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High levels of serum macrophage migration inhibitory factor and interleukin 10 are associated with a rapidly fatal outcome in patients with severe sepsis

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SUMMARY

Objectives: The aim of this study was to delineate the association between high macrophage migration inhibitory factor (MIF) and interleukin 10 (IL-10) levels in the early phase of sepsis and rapidly fatal outcome.
Methods: One hundred and fifty-three adult subjects with the main diagnosis of severe sepsis (including septic shock) admitted directly from the emergency department of two tertiary medical centers and one regional teaching hospital between January 2009 and December 2011, were included prospectively. MIF and IL-10 levels were measured and outcomes were analyzed by Cox regression analysis according to the following outcomes: rapidly fatal outcome (RFO, death within 48 h), late fatal outcome (LFO, death between 48 h and 28 days), and survival at 28 days.
Results: Among the three outcome groups, IL-10 levels were significantly higher in the RFO group ($p < 0.001$) and no significant differences were seen between the LFO and survivor groups. After Cox regression analysis, each incremental elevation of 1000 pg/ml in both IL-10 and MIF was independently associated with RFO in patients with severe sepsis. Each incremental elevation of 1000 pg/ml in IL-10 increased the RFO risk by a factor of 1.312 (95% confidence interval 1.094–1.575; $p = 0.003$); this was the group, and of the two, IL-10 was the most significant factor linked to RFO.

1. Introduction

Sepsis is one of the main causes of death among hospitalized patients, and current evidence suggests that sepsis care bundles can reduce mortality rates in septic patients.[1] However, identifying the risk factors for rapidly fatal outcome (RFO) in septic patients remains a great challenge. It has been suggested that during sepsis the immune response reflects an interaction between pro-inflammatory and anti-inflammatory mediators.[2] The early-phase systemic inflammatory response syndrome (SIRS) is characterized by the expression of pro-inflammatory mediators and is followed by the development of a compensatory anti-inflammatory response syndrome (CARS).

An exaggerated early-phase response, which occurs by the expression of pro-inflammatory mediators, can lead to early

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mortality as a result of shock.[3] This can be visualized by increased levels of macrophage migration inhibitory factor (MIF), a key mediator of the systemic inflammatory response, which predicts early death from severe sepsis.[4,5] In a previous study, we found that positive sputum *Klebsiella pneumoniae* cultures were able to predict RFO in septic patients from the medical intensive care unit (ICU).[6] Significantly elevated levels of interleukin 10 (IL-10), a potent anti-inflammatory mediator, were found in patients with bronchoalveolar lavage cultures positive for *K. pneumoniae* as compared to other pathogens.[7] In a mouse model of systemic *K. pneumoniae* infection, an elevation in IL-10 was observed within 24 h and peaked within 12 h after infection. Rukavina et al. speculated that in infected animals, the dynamics of the increased IL-10 production during the early phase was responsible for the inadequate inflammatory reaction and control of the infection.[8] All these findings suggest that the simultaneous appearance of high MIF (a pro-inflammatory mediator) and IL-10 (an anti-inflammatory mediator) levels in the early phase of sepsis could explain, in patients with severe sepsis, the association with RFO.

In the present study we investigated MIF and IL-10 concentrations in patients with severe sepsis to examine their association with RFO.

2. Materials and methods

2.1. Study population

Adult patients (>18 years of age) with the main diagnosis of severe sepsis (including septic shock) and admitted directly from the emergency department (ED) were recruited in this prospective observational study at the medical ICU of Taoyuan General Hospital (TYGH; a 20-bed unit in a 600-bed regional teaching hospital) between January 2009 and June 2010, at the National Taiwan University Hospital (NTUH; a 44-bed unit in a 1200-bed tertiary medical center), and at the Far-Eastern Memorial Hospital (FEMH; a 20-bed unit in a 1000-bed tertiary medical center) during the period October 2010 to December 2011. Patients transferred from other ICU units or from a general ward were excluded. Patients were enrolled in the study only after their legal representatives had signed informed consent forms and blood samples had been collected in the morning between 6 a.m. to 8 a.m. within 24 h of admission to the medical ICU. The study protocol complied with the Declaration of Helsinki and was approved by the ethics committees of TYGH (TYGH99024), NTUH (201104074RC), and FEMH (100043-E).

