

CORTISOL RESPONSE TO LOW-DOSE (1 µg) ACTH STIMULATION FOR THE PREDICTION OF OUTCOME IN PATIENTS WITH SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

ODGOVOR KORTIZOLA NA NISKODOZNI (1 µg) ACTH STIMULACIONI TEST U PREDIKCIJI ISHODA KOD BOLESNIKA SA SINDROMOM SISTEMSKOG INFLAMATORNOG ODGOVORA

Jelica Bjekić-Macut¹, Vojislav Radosavljević¹, Zoran Andrić¹, Dušan Ilić², Olivera Stanojlović³,
Danijela Vojnović Milutinović⁴, Ivana Božić Antić², Marija Zdravković¹, Saša Hinić¹,
Djuro Macut², Miloš Žarković²

¹UMC Bežanijska kosa, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

²Clinic of Endocrinology, Diabetes and Metabolic Diseases, Faculty of Medicine,
University of Belgrade, Belgrade, Serbia

³Institute of Medical Physiology, Faculty of Medicine,
University of Belgrade, Belgrade, Serbia

⁴Department of Biochemistry, Institute for Biological Research »Siniša Stanković«, University of Belgrade,
Belgrade, Serbia

Summary

Background: Systemic inflammatory response syndrome (SIRS) changes cortisol dynamics and indicates dissociation between the adrenal cortex and the hypothalamo-pituitary unit. The aim of this study was to assess the cortisol response after stimulation with ACTH₁₋₂₄ in patients with SIRS at admission to the Respiratory Intensive Care Unit (RICU) and seven days later.

Methods: Fifty-four subjects were included in the study, and SIRS was defined according to the Consensus Conference criteria from 1992. Severity of the disease was determined using the APACHE II score, and organ dysfunction using the SOFA score. Low-dose (1 µg) ACTH test (LDT) was performed in all patients, and cortisol was determined along with basal ACTH. Data were analyzed using parametric and nonparametric tests and regression analysis. The results are presented as mean ± standard deviation, and P<0.05 was considered statistically significant.

Results: There were no differences in cortisol values between the two LDTs. Cortisol increment lower than 250 nmol/L during the LDT was found in 14/54 (25.9%) subjects at the onset of SIRS. Five out of 54 (9.6%) patients died within 7 days from the onset of SIRS. Female sex and

Kratak sadržaj

Uvod: Sindrom sistemskog inflamatornog odgovora (SIRS) menja dinamiku kortizola, ukazujući na disocijaciju između adrenalnog korteksa i hipotalamo-hipofizne jedinice. Cilj ovog rada je da se proceni odgovor kortizola na stimulaciju sa ACTH₁₋₂₄ u bolesnika sa SIRS-om na prijemu u respiratornu jedinicu intenzivne nege (RICU) i sedam dana kasnije.

Metode: Pedeset četiri osobe su uključene u studiju, a SIRS je definisan prema kriterijumima Konsenzus konferencije iz 1992. Ozbiljnost bolesti je ustanovljena pomoću skora APACHE II, a organska disfunkcija pomoću skora SOFA. Niskodozni (1 µg) ACTH test (LDT) izveden je u svih bolesnika uz određivanje kortizola kao i bazalnog ACTH. Podaci su analizirani pomoću parametarskih i neparametarskih testova i regresione analize. Rezultati su predstavljeni kao srednje vrednosti ± standardna devijacija, a P<0,05 je smatrano statistički značajnim.

Rezultati: U ovom istraživanju nisu uočene razlike u nivou kortizola između dva LDT-a. Porast kortizola manji od 250 nmol/L tokom LDT-a nađen je kod 14/54 (25,9%) bolesnika prilikom postavljanja dijagnoze SIRS-a. Pet od 54 (9,6%) bolesnika je umrlo unutar sedam dana od početka SIRS-a.

Address for correspondence:

Jelica Bjekić-Macut, MD
UMC Bežanijska kosa,
Autoput b.b., 11080 Belgrade, Serbia
Phone: +381 11 3010777
e-mail: jbjekic@yahoo.com

List of abbreviations: SIRS, systemic inflammatory response syndrome; ACTH, adrenocorticotropin; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; ICU/RICU, intensive care unit / respiratory intensive care unit; LDT, low-dose test; ARDS, acute respiratory distress syndrome.

maximal cortisol response (Δ max) on LDT predicted the duration of hospitalization in RICU, while APACHE II and SOFA scores best predicted the duration of hospitalization, mortality outcome as well as overall survival outcome.

