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Association between DNA and RNA oxidative damage and mortality in septic patients

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ABSTRACT

Purpose: DNA and RNA oxidative damage occurs during sepsis. Higher urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels (from oxidation of guanosine from DNA) have been found in non-surviving patients than in surviving septic patients. However, the relation between DNA and RNA oxidative damage and mortality in septic patients has never been published; thus, the objective of this study was to determine the existence of this association.

Methods: This prospective and observational study including septic patients was conducted in 8 Spanish Intensive Care Units. Serum concentrations of the three oxidizied guanine species (OGS) (8-OHdG from DNA, 8-hydroxyguanosine from RNA, and 8-hydroxyguanine from DNA or RNA) were determined, and malondialdehyde (to estimate lipid peroxidation) in the diagnosis of sepsis. Mortality at 30 days was the end-point study.

Results: Non-surviving patients ($n = 78$) compared to surviving patients ($n = 139$) showed higher serum concentrations of OGS ($p = .004$) and malondialdehyde ($p < .001$). Simultaneously, an association between serum OGS concentrations and mortality in logistic regression analysis was found (OR = 1.105; 95% CI = 1.024–1.193; $p = .01$), and a positive correlation between serum levels of OGS and malondialdehyde (rho = 0.21; $p = .002$).

Conclusions: The new findings from our study were that oxidative DNA and RNA damage in septic patients was associated with mortality and lipid peroxidation.

1. Introduction

Sepsis entails a large number of deaths and costs to the healthcare system [1,2]. The hyperoxidative state that appears in sepsis can cause damage to proteins, lipids, deoxyribonucleic acid (DNA) [3-6] and ribonucleic acid (RNA) [7,8] caused by the action of reactive oxygen species (ROS) on them.

DNA and RNA are up of 4 types of nucleobases; Guanine, adenine, and cytosine are nucleobases found in both RNA and DNA, while thymine is only found in DNA and uracil only in RNA. Guanine is nucleobase most prone to oxidation due to its lowest redox potential [3-6]. The three species of guanine oxidized (OGS) are 8-oxo-deoxyguanosine (8-oxo-dG) also named 8-hydroxy-2′-deoxyguanosine (8-OHdG) from DNA, 8-oxo-guanosine (8-oxo-G) also called 8-hydroxyguanosine (8-OHG) from RNA, and 8-oxo-guanine (8-oxo-Gua) also called 8-hydroxyguanine (8-OHGua) from DNA or RNA and is ribose free base.

8-OHdG is the most studied OGS with some meta-analyses having found higher levels of it in patients with cardiovascular diseases, heart failure and periodontal disease than in healthy subjects [9-11].

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Furthermore, some studies found higher levels of 8-OHG [12-15] and 8-OHGua [16,17] in patients with different diseases.

Oxidative nucleic acid damage in septic patients has hardly been studied [18,19]. One study found higher plasma levels of 8-OHdG in 29 septic patients than in 22 healthy subjects [18]. Another study with 85 septic patients found higher urinary levels of 8-OHdG in non-surviving than in surviving patients [19]. However, no association has been published between oxidative DNA and RNA damage and mortality in septic patients; therefore, the objective of this study was to determine whether that association exists.

2. Methods

2.1. Design and subjects

This prospective and observational study including septic patients was conducted in 8 Spanish Intensive Care Units: H. Quirón Tenerife (Tenerife), H. General de La Palma (La Palma), H. Clínico Universitario de Valencia (Valencia), H. Universitario Nuestra Señora de Candelaria (Tenerife), H. San Jorge (Huesca), H. Universitario de Canarias (Tenerife), H. Insular (Gran Canaria), and H. Universitario Dr. Negrín (Gran Canaria). The study was approved by the Institutional Ethics Review Boards of all hospitals. Patients or their relatives signed the informed consent to participate in the study.

Patients with sepsis according to the Sepsis-3 Consensus criteria [20] were included. Patients with some of the following criteria were excluded: white blood cell count <1000/μl, human immunodeficiency virus (HIV), solid or hematological tumor, patients undergoing immunosuppressive therapy or steroid agents, radiation therapy, age <18 years, breastfeeding, or pregnancy.

Previously, serum malondialdehyde concentrations (as a biomarker of lipid peroxidation) were determined in some of the patients [21]; and in this work, serum OGS concentrations were determined.

