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Original Article

Serum neprilysin levels are elevated in preeclampsia

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ABSTRACT

Objective: To evaluate the possible associations between serum Neprilysin (NEP) levels and preeclampsia and mild and severe preeclampsia subgroups.
Materials and methods: Fifty-five consecutive women with mild preeclampsia and fifty-five consecutive women with severe preeclampsia were compared with 110 approximately gestational age-matched (±1 week) women with an uncomplicated pregnancy.
Results: Mean serum NEP was significantly higher in women with preeclampsia compared to that of the gestational age-matched-controls (231.62 ± 65.30 pg/mL vs. 187.75 ± 84.38 pg/mL, $p < 0.001$). Mean serum NEP was significantly higher in the mild preeclampsia group compared to its gestational age-matched control group (228.84 ± 67.26 pg/mL vs. 186.14 ± 85.09 pg/mL, $p = 0.008$); and in the severe preeclampsia group compared to its gestational age-matched control group (234.45 ± 63.85 pg/mL vs. 189.29 ± 84.59 pg/mL, $p = 0.004$). Serum NEP was positively correlated with systolic and diastolic blood pressure, BUN, uric acid, and creatinine.
Conclusion: Mean serum NEP was significantly higher in women with preeclampsia than women with an uncomplicated pregnancy. Further studies are needed to elucidate the possible therapeutic role of NEP inhibitors to treat preeclampsia.

Introduction

Preeclampsia, one of the hypertensive disorders of the pregnancy, which may be evaluated in two main stages. The first stage is the abnormal placental development and placenta-derived factors, and the second stage is endothelial dysfunction [1]. It may involve intrauterine growth and many maternal organ systems such as cardiovascular, renal, and neurological systems [2,3]. Many vasoactive peptides are known to be affected in preeclampsia [4–7].

Neprilysin (NEP), also known as endopeptidase-24.11, CD10, enkephalinase, neutral endopeptidase, and common acute lymphoblastic leukemia antigen (CALLA), is a zinc-metalloproteinase [8–10]. It was first isolated in the proximal tubules microvillus in the rabbit kidney, followed by the brain, lung, heart, testis, and adipocytes [11]. It has a molecular weight of 93 kDa and can be found as a free-circulating enzyme or attached to the plasma membrane of the neutrophils [12]. Insulin regulation, control of the inflammation, degradation of the natriuretic peptide in the heart and vessels, and degradation of beta-amyloid in the brain are among the biological effects of NEP [12]. NEP plays a role in the regulation of vasoactive peptides in patients with cardiovascular disease. These peptides include natriuretic peptides, angiotensin 2, bradykinin, substance P, adrenomedullin, and endothelin-1 [12]. Moreover, serum NEP levels are associated with acute and chronic heart failure [13,14].

There are many studies about the importance of the pathophysiological role of the peptides regulated by NEP in preeclampsia [15]. However, there is a scarcity of data about serum NEP levels in preeclampsia. There are only two studies about placental NEP expression in preeclampsia, which suggested controversial results [16,17].

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In this study, we aimed to evaluate the possible associations between serum NEP levels and preeclampsia and mild and severe preeclampsia subgroups for the first time in the literature.

Materials and methods

This cross-sectional study was carried out between February 2018 and March 2020 at Istanbul Cerrahpasa University, University Hospital for Obstetrics and Gynecology, Istanbul, Turkey, with the cooperation of Carl von Ossietzky University Oldenburg, University Hospital for Obstetrics and Gynecology in Klinikum Oldenburg AöR, Oldenburg, Germany. The study protocol was authorized by the Ethics Committee of Istanbul Cerrahpasa University, School of Medicine (Date: 04.04.2014 and 11.03.2019, Number: 8879 and 39712/83045809 - 604.01.02). All patients gave informed consent. The regulations of the World Medical Association Declaration of Helsinki were followed.

A pilot study was performed to specify the sample size using the primary outcome of our study, serum NEP. The serum NEP levels of twenty pregnant women (n = 10 with preeclampsia, n = 10 with uncomplicated pregnancy) were measured (232.66 ± 68.11 pg/mL vs. 181.27 ± 72.19 pg/mL, respectively). The alpha error was accepted as 0.05, and the target power was taken as 80%, which gave a minimum sample size of 28 women in each group. The patients in the pilot study were excluded from the statistical calculations.