Conclusions: A difference was found in Δ max at the diagnosis of SIRS and seven days later. Δ max, and primarily the clinical scores APACHE II and SOFA predicted the outcomes of hospitalization and overall survival.

Keywords: cortisol, systemic inflammatory response syndrome, ACTH test, APACHE II, SOFA

Introduction

Systemic inflammatory response syndrome (SIRS) represents an inflammatory condition that spreads all over the body. SIRS is most frequently caused by infection or sepsis. In comparison to the general term infection, sepsis is a condition in which the patient fulfills the criteria for SIRS, and has a known or very certain infection. SIRS is a serious condition that is related to systemic inflammation, organ dysfunction and complete cessation of functions. SIRS is considered a part of the »cytokine storm«, and is manifested by the dysregulation of different cytokines (1). The American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) defined SIRS using the following criteria: body temperature below 36 °C or over 38 °C, heart rate over 90 per minute, tachypnea over 20 respirations per minute or carbon dioxide partial pressure below 4.3 kPa (32 mmHg), and leukocyte number lower than 4000 cells/mm (4×10^9 cells/L) or over 12.000 cells/mm (12×10^9 cells/L), or presence of over 10% of immature neutrophils (1). The same societies accepted that severity of the disease is best predicted using the Acute Physiology and Chronic Health Evaluation II (APACHE II) score (2) while the patient's outcome, namely organ dysfunction/failure during Intensive Care Unit (ICU) monitoring, is predicted using the Sequential Organ Failure Assessment (SOFA) score (3).

Systemic inflammatory response leads to significant changes in the concentrations of different hormones. Among them, the most characteristic is the cortisol change dynamics indicating dissociation between the adrenal cortex and the hypothalamo-pituitary unit. This is manifested by very high concentrations of cortisol and ACTH immediately after the initiation of SIRS. A few days later, however, ACTH falls to a very low concentration while the concentration of cortisol remains high (4). This is explained by the direct effect of cytokines on the adrenal cortex stimulating glucocorticoid release (5).

Cortisol response to stimulation with ACTH was shown to be an important predictor in critically ill patients (6). Patients with an inadequate cortisol response to stimulation with ACTH had a high mortality rate. The use of ACTH test, however, has not

Ženski pol i maksimalni odgovor kortizola (Δ max) tokom LDT-a predviđaju trajanje hospitalizacije u RICU, dok skorovi APACHE II i SOFA najbolje predviđaju dužinu hospitalizacije, smrtni ishod kao i opšte preživljavanje.

Zaključak: Razlika u Δ max prilikom postavljanja dijagnoze SIRS-a i sedam dana kasnije a pre svega klinički skorovi APACHE II i SOFA predviđaju ishod hospitalizacije i opšte preživljavanje.

Ključne reči: kortizol, sindrom sistemskog inflamatornog odgovora, ACTH test, APACHE II, SOFA

been standardized till now and has not been performed over different periods beginning with the onset of an inflammatory condition. Stimulation with 250 μ g of ACTH is considered to be a standard test for the assessment of adrenal function (7, 8). The low-dose (1 μ g) test (LDT), however, represents a more sensitive method for detecting specific forms of adrenal insufficiency such as hypothalamo-pituitary dysfunction (9, 10). The use of LDT in critically ill patients is not clearly defined as the data on LDT are limited and insufficient for a clear recommendation (11–13).

It is known that the response of cortisol to ACTH stimulation depends on the period that has elapsed since the occurrence of inflammation (6). The aim of this study was to assess the response of the adrenal cortex after stimulation with synthetic ACTH in patients with SIRS at admission to the Intensive Care Unit (ICU) and seven days later, and to follow the outcome of ICU hospitalization.