The following data were collected: age, sex, creatinine, bilirubin, activated partial thromboplastin time (aPTT), international normalized ratio (INR), platelets, lactic acid, leukocytes, pressure of arterial oxygen (PaO_2), fraction inspired of oxygen (FIO_2), Acute Physiology and Chronic Health Evaluation (APACHE)-II score [22], Sepsis-related Organ Failure Assessment [SOFA] score [23], ischemic heart disease, chronic renal failure (defined as rate of glomerular filtration <60 ml/min/1.73m^2), chronic obstructive pulmonary disease (COPD), diabetes mellitus, bloodstream infection, microorganism responsible, site of infection, and empiric antimicrobial treatment. Mortality at 30 days was considered the endpoint of the study.

2.2. Determination of serum concentrations of OGS and malondialdehyde

Serum blood samples were collected on day 1 of the sepsis diagnosis and frozen at −80 °C until serum concentrations determinations. Serum concentration of the three OGS was measured using the DNA/RNA Oxidative Damage ELISA Kit® (Cayman Chemical Corporation, Ann Arbor, USA). The detection limit of the assay was 10 pg/mL, the intra-assay coefficient of variation (CV) was 4.7–11.6%, and the inter-assay CV was 4.5–10.7%. Those serum OGS concentration determinations were performed blindly to clinical data at the Laboratory Department of the Hospital Universitario de Canarias (Tenerife, Spain). Serum malondialdehyde concentrations were measured to assess lipid peroxidation [24,25], using the thiobarbituric acid-reactive substance method (TBARS) described by Kikugawa et al. [26]. The detection limit of the assay was 0.079 nmol/ml; and the inter- and intra- assay CV were 4.01% and 1.82%, respectively. Those serum MDA concentrations determinations were performed blindly to clinical data at the Department of Physiology of the Faculty of Medicine of the La Laguna (Tenerife, Spain).

3. Statistical methods

Continuous variables were reported as medians and interquartile ranges, and categorical variables as frequencies and percentages. Continuous variables for the surviving and non-surviving patients were compared by Mann-Whitney *U* test, and categorical variables by chi-square test. A receiver operating characteristic (ROC) curve analysis was performed using 30-day mortality as the classification variable and serum OGS concentrations as the prognostic variable (using Youden J index for selection of the optimal cut-off value).

A Kaplan-Meier survival analysis was performed to compare 30-day survival among patients with serum OGS levels ≤4.53 ng/mL and >4.53 ng/mL. The unadjusted hazard ratio (HR) with a 95% confidence interval (CI) comparing risks in the two groups of patients (serum OGS levels ≤4.53 ng/mL and >4.53 ng/mL) was included in the survival analysis. Unadjusted HR means no covariate was used. HR and CI are calculated according to Altman et al. [27]. All patients who reached 30 days were considered censored.

Multiple logistic regression analyses were performed to determine the association between serum OGS concentrations and 30-day mortality, controlling for possible confounds; and odds ratio (OR) and 95% CI were used to estimate the clinical impact of prognostic variables. Several logistic regression models were constructed with different predictor variables in each model. In the logistic regression models, variables showing a $p < .10$ value in comparison between surviving and non-surviving patients were introduced. In the first model, diabetes mellitus, lactic acid, INR, aPTT, age, SOFA, and OGS were included. The second model included the same variables; however, OGS was introduced as a binary variable (OGS levels > and <4.53 ng/mL). In the third model, diabetes mellitus, lactic acid, INR, aPTT, APACHE-II, and OGS were included. The fourth model included the same variables as the third model; however, OGS was introduced as a binary variable (OGS levels > and <4.53 ng/mL). Thus, in the third and fourth models SOFA was changed to APACHE-II. Both were not included, as they are both severity scores. Age was also excluded because it is included in the APACHE-II score. Therefore, all models were adjusted for diabetes mellitus, lactic acid, INR, and aPTT. In addition, the first model was also adjusted for age, SOFA, and continuous OGS; the second model for age, SOFA and binary OGS; the third model for APACHE-II and continuous OGS; and the fourth model for APACHE-II and binary OGS. The association between serum concentrations of OGS and malondialdehyde was determined using Spearman correlation coefficient. Statistically significant differences were considered when $p < .05$. Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and NCSS 2000 (Kaysville, Utah).

