemother. 2011;66 Suppl 2:ii33-40.
34. Martins GA, Kawamura MT, Carvalho Mda G. Detection of DNA in the plasma of septic patients. Ann N Y Acad Sci. 2000;906:134-40.
35. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. Nat Rev Immunol. 2006;6(11):813-22.
36. Keegan MT, Gajic O, Afessa B. Severity of illness scoring systems in the intensive care unit. Crit Care Med. 2011;39(1):163-9.
37. Afessa B, Gajic O, Keegan MT. Severity of illness and organ failure assessment in adult intensive care units. Crit Care Clin. 2007;23(3):639-58.
38. Afessa B, Keegan MT, Gajic O, Hubmayr RD, Peters SG. The influence of missing components of the Acute Physiology Score of APACHE III on the measurement of ICU performance. Intensive Care Med. 2005;31(11):1537-43.
39. Fairbanks RJ, Shah MN, Lerner EB, Ilangovan K, Pennington EC, Schneider SM. Epidemiology and outcomes of out-of-hospital cardiac arrest in Rochester, New York. Resuscitation. 2007;72(3):415-24.
40. Balthazar EJ. Staging of acute pancreatitis. Radiol Clin North Am. 2002;40(6):1199-209.
41. Chan SW, Chevalier S, Aprikian A, Chen JZ. Simultaneous quantification of mitochondrial DNA damage and copy number in circulating blood: a sensitive approach to systemic oxidative stress. Biomed Res Int. 2013;2013:157547.
42. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. 2010; 464(7285):104-7.
43. Kung CT, Hsiao SY, Tsai TC, Su CM, Chang WN, Huang CR, et al. Plasma nuclear and mitochondrial DNA levels as predictors of outcome in severe sepsis patients in the emergency room. J Transl Med. 2012;10:130.