Materials and Methods

Study population

Fifty-four consecutive patients (37 males, 17 females) were included in the study. All the investigated subjects were referred to the University Medical Center (UMC) »Bežanijska kosa«, Belgrade, due to the presence of fever, dyspnea and poor general condition. All of the patients were of Caucasian origin, with no history of malignant disease and previous therapy with ketoconazole, etomidate, and glucocorticoids. After the initial examination, most of the patients were admitted to the Respiratory Intensive Care Unit (RICU) of the UMC. If the patient was initially admitted to the Department of Pulmonology, but his clinical state soon deteriorated, he or she was transferred to the RICU. At admission to the RICU, vital status was assessed in all the patients, including state of consciousness, systemic arterial blood pressure and heart rate, and body temperature. In all the patients, community acquired pneumonia was confirmed.

SIRS was defined according to the 1992 ACCP/SCCM Consensus Conference (1) when the condition met two or more of the given criteria

(assessment of body temperature using the Celsius Scale, determination of heart rate and number of respirations per minute, and leukocyte concentration in the blood).

Data collection

Clinical evaluation. At the onset of SIRS, the following variables were recorded: (1) general characteristics including age and sex, date of RICU admission; (2) severity of disease as determined using the APACHE II score (age, history of severe organ insufficiency or being immunocompromised, rectal temperature, mean arterial pressure, heart rate, respiratory rate, oxygenation, arterial pH, serum sodium, potassium and creatinine, hematocrit, white blood cell count and Glasgow Coma Scale score); (3) mortality prediction based on the degree of dysfunction of six organ systems using the SOFA score (oxygenation, platelets count, Glasgow Coma Scale score, bilirubin, mean arterial pressure and creatinine); (4) need for and length of mechanical ventilation, number of days spent in the RICU, and the outcome of SIRS (survived or non-survived).

The APACHE II score is expressed in points from 0 to 71, and a higher score implies more severe disease and higher mortality risk. The SOFA score is used in monitoring patient status during the stay in the ICU. Both APACHE II and SOFA provide an estimate of disease severity and mortality during stay in the ICU based on a number of the abovementioned laboratory values and patient signs, taking both acute and chronic disease into account.

Laboratory variables. At admission to the hospital and the diagnosis of SIRS, hematologic and chemistry data, and blood gas determinations were done systematically. Low-dose ACTH test was performed in all patients immediately after the admission to the RICU and seven days later.

ACTH testing procedure. At admission to the RICU, an indwelling venous catheter was placed in the antecubital vein and, in all the patients, blood samples were drawn for the determination of standard biochemical analyses, cortisol and ACTH. At least 30 min after placing the catheter, the LDT was started with an IV injection of 1 µg ACTH₁₋₂₄ (Synacthen, Novartis Pharma Schweiz AG). Blood samples for cortisol determination were taken from the IV cannula at 0, 15 and 30 min. All blood samples were immediately separated and kept frozen at -80 °C until assayed. As previously described, for the low-dose ACTH test, a vial of 250 µg Synacthen was diluted in normal saline solution to a concentration of 0.5 µg/mL (10), and the solution was used immediately.

Study protocol was approved by the Ethical Committee of the Faculty of Medicine, University of

Belgrade, and informed consent was obtained from each patient or patient's next-of-kin.

Assays

Cortisol was determined using an immunoassay (Elecsys Cortisol II assay, Cobas e411 analyzer, Roche Diagnostics GmbH, Mannheim, Germany), with intra- and inter-assay CV of 1.8–7.1% and 2.7–12.7%, respectively. ACTH was also determined using an immunoassay (Elecsys ACTH assay, Cobas e411 analyzer, Roche Diagnostics GmbH, Mannheim, Germany) with intra- and inter-assay CV of 2.0–2.9% and 2.4–5.4%, respectively.

Statistical analysis

Statistical analysis was performed using R (14), Vienna, Austria (<https://www.R-project.org/>). For comparison between groups, Student t test was used for two independent samples for parametric data, and χ^2 test was employed for nonparametric data and binomial variables. Results are presented as mean \pm standard deviation ($\bar{x} \pm SD$) for the continuous variables, and as percentages for the binary variables.

Zero-inflated negative binomial regression was used for modeling count variables with excessive zeros, and for overdispersed count outcome variables. We investigated the possibility of predicting whether a patient will receive mechanical ventilation during his/her stay in the RICU and for how many days, based on patient characteristics collected at the onset of SIRS presented with APACHE II and SOFA scores.

