



# Perioperative hyperkalemia in hemodialysis patients undergoing parathyroidectomy for renal hyperparathyroidism

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## Abstract

The present study investigates the incidence of perioperative hyperkalemia and the influence factors of serum potassium levels during and after parathyroidectomy (PTX) in hemodialysis patients with renal hyperparathyroidism (rHPT). A total of 204 hemodialysis patients with refractory rHPT undergoing successful total parathyroidectomy with autotransplantation (tPTX + AT) were analyzed retrospectively. Hyperkalemia was defined as serum potassium levels  $\geq 5.5$  mmol/L. The preoperative baseline level of serum potassium ( $K_{\text{base}}^+$ ) was defined as a mean of the three preoperative prehemodialysis serum potassium levels. The higher levels of serum potassium during and immediately after surgery were recorded as  $K_{\text{d0}}^+$  and the peak prehemodialysis serum potassium levels 3 days after surgery as  $K_{\text{d3}}^+$ . 136/204 (66.7%) patients suffered from hyperkalemia during or immediately after surgery and 65/204 (31.9%) patients were affected with prehemodialysis hyperkalemia 3 days after surgery.  $K_{\text{base}}^+$  was the only influencing factor for  $K_{\text{d0}}^+$ . Serum  $K_{\text{base}}^+$ , preoperative serum alkaline phosphatase, and total calcium supplement dosage during intravenous calcium supplement were the influencing factors for  $K_{\text{d3}}^+$ . In the case of PTX, the serum potassium levels of patients with higher serum  $K_{\text{base}}^+$  and severe postoperative hypocalcemia need to be monitored with extended attention perioperatively.

**Keywords** Chronic kidney disease-mineral and bone disorder · Hemodialysis · Hyperkalemia · Hypocalcemia · Parathyroidectomy

## Introduction

Renal hyperparathyroidism (rHPT) is a common complication in hemodialysis patients. Parathyroidectomy (PTX) with a surgical rate of 2–8 per 1000 patients in Dialysis Outcomes Practice Patterns Study (DOPPS) [1] remains a valid treatment option for refractory rHPT patients [2, 3]. Hungry-bone

syndrome is a well-described phenomenon, following PTX, resulting in hypocalcemia [4, 5]; a significant variation in serum potassium level may also be observed in this cohort [6–10]. Hyperkalemia during and immediately after PTX, with an incidence of 25–80%, has been reported and may lead to devastating consequences, especially various severe dysrhythmia and cardiac arrest [7–10]. However, the mechanism and risk factors underlying perioperative hyperkalemia have not yet been clearly defined. Thus, the present study aims to investigate the incidence of hyperkalemia and potential influencing factors of perioperative serum potassium levels in PTX patients.

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## Materials and methods

### Patient population

A single-center retrospective study on a cohort of 204 maintenance hemodialysis patients undergoing successful total parathyroidectomy with autotransplantation

(tPTX + AT) due to refractory rHPT in our hospital between October 2015 and March 2018 was conducted. These patients came from different hemodialysis centers in east and south China. Inclusion criteria were as follows: age > 18 years, maintenance hemodialysis for a minimum of 3 months, refractory rHPT, and having undergone successful tPTX + AT. According to KDIGO guidelines [2] and our previous study [11–13], the surgical indications for tPTX + AT include: (1) iPTH > 800 pg/mL with hypercalcemia or hyperphosphatemia; (2) clinical manifestations such as severe bone ache, skin itching, external calcification, and deformity of bone; (3) drug resistance; (4) examination of images, including neck ultrasonography and parathyroid scintigraphy technetium-99 m methoxy isobutyl isonitrile (99mTc-MIBI) that identifies at least one enlarged parathyroid gland. tPTX + AT can be considered while fulfilling any one of the above first to third items and the fourth item. Surgical contraindications to PTX include: (1) blood platelets <  $80 \times 10^9/L$ ; (2) prothrombin time > 15 s or activated partial thromboplastin time > 40 s; (3) left ventricular ejection fraction < 30%; (4) pulmonary arterial systolic pressure > 70 mmHg; (5) acute coronary syndrome; or (6) malignant dysrhythmia including supraventricular tachycardia, multiform premature ventricular contraction, ventricular tachycardia, and ventricular fibrillation. The exclusion criteria were as follows: (1) liver, biliary or pancreatic diseases; (2) medication of cinacalcet hydrochloride within 6 months before surgery; (3) peritoneal dialysis; (4) unsuccessful tPTX + AT.