After the pilot study, in order to assess the mild and severe preeclampsia subgroups, fifty-five consecutive women with mild preeclampsia and fifty-five consecutive women with severe preeclampsia were included. In order to eliminate potential bias regarding the effect of gestational age on our results, gestational age matching (±1 pregnancy week) was performed for the control group. The gestational age at the time of diagnosis of preeclampsia was recorded for every preeclamptic woman so that a non-preeclamptic pregnant woman approximately in this pregnancy-week (±1) with an appropriate for gestational age (AGA) fetus was included in our study. The blood samples were obtained at the time of recruitment.

The inclusion criteria were age between 18 and 40 and singleton pregnancy. The exclusion criteria included fever, any known disorders associated with inflammatory response, hypertension, maternal systemic diseases, and premature rupture of membranes or onset of labor.

Preeclampsia was defined as the arterial blood pressure (ABP) above 140/90 mmHg and measured twice at least four hours apart after the 20. GW in a pregnant woman with no history of hypertension and proteinuria (>300 mg/d or a protein/creatinine ratio of ≥30 mg/ml in spot urine).

Severe preeclampsia defining criteria included an ABP ≥160 mmHg/110 mmHg, thrombocytopenia; liver function impairment; the right upper quadrant or epigastric pain; renal insufficiency; pulmonary edema; new-onset idiopathic headache; visual disturbances or signs of placental insufficiency such as intrauterine growth retardation.

Serum NEP being the primary outcome; complete blood count (CBC), serum creatinine, total bilirubin, uric acid, blood urea nitrogen (BUN), alanine and aspartate aminotransferase were the secondary outcomes of the study.

The venous blood samples were collected and readily centrifugated at 1000 g for 15 min at 2–8 °C. The plasma phase was obtained and stored at −80 °C until quantitative measurement using the Human Neprilysin ELISA kit (SL2398Hu, Sunlong Biotech, Hangzhou, China), according to the manufacturer's instructions. A standard automated plate reader (Thermo Scientific Microplate Reader, MA, USA) was used for the optical density (wavelength of 450 nm). The detection range of kits was 16 pg/ml-1000 pg ml, and the sensitivity was 4.5 pg/ml.

The clinical characteristics at the time of recruitment as well as the laboratory results were recorded. The mild and severe preeclampsia subgroups and the gestational age-matched control groups were compared using the T-test for the comparison of the homogenous continuous variables. The possible correlations between the homogenous continuous variables were tested by Pearson's correlation test. <0.05 was accepted as the level of statistical significance.

Results

All the distributions of continuous variables were homogenous. There were no significant differences in demographic data (age, BMI, parity) between the preeclampsia and non-preeclampsia groups (see Table 1). Mean serum NEP was significantly higher in women with preeclampsia compared to that of the gestational age-matched-controls (231.62 ± 65.30 pg/mL vs. 187.75 ± 84.38 pg/mL, $p < 0.001$). Serum NEP levels in the control and preeclampsia groups according to the gestational age at sampling have been shown (Fig. 1). There were no significant differences in white blood cell (WBC) count and total bilirubin between the groups. Mean serum creatinine, uric acid, blood urea nitrogen (BUN), alanine, and aspartate aminotransferase were significantly higher, and the platelet count was significantly lower in the preeclampsia group than that of the controls (Table 1).

The demographic characteristics and biochemical parameters in the mild and severe preeclampsia groups and their gestational age-matched control groups are presented in Tables 2 and 3, respectively. Mean serum NEP was significantly higher in the mild preeclampsia group compared to its gestational age-matched control group (228.84 ± 67.26 pg/mL vs. 186.14 ± 85.09 pg/mL, $p = 0.008$); and in the severe preeclampsia group compared to its gestational age-matched control group (234.45 ± 63.85 pg/mL vs. 189.29 ± 84.59 pg/mL, $p = 0.004$) (Fig. 2a and b).

There was no significant difference in mean serum NEP levels between the mild and severe preeclampsia groups (228.84 ± 67.26 pg/mL vs. 234.45 ± 63.85 pg/mL, $p = 0.677$) (Table 4).