Poisson regression model was used for prediction of days spent in the RICU based on sex and maximal cortisol increase during the ACTH stimulation test.

Logistic regression analysis was performed to predict overall outcome (survival or death). Calibration of the logistic model was assessed using the Hosmer-Lemeshow goodness-of-fit test to evaluate the importance of the discrepancy between observed and expected mortality.

For all tests, $P < 0.05$ was considered statistically significant.

Results

The clinical and biochemical characteristics of SIRS patients are presented in *Table 1*. Respiratory infections were confirmed in all patients. Of 54 patients, 5 (9.6%) died within the 7-day period following the onset and management of SIRS in the RICU.

In respect to hormonal analysis, we evaluated both the basal values of ACTH and cortisol, and cortisol values at each time point of the ACTH test. Basal ACTH concentrations did not differ between admis-

Table I Clinical and biochemical characteristics of the whole group of patients at baseline and after 7 days in RICU.

Variable		
N	54	
Age (years)	45.25±5.45	
Sex		
Male, N (%)	37 (67.3)	
Female, N (%)	17 (32.7)	
Onset of disease (days)	4.25±1.31	
Underlying disease		
Community acquired pneumonia, N (%)	48 (92.3)	
ARDS, N (%)	3 (5.8)	
Sepsis, N (%)	1 (1.9)	
APACHE II		
Point	15.79±6.16	
%	25.19±17.50	
SOFA		
Point	3.48±2.04	
%	11.92±7.93	
Mechanical ventilation		
Yes / No	9 (16.7) / 45 (83.3)	
Duration (patients on ventilation) (days)	5.14±4.49	
Duration (whole group) (days)	0.69±2.35	
Outcome		
Survived, N (%)	49 (90.4)	
Died, N (%)	5 (9.6)	
	Day 0	Day 7
ACTH (ng/dL)	6.57±7.08	6.46±8.72
Synacthen test		
Cortisol (nmol/L), 0 min	633.22±362.30	582.62±341.56
Cortisol (nmol/L), 15 min	832.25±311.89	805.35±328.51
Cortisol (nmol/L), 30 min	896.57±305.36	854.54±313.16
Cortisol (nmol/L), 60 min	884.69±339.29	841.11±363.53
Maximal cortisol (mean)	967.75±321.02	932.65±348.12
Difference cortisol (maximal value – basal value)	353.47±193.72	350.04±262.96
Difference cortisol (maximal value – 15 min value)	220.92±133.36	222.73±152.52
Difference cortisol (maximal value – 30 min value)	285.25±147.13	271.93±175.85
Difference cortisol (maximal value – 60 min value)	270.41±222.04	258.49±289.77
Maximal cortisol (Δ max) (Day 0 – Day 7)	582.62±341.56	

RICU, respiratory intensive care unit; ARDS, acute respiratory distress syndrome; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; ACTH, adrenocorticotropin.

Table II Inflated Negative Binomial Regression analysis for the prediction of mortality using APACHE II score.

Variable	Regression Coefficient (β)	SE	Odds Ratio (95% Confidence Interval)	P Value
Count Model Coefficients				
Intercept	-0.085	0.658	-0.130	0.896
Sex (female)	-0.734	0.504	-1.467	0.145
Maximal cortisol difference	0.005	0.001	3.286	0.001
Zero-Inflation Model Coefficients				
Intercept	19.696	9.285	2.121	0.033
APACHE II	-0.931	0.470	-1.981	0.047

APACHE, acute physiology and chronic health evaluation.

Table III Inflated Negative Binomial Regression analysis for the prediction of mortality using SOFA score.

Variable	Regression Coefficient (β)	SE	Odds Ratio (95% Confidence Interval)	P Value
Count Model Coefficients				
Intercept	-0.969	1.010	-0.960	0.337
Sex (female)	-4.122	1.352	-3.048	0.002
Maximal cortisol difference	0.004	0.001	3.348	<0.001
Zero-Inflation Model Coefficients				
Intercept	7.097	3.090	2.296	0.021
SOFA	-1.335	0.732	-1.824	0.068

SOFA, sequential organ failure assessment.