In the current study, the relative definitions were as follows. Refractory rHPT indicates that drug-resistant patients meet the surgical indications for tPTX + AT. Drug resistance means ineffectiveness of phosphate binder, calcimimetics, and vitamin D analogs in the recommended dose for SHPT over 6 months [2]. Successful tPTX + AT is defined as a number of resected parathyroid glands  $\geq 3$ , and an iPTH value of < 60 pg/mL 24 h post-surgery [14, 15]. Hyperkalemia is defined as serum potassium levels  $\geq 5.5$  mmol/L. The preoperative baseline level of serum potassium ( $K_{base}^+$ ) is defined as a mean of the three preoperative serum potassium levels just before the start of the hemodialysis session. The preoperative baseline level of serum urea was also recorded similarly. Kt/V at baseline is calculated according to the second-generation formula of Daugirdas [16]. In the current study, serum calcium levels mean serum-corrected calcium levels instead of serum total calcium levels. The serum values of corrected calcium are calculated using the following formula: serum-corrected calcium value (mmol/L) = serum total calcium value (mmol/L) +  $[40 - \text{serum albumin (g/L)}] \times 0.025$ .

## Anesthesia and surgery

The drugs used for inducing anesthesia were propofol, fentanyl, ondansetron, and atracurium. The condition was maintained with oxygen/nitrous oxide/sevoflurane mixture, followed by morphine intraoperatively for further analgesia, and finally reversal of neuromuscular blockade was effectuated with glycopyrrolate/neostigmine. The enrolled patients did not receive succinylcholine for induction of anesthesia. The surgical procedures were the same as described in our previous study [17].

## Perioperative monitoring and treatment

All enrolled patients were treated with heparin-free hemodialysis 12–18 h before surgery. The levels of serum potassium and blood base excess were monitored immediately before, during, and immediately after surgery. If the serum potassium levels immediately before surgery were  $\geq 5.5$  mmol/L, the planned surgery was canceled. The serum calcium and potassium levels were monitored every 4 h after surgery until 72 h postoperatively and then every 24 h until complete withdrawal of intravenous calcium supplement and no variation of oral calcium dose. When perioperative hyperkalemia happened, an electrocardiogram (ECG) was routinely obtained.

To control intraoperative hyperkalemia, patients with serum potassium level from 5.5 to 6.5 mmol/L required intravenous insulin/dextrose, while those > 6.5 mmol/L required emergency hemodialysis. Patients with immediate postoperative hyperkalemia of serum potassium levels > 5.5 mmol/L required emergency hemodialysis. In addition, all enrolled patients received heparin-free hemodialysis on days 1 and 3 post-surgery to avoid or treat hyperkalemia.

The calcium supplement program aimed to sustain the serum calcium levels at the normal range of 2.10–2.50 mmol/L (8.4–10.0 mg/dL) [18]. In the case of patients with postoperative serum calcium levels never < 2.0 mmol/L, when the values of postoperative serum calcium reduction were > 0.25 mmol/L and the levels of postoperative serum calcium were < 2.50 mmol/L, oral calcium carbonate was administered at an initial daily dose of 18.0 g, divided into three times and in-between meals. Calcitriol was administered at a daily dose of 0.25  $\mu\text{g}$  simultaneously. In subsequent serum calcium monitoring, a daily dose of oral calcium carbonate was adjusted to regulate the postoperative serum calcium levels in the normal range 2.10–2.50 mmol/L. For patients with postoperative serum calcium levels < 2.0 mmol/L, oral calcium and intravenous calcium would be supplemented.

Intravenous 20 mL/h of 5% calcium gluconate solution was initiated when the value of postoperative serum calcium was  $< 2.0$  mmol/L. The program of oral calcium supplement was similar to those patients, whose postoperative serum calcium levels never fell below 2.0 mmol/L; especially, during the administration of intravenous calcium supplement, the dose of oral calcium carbonate was not decreased. In subsequent monitoring, the transfusion speed of 5% calcium gluconate solution was adjusted so as to regulate the postoperative serum calcium level in the range of 2.10–2.50 mmol/L, until complete withdrawal of intravenous calcium supplement.