2.2. Clinical information

Gender, age, and underlying diseases of the patients were recorded. Clinical variables that were recorded during the first 24 h in the medical ICU included the presence of an infection focus such as pneumonia, urinary tract infection (UTI), or intra-abdominal infection (IAI), organ dysfunction, coagulation profile, the Acute Physiology and Chronic Health Evaluation (APACHE) II score, and the results of various cultures.

2.3. Definitions

The rapidly fatal outcome (RFO) group was defined as patients who died within the first 48 h after medical ICU admission. The late fatal outcome (LFO) group was defined as patients who died more than 48 h after medical ICU admission. The survivor group was defined as patients who lived more than 28 days after medical ICU admission. A clinical diagnosis of pneumonia, UTI, or IAI was made at the time of medical ICU admission if the patient presented with associated symptoms, laboratory data, or radiographic evidence.[9] Specimens for microbiological studies, including sputum, urine, body fluid, and at least two sets of blood cultures, were obtained according to clinician judgment before the administration of antibiotics in the ED or medical ICU. Blood culture specimens were inoculated into BACTEC culture bottles using the BACTEC 9240 system (Becton Dickinson, Cockeysville, MD, USA). The adequacy of empirical antibiotics was determined in patients with microbiological diagnoses according to the in vitro sensitivities of microorganisms. Severe sepsis was defined as sepsis associated with acute organ dysfunction, including the following: cardiovascular dysfunction (systolic blood pressure <90 mmHg, mean arterial pressure <65 mmHg, or a reduction in systolic blood pressure >40 mmHg), respiratory dysfunction (bilateral pulmonary infiltrates with a ratio of partial pressure of arterial oxygen to fraction of inspired oxygen <300 mmHg), renal dysfunction (serum creatinine levels >2 mg/dl or urine output <0.5 ml/kg/h for 2 h), hepatic dysfunction (total serum bilirubin levels >2 mg/dl), hematologic dysfunction (platelet counts $<100 \times 10^9$/l), and metabolic dysfunction (serum lactate levels >2 mmol/l).[10,11]

2.4. Data collection

Blood was collected in sterile test tubes containing heparin and centrifuged at $1000 \times g$ for 10 min. Plasma was aliquoted into Eppendorf tubes and stored at $-70\,^{\circ}C$ until analysis. Plasma MIF and IL-10 concentrations were measured using sandwich enzyme-linked immunosorbent assays (ELISA) in accordance with the manufacturers' instructions (RayBiotech, Norcross, GA, USA for MIF; Biolegend, San Diego, CA, USA for IL-10). The minimum detectable concentration was <6 pg/ml for MIF and 2 pg/ml for IL-10. The intra-assay variation was less than 10% for MIF and between 5.2% and 6.1% for IL-10. The inter-assay variation was <12% for MIF and between 6.7% and 7.8% for IL-10. These data were provided by the manufacturers.

2.5. Statistical analysis

The data were compiled and analyzed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Comparisons among the three outcome groups were assessed using the Kruskal–Wallis test for continuous variables and the Chi-square or Fisher's exact test for categorical variables when appropriate. Multivariate Cox regression analysis, after adjustment for age and gender, was used to identify the significant independent factors predicting RFO. Odds ratios and the corresponding 95% confidence intervals (95% CI) were calculated. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 153 patients with the main diagnosis of severe sepsis were enrolled in the medical ICU during the study period. Ninety-five of these patients were male, and the mean age of all patients was 71 years (range 29–95 years). Forty-four patients (28.7%) died within 28 days and 12 patients (27.3%) had RFO. No significant differences existed among the three outcome groups for age, gender, or Charlson co-morbidity index. There were significant differences among the three groups with regard to organ dysfunction, including cardiovascular, metabolic, and hematologic dysfunction, APACHE II scores, and Glasgow coma scale scores (Table 1). In the post-hoc analysis, the RFO group had a significantly higher incidence of cardiovascular, metabolic, and hematologic dysfunction as compared to the survivor group. However, no differences were seen between the RFO and LFO groups in organ dysfunction of the above-mentioned organs.