Table IV Poisson regression analyses for the prediction of the duration of stay in RICU.

Variable	Regression Coefficient (β)	SE	Odds Ratio (95% Confidence Interval)	P Value
Intercept	0.986	0.174	5.653	<0.001
Sex (female)	-0.106	0.190	-0.559	0.576
Maximal cortisol difference	0.0004	0.0002	1.967	0.049
Inclusion of APACHE II score				
Intercept	0.400	0.233	1.715	0.086
Sex (female)	-0.087	0.192	-0.453	0.650
Maximal cortisol difference	0.0001	0.0002	0.391	0.696
APACHE II	0.048	0.013	3.594	<0.001
Inclusion of SOFA score				
Intercept	0.643	0.207	3.101	0.001
Sex (female)	-0.065	0.191	-0.343	0.731
Maximal cortisol difference	0.0002	0.0002	1.237	0.216
SOFA	0.113	0.035	3.149	0.001

RICU, respiratory intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment.

Table V Logistic regression analysis for the prediction of overall survival outcome.

Variable	Regression Coefficient (β)	SE	Odds Ratio (95% Confidence Interval)	P Value
Inclusion of APACHE II score				
Intercept	-6.624	1.817	-3.645	<0.001
APACHE II	0.229	0.079	2.874	0.004
Inclusion of SOFA score				
Intercept	-7.579	2.327	-3.256	0.001
SOFA	1.095	0.401	2.729	0.006

APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment.

sion time to the RICU and at follow-up seven days later. Basal cortisol concentrations and cortisol values during the LDT did not differ at the two time points during follow-up. The cortisol increment defined as a difference between maximal value and the values at each sampling time point did not differ between the two tests 7 days apart. In the whole group of patients, cortisol increment lower than 250 nmol/L during the ACTH test was found in 14 (25.9%) subjects on Day 0, and in 13 (24%) subjects on Day 7. In patients who died during the follow-up period, cortisol increment lower than 250 nmol/L was recorded in 3/5 subjects (60%).

APACHE II and SOFA scores were significantly associated with prediction of mortality during hospitalization in the RICU, and are related to the maximal cortisol increase during ACTH testing (*Table II* and *Table III*). Female sex and maximal cortisol response on ACTH testing predicted duration of hospitalization in the RICU, namely, the higher the maximal cortisol response, the longer the period of hospitalization in the RICU (*Table IV*). In the logistic regression, APACHE II and SOFA scores were the only significant variables associated with prediction of the overall survival outcome (*Table V*).

Discussion

Our study was designed to assess the response of cortisol after low-dose ACTH stimulation in patients admitted to the RICU because of a fully clinically defined state of SIRS due to a respiratory infection. Although we did not find differences in either basal cortisol values or at each time point of the ACTH test, a difference in the maximal cortisol response (Δ max) during the ACTH test before and after 7 days of follow-up was clearly shown. Moreover, Δ max predicted the duration of hospitalization in the RICU and in-hospital mortality outcome. The clinical scores APACHE II and SOFA, however, were shown to be the best predictors of duration of hospitalization, mortality outcome as well as overall survival outcome.

SIRS implies a clinical response to a nonspecific insult characterized with two or more of the defined variables, while sepsis represents SIRS with a documented infection. Consequently, multiple organ dysfunction syndrome (MODS) represents a sequel of SIRS and/or sepsis, and failure to maintain homeostasis without intervention. Hence, MODS is recognized as a sequel to SIRS, a continuum that includes the development of SIRS, onset of sepsis and progression to septic shock, and multiple organ dysfunction (15), and it is related to high mortality (16). However, data on the prevalence of SIRS and sepsis are still lacking. According to some European analyses, severe infection is diagnosed in 16% of patients hospitalized in the ICU due to respiratory complications as the most common feature, with deadly outcome in approximately half of them (17).

Our group of patients had SIRS caused by a respiratory infection, and all of them continue their monitoring and treatment in the RICU. The mortality rate calculated from the APACHE II score was higher than the mortality recorded in our study. A possible explanation could be the design of the study that followed patients for seven days in comparison to other studies that followed patients for 28 days after the onset of SIRS or sepsis. Moreover, estimated organ dysfunction predicted by the SOFA score and the mortality rate predicted by the APACHE II score suggest possibly milder forms of SIRS at presentation within our group of patients.