There was no correlation between serum NEP and age, BMI, parity, GA at sampling, WBC, platelet count, total bilirubin, and transaminases. Serum NEP was positively correlated with systolic and diastolic blood pressure, BUN, uric acid, and creatinine (Table 5).

Discussion

NEP has more than 50 peptide substrates. These peptides are found mainly in the heart, vessels, kidney, nerve cells, gastrointestinal and respiratory tracts, as well as inflammatory medium [18].

In the cardiovascular system, NEP plays a role in the hydrolysis of vasodilator substrates such as natriuretic peptides, bradykinin, substance P and adrenomedullin, and in the hydrolysis of vasoconstrictive substrates such as angiotensin 2 and endothelin 1. The most important ones are natriuretic peptides. The net effect of NEP on the cardiovascular system is determined by its affinity to these substrates [19–21].

The fact that NEP is widely distributed in the body and interacts with numerous substrates suggests that it may play a role in various physiological and pathological processes. The most critical investigations regarding the pathological conditions were performed on patients with heart failure, and the NEP levels were found high in these patients. These high levels were associated with complications in patients with heart failure with low ejection fraction [14]. The autonomic nervous system, renin-angiotensin-aldosterone system, and natriuretic peptide system have essential roles in the development of heart failure [22–24]. Recently, the combination of

Table 1
Demographic, clinical and laboratory features of the women with and without preeclampsia.

	Preeclampsia (n = 110)		Control Group gestational age matched for preeclampsia (n = 110)		p
	Mean	SD	Mean	SD	
Age (years)	29.90	5.38	28.61	5.15	0.085
BMI (kg/m^2)	30.90	4.69	29.89	4.41	0.120
Parity (n)	0.92	1.32	1.10	1.01	0.281
Systolic arterial blood pressure (mm/Hg)	156.3	14.6	113.6	14.0	<**0.001**
Diastolic arterial blood pressure (mm/Hg)	99.8	10.0	73.7	10.6	<**0.001**
GA at sampling (w)	32.15	4.88	32.46	4.82	0.636
GA at birth (w)	34.10	4.39	38.42	1.33	<**0.001**
WBC (10^9/L)	10.66	2.77	10.38	2.90	0.524
Platelet count (10^3/μL)	207.44	71.77	231.19	62.02	**0.017**
Creatinine (mg/dL)	0.60	0.11	0.50	0.10	<**0.001**
Total bilirubin (mg/dL)	0.41	1.09	0.37	0.27	0.734
Uric acid (mg/dL)	5.59	1.26	4.22	0.94	<**0.001**
BUN (mg/dL)	23.05	8.57	15.57	4.93	<**0.001**
AST (IU/L)	28.98	33.68	15.97	6.20	**0.001**
ALT (IU/L)	25.30	42.81	11.91	7.08	**0.005**
Neprilysin (pg/mL)	231.62	65.30	187.75	84.38	<**0.001**

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body-mass-index; BUN, blood urea nitrogen; GA, gestational age; SD, standard deviation; WBC, white blood cells.
P < 0.05 is significant (marked with bold).

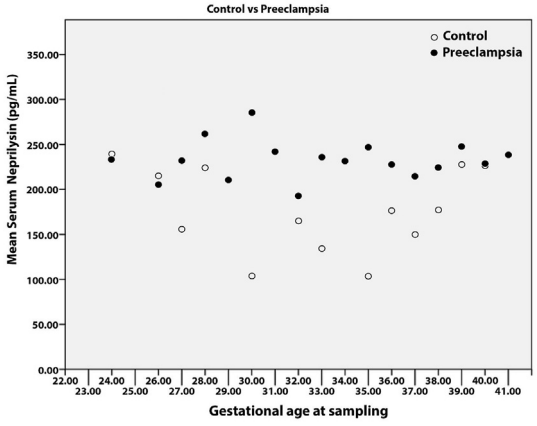

Fig. 1. Serum NEP levels in the control and preeclampsia groups according to the gestational age at sampling.

Table 2
Demographic, clinical and laboratory features of the women with mild preeclampsia and women in the control group matched for mild preeclampsia.