4. Results

Table 1 shows comparisons between non-surviving ($n = 78$) and surviving patients ($n = 139$) on demographic and clinical characteristics. There were no statistically significant differences between non-surviving and surviving septic patients regarding sex, leukocytes, bilirubin, $Pa0_2/FI0_2$ ratio, diabetes mellitus, ischemic heart disease, chronic renal failure, COPD, microorganism responsible of the infection, bloodstream infection, site of infection, and empiric antimicrobial treatment. However, non-surviving patients were found to have a lower platelet count and a higher age, APACHE-II score, aPTT, creatinine, INR, lactic acid, and SOFA compared to surviving patients. In addition, non-surviving patients showed higher serum concentrations of OGS ($p = .004$) and malondialdehyde ($p < .001$) than surviving patients.

In the logistic regression analysis serum OGS levels were associated with 30-day mortality (OR = 1.105; 95% CI = 1.024–1.193; $p = .01$) after control for lactic acid, SOFA, diabetes mellitus, age, aPTT and INR (Table 2).

Area under the curve (AUC) to predict 30-day mortality for serum OGS levels was 62% (95% CI = 55%–68%; $p = .002$) (Fig. 1).

Table 1
Comparisons between non-surviving and surviving severe septic patients on demographic and clinical characteristics at moment of severe sepsis diagnosis.

	Non-surviving (n = 78)	Surviving (n = 139)	*p*-Value
Age - median years (p 25–75)	64 (56–74)	55 (44–67)	<0.001
APACHE-II score - median (p 25–75)	23 (19–28)	18 (14–23)	<0.001
aPTT (seconds) - median (p 25–75)	36 (30–45)	32 (28–41)	0.01
Bilirubin (mg/dl) - median (p 25–75)	0.80 (0.50–2.30)	0.90 (0.44–1.50)	0.50
Creatinine (mg/dl) - median (p 25–75)	1.60 (1.00–3.00)	1.10 (0.80–1.90)	0.003
INR - median (p 25–75)	1.42 (1.15–1.93)	1.27 (1.10–1.53)	0.01
Lactic acid (mmol/L) - median (p 25–75)	3.45 (1.58–6.13)	1.80 (1.10–3.50)	<0.001
Leukocytes (cells/mm^3) - median*10^3 (p 25–75)	15.1 (7.4–20.6)	14.5 (9.7–18.7)	0.83
PaO_2/FIO_2 ratio - median (p 25–75)	173 (97–240)	177 (127–265)	0.25
Platelets (cells/mm^3) - median*10^3 (p 25–75)	139 (74–220)	197 (133–279)	<0.001
SOFA score - median (p 25–75)	11 (9–14)	9 (7–11)	<0.001
Bloodstream infection - n (%)	11 (14.1)	21 (15.1)	0.99
Chronic renal failure – n (%)	8 (10.3)	8 (5.8)	0.28
COPD – n (%)	12 (15.4)	15 (10.8)	0.39
Diabetes mellitus – n (%)	31 (39.7)	38 (27.3)	0.07
Ischemic heart disease - n (%)	5 (6.4)	12 (8.6)	0.61
Sex female – n (%)	28 (35.9)	51 (36.7)	0.99
Site of infection			0.72
Respiratory - n (%)	44 (56.4)	76 (54.7)	
Abdominal - n (%)	22 (28.2)	39 (28.1)	
Neurological	0	3 (2.2)	
Urinary - n (%)	4 (5.1)	7 (5.0)	
Skin - n (%)	3 (3.8)	8 (5.8)	
Endocarditis - n (%)	4 (5.1)	6 (4.3)	
Osteomyelitis	1 (1.3)	0	
Microorganism responsibles			
Unknwon - n (%	42 (53.8)	73 (52.5)	0.89
Gram-positive - n (%)	20 (25.6)	31 (22.3)	0.62
Gram-negative - n (%)	16 (20.5)	35 (25.2)	0.51
Fungii- n (%)	4 (5.1)	3 (2.2)	0.25
Anaerobe - n (%)	1 (1.3)	1 (0.7)	0.99
Adequate empiric antimicrobial treatment			0.95
Unknown due to negative cultures - n (%)	42 (53.8)	73 (52.5)	
Adequate- n (%)	30 (38.5)	57 (41.0)	
Inadequate- n (%)	2 (2.6)	3 (2.2)	
Unknown due to antigenuria diagnosis- n (%)	4 (5.1)	6 (4.3)	
OGS (ng/mL) - median (p 25–75)	6.22 (4.62–9.04)	4.92 (3.37–7.57)	0.004
Malondialdehyde (nmol/mL) - median (p 25–75)	3.94 (2.75–7.51)	2.77 (2.00–4.05)	<0.001

APACHE = Acute Physiology and Chronic Health Evaluation; aPTT = Activated partial thromboplastin time; INR = International normalized ratio; PaO_2/FIO_2 = pressure of arterial oxygen/fraction inspired oxygen; SOFA = Sepsis-related Organ Failure Assessment; COPD = Chronic Obstructive Pulmonary Disease; OGS = oxidizied guanine species; data are presented as number (percentage) or median (interquartile range).