It was observed that increased levels of plasma cortisol are related to higher risk of death in critically ill humans. Inappropriately low cortisol concentrations, however, were shown to be related to increased mortality as well (18). A new clinical description of 'critical illness-related corticosteroid insufficiency' (CIRCI) was used to define higher cortisol levels in this condition in comparison to healthy status (19). Diagnostic criteria for CIRCI in the critically ill were based on a cortisol incremental response of less than 9 mg/dL (250 nmol/L) after adrenal cortex stimulation with 250 μ g ACTH, and this was considered the

most discriminative for increased risk of death (20). During the ACTH test, a cortisol increment lower than 250 nmol/L was observed in 14/54 (25.9%) of our patients. Similar prevalence of relative adrenal insufficiency or dysfunction (less than 25%) was observed in ICU patients with diagnosis other than septic shock (13). In our group of patients, however, death as outcome was recorded in 3/5 (60%) of those with a cortisol increment lower than 250 nmol/L. Relatively lower values for deadly outcome in our patients could be related to the SIRS condition, in comparison to other studies that recorded deadly outcomes in 100% of patients with sepsis (21). Moreover, the Δ maximal cortisol increment obtained in our study far exceeds the cutoff point increment proposed for the assessment of outcome of the critically ill (20, 21) and is in line with the low prevalence of relative adrenal insufficiency in our patients with SIRS. The Δ maximal cortisol increment could be used for the prediction of length of hospitalization in the RICU and mortality outcome. However, when included into the respective analyses, the scores APACHE II and SOFA best predicted the duration of hospitalization in the RICU, mortality outcome and overall survival outcome, and the same has been shown elsewhere (2–3, 23).

Taking into consideration the above facts about the adrenal axis changes during SIRS, a high sensitivity test should be performed for the diagnosis of adrenal cortex dysfunction. It was shown that LDT with 1 µg of ACTH is more sensitive for detecting mild secondary adrenal insufficiency, and is superior to the standard stimulation test with 250 µg of ACTH (10). The patients we investigated represented a natural model of secondary adrenal insufficiency. As expected, we showed low normal levels of ACTH at both time points (Day 0 and Day 7). Although cortisol concentrations during the ACTH test showed a trend towards decrease after seven days, they did not reach statistical significance. In the repeated test, however, only the maximal cortisol response during the ACTH test showed a clear decrease. Our results are in line with a recent study on ACTH and cortisol concentrations during the first seven days of ICU monitoring. Namely, morning ACTH was suppressed from admission to the intensive care unit and remained below the limit of normality during the first week of critical illness (24). Although our results showed a similar pattern of low ACTH from the beginning of the fol-

low-up period, it could be assumed that we had missed the initial ACTH response to stress prior to admission to the ICU, and the same was supposed by others as well (24). This possibility was recently supported by the detection of low orexin activity in the hypothalamus during a prolonged phase of critical illness that happened 48 hours after the initial rise in ACTH (25).

Besides ACTH, the cortisol production rate in ICU patients was moderately increased but without the doubling of values that was observed in healthy subjects (24). Moreover, cortisol production was elevated only in patients with SIRS, possibly mediated by the activity of cytokines (1, 24). Accordingly, low ACTH and high cortisol suggest that mechanisms other than those dependent on ACTH are responsible for the high cortisol concentrations during critical illness, namely, growth factors, corticotrophin releasing hormone and ACTH loop, immune system or the local vascular system characteristics (26, 27). Nevertheless, other possibilities such as suppressed cortisol clearance with consequently elevated plasma cortisol concentrations and suppressed ACTH release should also be considered (24).

Conclusion

We showed a difference in the maximal cortisol response (Δ max) between two LDTs performed at the diagnosis of SIRS and seven days later. The Δ max, and primarily the clinical scores APACHE II and SOFA predicted clinical outcomes of hospitalization in the RICU, while the clinical scores also successfully predicted overall survival outcome. LDT represents a sensitive tool for detecting specific forms of adrenal insufficiency as it could be induced by the state of SIRS.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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