	Mild preeclampsia (n = 55)		Control Group gestational age matched for mild preeclampsia (n = 55)		p
	Mean	SD	Mean	SD	
Age (years)	29.58	5.28	28.46	5.09	0.283
BMI (kg/m^2)	31.51	5.16	30.45	4.32	0.271
Parity (n)	0.92	1.46	1.14	1.03	0.388
Systolic ABP (mm/Hg)	145.7	8.8	116.3	14.0	<**0.001**
Diastolic ABP (mm/Hg)	93.0	7.0	75.7	9.7	<**0.001**
GA at sampling (w)	33.76	4.49	34.04	4.68	0.749
GA at birth (w)	35.45	3.84	38.34	1.23	<**0.001**
WBC (10^9/L)	10.57	2.98	10.81	3.36	0.712
Platelet count (10^3/μL)	223.0	72.8	220.0	60.8	0.830
Creatinine (mg/dL)	0.57	0.10	0.50	0.09	<**0.001**
Total bilirubin (mg/dL)	0.33	0.26	0.45	0.36	0.076
Uric acid (mg/dL)	5.37	1.17	4.11	0.76	<**0.001**
BUN (mg/dL)	20.70	6.73	15.13	3.84	<**0.001**
AST (IU/L)	21.12	13.70	16.06	6.37	**0.025**
ALT (IU/L)	14.26	11.90	11.43	8.10	0.180
Neprilysin (pg/mL)	228.84	67.26	186.14	85.09	**0.008**

ABP, arterial blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body-mass-index; BUN, blood urea nitrogen; GA, gestational age; WBC, SD, standard deviation; white blood cells.
P < 0.05 is significant (marked with bold).

angiotensin-2 receptor antagonist valsartan and NEP inhibitor sacubitril, Ang receptor NEP inhibitor (ARNI), was introduced for the treatment of hypertension and heart failure [23,25]. It is known that the processes, which play a role in the development of hypertension and heart failure, are also crucial in the pathophysiology of preeclampsia [26–28]. The investigation of serum NEP levels is crucial in preeclampsia patients so that NEP inhibitors, which affect the natriuretic peptide levels, may eventually be used in the treatment of preeclampsia. In 1994, various peptidases, including neprilysin, were shown to be expressed in the healthy human placenta and fetal membranes, having important functions in the maternofetal interface [29].

In a study carried out with placentas of normotensive, chronic hypertensive, superimposed preeclampsia, and preeclampsia patients, it was shown that the NEP staining was intense in extravillous trophoblasts, chorionic plate, and stem villus in all patient groups while staining of villous trophoblasts was found to be high only in the preeclampsia patients and mild-moderate in other patient groups. It has been suggested that the NEP expression increased in villous trophoblasts, causing preeclampsia through disturbing local regulation of vasoactive molecules in the maternal–fetal interface [16]. In another study on the placenta conducted in 2019, it was found that in the preeclamptic patients, the NEP levels increased not only in the placenta but also in syncytiotrophoblast-derived extracellular vesicles isolated from both placenta and peripherical circulation [30]. Our study also shows that the serum NEP levels were high other than placental villous trophoblasts and syncytiotrophoblast-derived extracellular vesicles isolated from both placenta and peripherical circulation in the preeclamptic patients. NEP seems to cause hypertension, the primary symptom of preeclampsia, by decreasing natriuretic peptides with vasodilatory effects mainly associated with the cardiovascular system. This was supported by the fact that the serum NEP

Table 3
Demographic, clinical and laboratory features of the women with severe preeclampsia and women in the control group matched for severe preeclampsia.