The Kaplan-Meier analysis found that patients with serum OGS levels >4.53 pg/mL showed increased mortality at 30 days (Hazard ratio = 2.2; 95% CI = 1.42–3.55; p < .001) (Fig. 2).

A positive correlation was found between serum OGS and malondialdehyde levels (rho = 0.21; p = .002). A statistically significant correlation was found between serum OGS levels and APACHE-II score (rho = 0.14; *p* = .02), and a near to be statistically significant correlation between serum OGS levels and SOFA score (rho = 0.09; *p* = .08).

5. Discussion

To the best of our knowledge, our study is the largest series reporting data on oxidative DNA damage in septic patients and the first to report

Table 2
Multiple logistic regression analyses to predict mortality at 30 days.

	Odds ratio	95% confidence interval	*p*-Value
First model			
Age (years)	1.036	1.012–1.062	0.004
Diabetes Mellitus (yes vs non)	1.039	0.519–2.081	0.91
Lactic acid (mmol/L)	1.068	0.946–1.205	0.29
SOFA score (points)	1.175	1.067–1.295	0.001
OGS (ng/mL)	1.115	1.029–1.208	0.008
aPTT (sg)	1.008	0.984–1.032	0.53
INR	1.085	0.683–1.724	0.73
Second model			
Age (years)	1.036	1.011–1.062	0.004
Diabetes Mellitus (yes vs non)	1.120	0.565–2.221	0.75
Lactic acid (mmol/L)	1.070	0.948–1.208	0.27
SOFA score (points)	1.163	1.056–1.280	0.002
OGS > 4.53 ng/mL (yes vs non)	2.739	1.354–5.541	0.005
aPTT (sg)	1.009	0.984–1.034	0.49
INR	1.068	0.670–1.703	0.78
Third model			
Diabetes Mellitus (yes vs non)	1.010	0.503–2.026	0.98
Lactic acid (mmol/L)	1.084	0.961–1.223	0.19
APACHE-II score (points)	1.100	1.045–1.158	<0.001
OGS (ng/mL)	1.108	1.021–1.204	0.01
aPTT (sg)	1.016	0.992–1.041	0.19
INR	1.096	0.688–1.748	0.70
Fourth model			
Diabetes Mellitus (yes vs non)	1.099	0.555–2.174	0.79
Lactic acid (mmol/L)	1.090	0.965–1.231	0.17
APACHE-II score (points)	1.102	1.047–1.160	<0.001
OGS > 4.53 ng/mL (yes vs non)	2.812	1.396–5.666	0.004
aPTT (sg)	1.016	0.992–1.042	0.19
INR	1.078	0.672–1.728	0.76

SOFA = Sepsis-related Organ Failure Assessment; OGS = oxidizied guanine species; aPTT = Activated partial thromboplastin time; INR = International normalized ratio; APACHE = Acute Physiology and Chronic Health Evaluation.

data on the three OGS. The new findings from our study were that serum OGS levels in septic patients were higher in non-surviving than in surviving patients, and that were associated with mortality and with serum malondialdehyde levels.

Previously, higher plasma levels of 8-OHdG had been found in 29 septic patients than in 22 healthy subjects [18], and higher urinary levels of 8-OHdG in 22 non-surviving than in 63 surviving septic patients [19]. In our study serum levels of 8-OhdG were determined, as