Severe sepsis was caused by Gram-negative bacteria in 79 patients (51.6%), Gram-positive bacteria in 26 patients (16.9%), and

Table 1
Patient characteristics on the day of ICU admission[a]

Characteristics	RFO group (n = 12)	LFO group (n = 32)	Survivor group (n = 109)	p-Value
Age, years	67.5 ± 17.8	73.8 ± 11.8	71.4 ± 14.7	0.599
Gender, male/female	6/6	21/11	68/41	0.598
Charlson co-morbidity index	1.8 ± 1.0	2.2 ± 2.0	2.5 ± 2.3	0.781
Underlying medical conditions				
Alcohol intake	2 (16.7%)	3 (9.4%)	14 (12.8%)	0.708
Diabetes mellitus	6 (50.0%)	13 (40.6%)	38 (34.9%)	0.525
Liver cirrhosis	2 (16.7%)	5 (15.6%)	6 (5.5%)	0.074
Uremia	1 (8.3%)	2 (6.2%)	8 (7.3%)	0.999
Malignancy	1 (8.3%)	5 (15.6%)	14 (12.8)	0.921
Acute organ dysfunction				
APACHE II score	26.8 ± 6.7	25.1 ± 7.6	22.0 ± 7.5	0.046
Glasgow coma scale score	5.7 ± 3.4	7.8 ± 3.7	8.6 ± 3.7	0.033
Cardiovascular dysfunction	10 (83.3%)[b]	26 (81.2%)	66 (60.6%)	0.042
Respiratory dysfunction	6 (50.0%)	12 (37.5%)	44 (40.4%)	0.762
Renal dysfunction	7 (63.6%)	16 (51.6%)	55 (52.9%)	0.789
Hepatic dysfunction	2 (16.7%)	5 (15.6%)	12 (11.0%)	0.645
Metabolic dysfunction	11 (91.7%)[b]	25 (80.6%)	61 (58.1%)	0.008
Hematologic dysfunction	7 (58.3%)[b]	11 (34.4%)	20 (18.3%)	0.004

ICU, intensive care unit; RFO, rapidly fatal outcome; LFO, late fatal outcome; APACHE, Acute Physiology and Chronic Health Evaluation.
[a] All data are presented as the mean ± standard deviation, or number (%).
[b] Comparison between the RFO and survival groups with $p < 0.05$.

fungi in eight patients (5.2%) (Table 2). Mixed infections occurred in 23 patients (15.0%); one of them had two different Gram-positive bacteria, 11 had at least two different Gram-negative bacteria, and 11 had both Gram-positive and Gram-negative bacteria. The remaining 51 patients had negative culture results. A total of 129 pathogens were isolated from 102 patients in this study. Blood cultures were positive in 44 patients (28.7%). *K. pneumoniae* was the most common pathogen in all positive cultures (23.2%, 30/129) and in positive blood cultures (25.0%, 11/44). A total of 12 pathogens with antimicrobial resistance were isolated from 11 patients: eight were methicillin-resistant *Staphylococcus aureus*, two were extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, and two were ESBL-producing *K. pneumoniae*. There were no significant differences in infection foci, pathogens, bacteremia, resistant pathogens, and antibiotic adequacy among the three groups.

The MIF levels were highest in the RFO group and lowest in the survivor group; however, no significant differences were found among the three groups (median 5616.4 pg/ml, interquartile range (IQR) 1547.9–10275.4 pg/ml in the RFO group; median 1688.6 pg/ml, IQR 1030.5–13161.0 pg/ml in the LFO group; median 1439.6 pg/ml, IQR 726.3–6619.6 pg/ml in the survivor group) (Figure 1, p = 0.081). The IL-10 levels differed significantly among the three outcome groups (Figure 2, $p < 0.001$). The post-hoc analysis also showed significant differences in the IL-10 levels between the RFO (median 1926.6 pg/ml, IQR 166.5–3120.2 pg/ml) and LFO (median 71.7 pg/ml, IQR 28.8–371.0 pg/ml; p = 0.031) groups and between the RFO and survivor (median 35.4 pg/ml, IQR 10.8–136.4 pg/ml; $p < 0.001$) groups, but no differences in the IL-10 levels existed between the LFO and the survivor groups.