	Severe preeclampsia (n = 55)		Control Group gestational age matched for severe preeclampsia (n = 55)		p
	Mean	SD	Mean	SD	
Age (years)	30.22	5.51	28.76	5.26	0.179
BMI (kg/m^2)	30.33	4.18	29.33	4.47	0.253
Parity (n)	0.92	1.17	1.06	1.00	0.523
Systolic ABP (mm/Hg)	166.4	11.7	110.8	13.7	<**0.001**
Diastolic ABP (mm/Hg)	106.3	7.9	71.7	11.2	<**0.001**
GA at sampling (w)	30.54	4.76	30.88	4.93	0.713
GA at birth (w)	32.75	4.52	38.51	1.46	<**0.001**
WBC (10^9/L)	10.75	2.58	9.89	2.19	0.108
Platelet count (10^3/μL)	191.8	67.8	244.2	61.5	<**0.001**
Creatinine (mg/dL)	0.63	0.12	0.50	0.11	<**0.001**
Total bilirubin (mg/dL)	0.48	1.52	0.29	0.13	0.412
Uric acid (mg/dL)	5.80	1.33	4.35	1.10	<**0.001**
BUN (mg/dL)	25.40	9.58	16.10	6.00	<**0.001**
AST (IU/L)	36.84	44.48	15.87	6.07	**0.005**
ALT (IU/L)	36.34	57.55	12.50	5.64	**0.013**
Neprilysin (pg/mL)	234.45	63.85	189.29	84.59	**0.004**

ABP, arterial blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body-mass-index; BUN, blood urea nitrogen; GA, gestational age; SD, standard deviation; WBC, white blood cells.
P < 0.05 is significant (marked with bold).

Table 4
Demographic, clinical and laboratory features of the women with mild and severe preeclampsia.

	Mild preeclampsia (n = 55)		Severe preeclampsia (n = 55)		p
	Mean	SD	Mean	SD	
Age (years)	29.58	5.28	30.22	5.51	0.555
BMI (kg/m^2)	31.51	5.16	30.33	4.18	0.215
Parity (n)	0.92	1.46	0.92	1.17	1.000
Systolic ABP (mm/Hg)	145.7	8.8	166.4	11.7	<**0.001**
Diastolic ABP (mm/Hg)	93.0	7.0	106.3	7.9	<**0.001**
GA at sampling (w)	33.76	4.49	30.54	4.76	**0.001**
GA at birth (w)	35.45	3.84	32.75	4.52	**0.002**
WBC (10^9/L)	10.57	2.98	10.75	2.58	0.745
Platelet count (10^3/μL)	223.0	72.8	191.8	67.8	**0.030**
Creatinine (mg/dL)	0.57	0.10	0.63	0.12	**0.012**
Total bilirubin (mg/dL)	0.33	0.26	0.48	1.52	0.488
Uric acid (mg/dL)	5.37	1.17	5.80	1.33	0.096
BUN (mg/dL)	20.70	6.73	25.40	9.58	**0.006**
AST (IU/L)	21.12	13.70	36.84	44.48	**0.019**
ALT (IU/L)	14.26	11.90	36.34	57.55	**0.009**
Neprilysin (pg/mL)	228.84	67.26	234.45	63.85	0.677

ABP, arterial blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body-mass-index; BUN, blood urea nitrogen; GA, gestational age; SD, standard deviation; WBC, white blood cells.
P < 0.05 is significant (marked with bold).

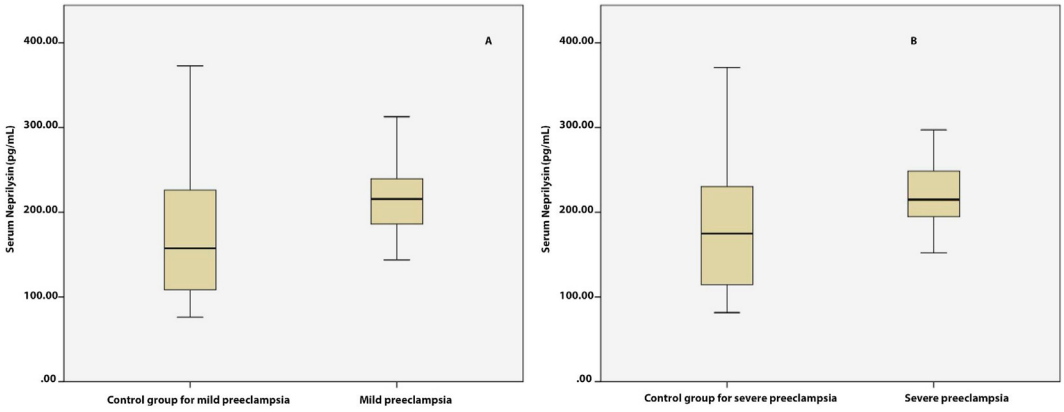

Fig. 2. Box-Plot analysis of the serum neprilysin levels in mild (A) and severe (B) preeclamptic women with control groups.

levels were correlated with the systolic and diastolic blood pressure of the patients in this study. Although the NEP increase was expected to be more evident in severe preeclampsia than mild preeclampsia, this could not be detected. The presence of hypertension is probably more significant than being mild or severe.