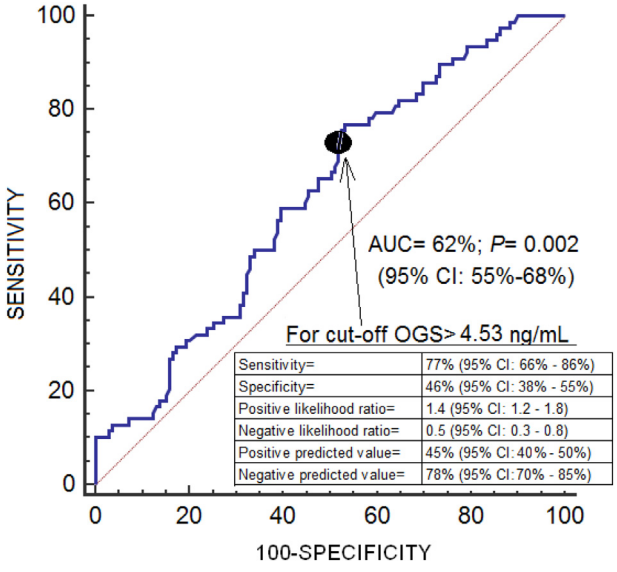

Fig. 1. Receiver operating characteristic analysis using serum oxidizied guanine species (OGS) levels as a predictor of mortality at 30 days.

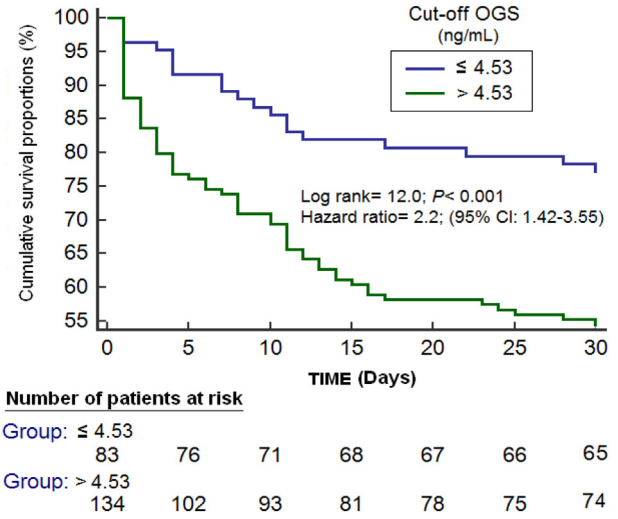

Fig. 2. Survival curves at 30 days using serum oxidizied guanine species (OGS) levels lower or equal vs higher than 4.53 ng/mL.

well as of the other two OGS (8-oxo-guanosine or 8-hydroxyguanosine from RNA, and 8-oxo-guanine or 8-hydroxyguanine from DNA or RNA). An association between serum OGS concentrations and mortality in septic patients has been found for the first time.

Another interesting new finding of our study was the positive association between serum OGS and malondialdehyde levels and therefore between oxidative DNA/RNA damage and lipid peroxidation.

We must recognize some limitations of our study. Firstly, we only determined serum OGS levels at the time of sepsis diagnosis and we cannot report serum levels during follow-up. Second, we have not determined serum OGS levels in other critically non-septic patients or in healthy subjects. However, we believe that the strengths of our study are the reported serum levels of the three OGS and not only of the 8-OhdG levels, as well as being the largest series reporting data on oxidative nucleic acid damage in septic patients.

We believe that the new findings of our study with septic patients and the findings of animal septic models on the benefits of administering antioxidant agents that reduce oxidative DNA damage and lipid peroxidation [28-31] may motivate the interest in research on the use of serum levels of oxidative DNA and RNA damage as biomarkers for predicting mortality, as well as research on the use of antioxidants agents in septic patients.

6. Conclusions

To our knowledge, our study is the largest series reporting data on oxidative DNA damage in septic patients and the first to report data on oxidative DNA and RNA damage in septic patients. The new findings from our study were that oxidative DNA and RNA damage in septic patients was associated with mortality and lipid peroxidation.

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Author contributions

- LLo conceived, designed and coordinated the study, participated in acquisition and interpretation of data, and drafted the manuscript.
- MMM, ROL, JF, JSV, LLa, CD, SP participated in acquisition of data.
- AGC and APC carried out the analyses of DNA and RNA oxidative damage.
- PAG carried out the analyses of malondialdehyde concentrations.
- AJ participated in the interpretation of data.

All authors revised the manuscript critically for important intellectual content and made the final approval of the version to be published.

Key messages

- There is a significant univariate association between serum levels of oxidizied guanine species levels and mortality in septic patients which remains significant after adjusting for potential confounds.
- There is an association between serum levels of oxidizied guanine species levels and lipid peroxidation in septic patients.

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Declaration of Competing Interest

None.

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