The results of the Cox regression analysis for factors associated with RFO are summarized in Table 3. Univariate analysis revealed several factors that were associated with RFO, and these included APACHE II score, Glasgow coma scale score, cardiovascular, metabolic, and hematologic dysfunction, and each 1000 pg/ml incremental elevation in IL-10 levels. Multivariate analysis, after adjustment for age and gender, revealed that cardiovascular,

Table 2
Infection foci and microbiological data obtained during admission to the ICU[a]

	RFO group (n = 12)	LFO group (n = 32)	Survivor group (n = 109)	p-Value
Infection focus				
Pneumonia	7 (58.3%)	15 (46.9%)	61 (56.0%)	0.663
Urinary tract infection	4 (33.3%)	5 (15.6%)	28 (25.7%)	0.396
Intra-abdominal infection	2 (16.7%)	6 (18.8%)	13 (11.9%)	0.567
Other	2 (16.7%)	7 (21.9%)	9 (8.3%)	0.079
Pathogen				
Gram-positive bacteria	3 (25.0%)	4 (12.5%)	19 (17.4%)	0.510
Gram-negative bacteria	6 (50.0%)	20 (62.5%)	53 (48.6%)	0.375
Fungi	0 (0.0%)	4 (12.5%)	4 (3.7%)	0.139
Bacteremia				
Gram-positive bacteremia	1 (8.3%)	2 (6.3%)	6 (5.5%)	0.730
Gram-negative bacteremia	3 (25.0%)	9 (28.1%)	19 (17.4%)	0.322
Fungemia	0 (0.0%)	3 (9.4%)	1 (0.9%)	0.060
Resistant pathogen				
Gram-positive resistant strain	2 (16.7%)	2 (6.3%)	4 (3.7%)	0.255
Gram-negative resistant strain	0 (0.0%)	1 (3.1%)	3 (2.8%)	0.706
Microbiological diagnosis and antibiotic adequacy				
With microbiological diagnosis				0.315
Adequate antibiotics	4 (50.0%)	20 (76.9%)	45 (66.2%)	
Inadequate antibiotics	4 (50.0%)	6 (23.1%)	23 (33.8%)	
Without microbiological diagnosis	4 (33.3%)	6 (18.8%)	41 (37.6%)	

ICU, intensive care unit; RFO, rapidly fatal outcome; LFO late fatal outcome.
[a] All data are presented as numbers (%).

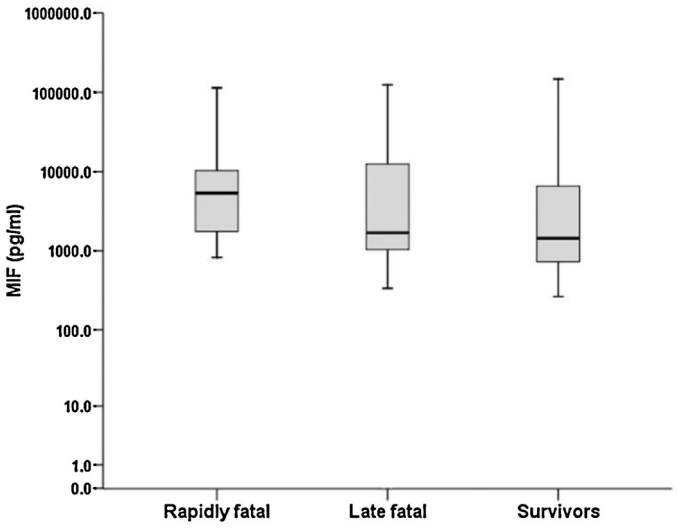

Figure 1. Box plots of macrophage migration inhibitory factor (MIF) levels among patients. Box plots show the median (center line), interquartile range (the 25th to the 75th percentile; box), and the 5th and 95th percentiles (whiskers). *p*-Values determined by the Kruskal–Wallis test for comparisons among groups were 0.081.