NEP also has substrates in the renin-angiotensin-aldosterone system with vasoconstrictor peptides as well as vasodilator natriuretic peptides. Natriuretic peptides system and renin-angiotensin-aldosterone system act opposite to each other. It is known that the renin-angiotensin-aldosterone system is active during pregnancy, but its status in the placental chorionic villus is unknown. Although the preeclamptic patients had elevated angiotensin-2 levels in the placental chorionic villus, their angiotensin-1 and angiotensin (1–7) levels did not increase. Furthermore, being effective in this system, the gene expressions of renin, ACE1, ACE2, and NEP did not change [30]. Based on the fact that placental renin-angiotensin-aldosterone system dysfunction is observed in preeclampsia patients, the renin-angiotensin-aldosterone system has been examined in the placentas of patients with IUGR, and it was found that angiotensin-2 levels increased while angiotensin (1–7) levels decreased. However, it was noted that the NEP gene expression also decreased [17]. Since the NEP levels in the chorionic villus of the patients with preeclampsia and IUGR were not measured, it may be difficult to make any decision on the NEP levels based on gene expression. Nevertheless, the increased NEP expression in placental syncytiotrophoblast-derived extracellular vesicles in the maternal circulation accord with the increased maternal serum NEP levels in our study. A possible explanation could be that NEP expression increases in chorionic villi during the abnormal placental development in preeclampsia, causing increased serum NEP, leading to the systemic endothelial dysfunction and clinical features of preeclampsia by interfering with the regulation of the vasoactive factors.

Patak et al. [31] have shown that NEP mRNA levels were 14-fold higher in the uteri from pregnant women than those from nonpregnant women. Pennfather et al. [32] have shown that the effects of the mammalian tachykinins neurokinin A (NKA) and

Table 5
The Pearson correlation analysis of neprilysin and various parameters in all participants.

	r	p
Age	−0.033	0.650
BMI	0.081	0.275
Parity	−0.144*	0.051
Systolic arterial blood pressure	0.238**	**0.001**
Diastolic arterial blood pressure	0.194**	**0.009**
GA at sampling	−0.144*	0.051
WBC	−0.114	0.144
Platelet count	0.018	0.815
Creatinine	0.181*	**0.018**
Total bilirubin	−0.063	0.418
Uric acid	0.319**	**<0.001**
BUN	0.269**	**<0.001**
AST	0.026	0.732
ALT	0.012	0.872

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body-mass-index; BUN, blood urea nitrogen; GA, gestational age; WBC, white blood cells.
P < 0.05 is significant (marked with bold).

human hemokinin-1 (hHK-1) and the nonmammalian tachykinin eledoisin are enhanced by the inhibition of neprilysin in myometrial tissues from pregnant women and the inhibitors of neprilysin are without effect on responses to NKA and hHK-1 in tissues from nonpregnant women. There is a scarcity of human-studies comparing serum NEP levels in uncomplicated pregnancies or preeclampsia and pregnancy-induced hypertension or nonpregnant hypertension.

The shortcomings of this study include a limited number of patients, the fact that only NEP levels in serum were determined rather than the placental NEP levels, and the fact that natriuretic peptides system and renin-angiotensin-aldosterone system, where the NEP has substrates are, were not evaluated.

In our study, mean serum NEP was significantly higher in women with preeclampsia, in women with mild preeclampsia, and in women with severe preeclampsia compared to the gestational age-matched controls. These results give hope that NEP inhibitors may eventually be used to treat preeclampsia in the future, as in heart failure patients with increased NEP levels. There is a need for more comprehensive, prospective, and randomized studies for better evaluations.

Financial disclosure

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Declaration of competing interest

The authors have no conflict of interest.

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