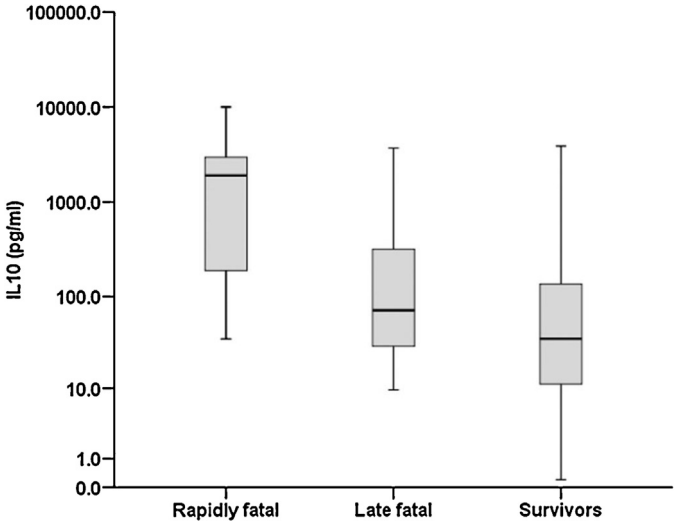

Figure 2. Box plots of interleukin 10 (IL-10) levels among patients. Box plots show the median (center line), the interquartile range (the 25th to the 75th percentile; box), and the 5th and 95th percentiles (whiskers). *p*-Values determined by the Kruskal–Wallis test for comparisons among groups were less than 0.001. For participants who had a rapidly fatal outcome vs. survivors, the *p*-value was less than 0.001. For those who had a rapidly fatal outcome vs. late fatal outcome, the *p*-value was 0.031.

metabolic, and hematologic dysfunction, and each incremental elevation of 1000 pg/ml in IL-10 and MIF levels were independently associated with RFO in patients with severe sepsis. Each incremental elevation of 1000 pg/ml in IL-10 increased the risk of RFO by a factor of 1.312 (95% CI 1.094–1.575; $p = 0.003$) and this was the most significant factor leading to RFO in patients with severe sepsis.

4. Discussion

Analysis of the clinical data and MIF and IL-10 levels in 153 patients with severe sepsis revealed that the secretion of pro-inflammatory mediators such as MIF and anti-inflammatory mediators such as IL-10 occurred spontaneously in the early phase of severe sepsis. We also found that each incremental elevation of 1000 pg/ml in IL-10 and MIF levels was independently associated with RFO in patients with severe sepsis. These findings are consistent with our theory that a simultaneous increase in MIF and IL-10 levels in patients with severe sepsis could be associated with RFO.

Classically the two-phase model of sepsis consists of an initial pro-inflammatory phase followed by an anti-inflammatory phase. Our study clearly demonstrated that both pro- and anti-inflammatory cytokines such as MIF and IL-10 occurred simultaneously in patients with severe sepsis. Tamayo et al. reported similar results, with both pro- and anti-inflammatory responses being simultaneously regulated in the early phase of severe sepsis.[12] IL-10 has been found to be a powerful predictor of 28-day and hospital mortality in patients with severe sepsis at admission, 48 h,[13] 72 h,[14] and even up to 15 days.[15] In three recent studies using multiplex analysis of cytokines, IL-10 was not a significant predictor of early (<48 h) mortality[16] or even mortality at 28 days[16,17] in two of the studies, but in the other it emerged as a significant predictor of early mortality (<72 h) and mortality at 28 days.[18] Our findings also demonstrated that an incremental elevation in IL-10 levels was the most powerful predictor of RFO in patients with severe sepsis. There are several possible reasons to explain these discrepant findings. First, the sources of patients were different. None of the three studies mentioned the source of the patients.[16–18] Our study clearly stated that the patients had been transferred from the ED to the medical ICU, thus ensuring unified clinical characteristics of the participants. Second, the timing of blood sample collections was different. One study mentioned that blood samples were collected between 10:00 and 12:00 a.m.,[16] while another study mentioned that they were collected at the time of admission.[17] Both our study and that of Andaluz-Ojeda et al. collected blood samples within the first 24 h following ICU admission.[18] Third, these studies used different assays to measure cytokine levels. Three studies used multiplex cytokine assays, while our study

Table 3
Multivariate Cox proportion hazard model of prognostic factors associated with rapidly fatal outcome in patients with severe sepsis

Factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age (years)	1.002	0.981–1.023	0.853	1.019	0.991–1.047	0.180
Gender (male)	0.921	0.502–1.690	0.791	0.844	0.418–1.704	0.637
Cardiovascular dysfunction	2.541	1.181–5.468	0.017[a]	2.497	1.104–5.646	0.028[a]
Metabolic dysfunction	3.152	1.402–7.087	0.005[a]	2.827	1.119–7.142	0.028[a]
Hematologic dysfunction	2.729	1.494–4.988	0.001[a]	2.849	1.343–6.047	0.006[a]
MIF (each increment of 1000 pg/ml)	1.009	1.000–1.019	0.053	1.013	1.003–1.023	0.012[a]
IL-10 (each increment of 1000 pg/ml)	1.407	1.217–1.626	<0.001[a]	1.312	1.094–1.575	0.003[a]

HR, hazard ratio; CI, confidence interval; MIF, macrophage migration inhibitory factor; IL-10, interleukin 10.
[a] Significant; $p < 0.05$.

used ELISA kits for the quantitative determination of cytokines.[16–18] While cytokine detection by Luminex xMAP technology is comparable to ELISA measurements, inter-institutional variations could still exist.

Previous studies suggested that high MIF levels and an inappropriate adrenal response were associated with early death in patients with severe sepsis.[4,5] Our results also demonstrated incremental elevations in MIF levels to be one of the independent predictors of RFO in patients with severe sepsis. These findings support the initial pro-inflammatory phase that classically occurs in the two-phase model of sepsis. The intraperitoneal injection of recombinant MIF in a rat model of sepsis induced disseminated intravascular coagulation (DIC).[19] Continuously high levels of MIF, soluble fibrin, and low protein C activity were also found in septic patients with DIC.[20] There was a weak but significant correlation between both peak MIF/peak protein C ($r = 0.302$, $y = -0.74x + 45.8$; $p < 0.0001$) and total MIF/total protein C ($r = 0.278$, $y = -0.218x + 70.0$; $p < 0.0001$) in a study conducted by Gando et al. (personal communication). We postulated a significantly negative correlation between MIF and protein C activity levels that induced DIC in patients with RFO, but this needs to be further explored in our future work.

IL-10 is a potent endogenous immunosuppressant cytokine that is secreted by a variety of cell types, including T helper (Th) 2 cells, monocytes/macrophages, dendritic cells, and epithelial cells. It inhibits Th1 pro-inflammatory cytokines, including interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-1β, IL-8, and IL-12.[21] MIF is a key mediator of systemic inflammatory responses that is secreted by the anterior pituitary gland and immune cells, mainly T cells. It stimulates the expression and secretion of pro-inflammatory cytokines including TNF-α, IFN-γ, IL-1β, IL-6, and IL-8.[22] Our study confirmed that a group of patients who died rapidly due to severe sepsis had significant incremental elevations in MIF and IL-10 levels. In future studies, it will be our highest priority, and interesting to investigate, whether pro-inflammatory cytokines secreted by Th1 cells are produced under the dual effects of high MIF and IL-10 levels in severely septic patients with RFO. The differential expression profile of Th1/Th2 cells in patients with severe sepsis and the different outcomes will also be included in future studies.

Several limitations of this study should be mentioned. First, the number of patients in the RFO group was limited due to difficulties in obtaining informed consent signed by legal representatives, especially for patients whose condition was rapidly deteriorating. Second, the role of other cytokines that have been studied at the time of sepsis using cytokine multiplex analysis[16,17] could not be determined in this study because of the single-point blood sampling design. Third, the lymphocytes from our patients could not be further evaluated because, due to the design of this study, we did not collect the peripheral blood mononuclear cells. Further prospective studies are needed to explore the relationships between serial multiple cytokine levels and lymphocyte expression in severe septic patients with different outcomes. In summary, our study revealed both MIF and IL-10 to be spontaneously produced in the early phase of severe sepsis, and incremental elevations in these cytokines were found to be independently associated with RFO in patients with severe sepsis. Whether the spontaneous appearance of both MIF and IL-10 affects the expression of Th1/Th2-related cytokines in patients with severe sepsis, and thereby shapes the different outcomes, deserves further investigation.

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Conflict of interest: All authors have no conflicts or financial interests to declare.

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