### Data collection

Baseline data, including age, gender, body weight, body mass index (BMI), hypertension, renin–angiotensin–aldosterone system (RAAS) inhibitors therapy, diabetes history, smoking history, dialysis age, preoperative laboratory parameters including  $K_{\text{base}}^+$  and preoperative prehemodialysis serum levels of calcium, phosphorus, albumin (Alb), hemoglobin (Hb), intact parathyroid hormone (iPTH), and alkaline phosphatase (ALP), total volume of parathyroid glands at ultrasounds, surgical duration, and total mass of parathyroid glands surgically removed were recorded. The higher levels of serum potassium during and immediately after surgery were recorded as  $K_{\text{d}0}^+$ , and peak serum potassium levels before the start of hemodialysis session 3 days post-surgery as  $K_{\text{d}3}^+$ . The serum calcium levels,  $\text{Ca}_{\text{d}0}^{2+}$  and  $\text{Ca}_{\text{d}3}^{2+}$ , were also recorded similarly. In addition, the levels of blood base excess immediately before, during, and immediately after surgery were recorded. The postoperative serum iPTH levels were tested at 24 h after surgery. The calcium supplement after surgery was recorded as calcium supplement procedures (intravenous combined with oral, only oral), intravenous calcium supplement holding time, intravenous calcium supplement dosage, and calcium supplement dosage (equal to intravenous plus oral calcium supplement dosage).

### Statistical methods

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean  $\pm$  SD or median (interquartile range), and categorical variables were presented as number and proportion. The differences between groups were compared using an independent samples *t* test or Wilcoxon rank sum test for continuous variables and a Chi squared or Fisher's exact test for categorical variables. After the highly collinear variables included in multiple linear regression analyses such as serum calcium before surgery, serum calcium at 3 days after surgery, and duration of intravenous calcium were

discriminated and deleted, a multivariate linear stepwise regression analysis model was performed for identifying the independent influencing factors for postoperative serum potassium levels. A *P* value  $< 0.05$  was considered as statistically significant.

## Results

### Description of the study population

A total of 204 hemodialysis patients were enrolled (Table 1), and 74/204 (36.3%) patients suffered from serum  $K_{\text{base}}^+ \geq 5.5$  mmol/L. None of the enrolled patients presented serum potassium levels  $\geq 5.5$  mmol/L immediately before surgery. The levels of blood base excess immediately before, during, and immediately after surgery ranged from  $-1.02$  to  $3.57$ ,  $-2.23$  to  $3.37$ , and  $-2.58$  to  $1.98$ , respectively.

### Incidence of hyperkalemia and electrocardiogram changes during or immediately after surgery

136/204 (66.7%) patients were affected with hyperkalemia during or immediately after surgery. In ECG test for perioperative hyperkalemia patients, emerging change was observed as follows. (1) 108/136 (79.4%) patients had tall, peaked, narrow-based T waves, (2) 72/136 (52.9%) patients had first-degree atrioventricular block, (3) 9/136 (6.6%) patients had secondary-degree atrioventricular block, and (4) 4/136 (2.9%) patients had prolongation of QRS. No patient suffered from tertiary-degree atrioventricular block, ventricular tachycardia, ventricular fibrillation, prolongation of QTc interval, or cardiac arrest.

### Incidence of hyperkalemia and electrocardiogram changes 3 days after surgery

65/204 (31.9%) patients suffered from prehemodialysis hyperkalemia 3 days after surgery. In ECG test for hyperkalemia patients, 36/65 (55.4%) patients had tall, peaked, narrow-based T waves and 12/65 (18.5%) patients had first-degree atrioventricular block. No patient suffered from secondary- or tertiary-degree atrioventricular block, prolongation of QRS, ventricular tachycardia, ventricular fibrillation, prolongation of QTc interval, or cardiac arrest.

### Influencing factors for serum potassium level during and immediately after surgery ( $K_{\text{d}0}^+$ )

Multivariate regression analysis finds that  $K_{\text{base}}^+$  is the independent influencing factor for  $K_{\text{d}0}^+$  (Table 2).

**Table 1** Comparison of demographic and laboratory data between patients with and without hyperkalemia during or immediately after surgery

	Patients with serum $K_{d0}^+ \geq 5.5$ mmol/L		P value
	Yes [N=136 (66.7%)]	No [N=68 (33.3%)]	
Age (years)	46.59 ± 9.74	48.38 ± 10.86	0.234
Female:male	58:78	27:41	0.688
Dialysis age (month) (IQR)	96.00 (63.00, 120.00)	102.00 (72.75, 120.00)	0.136
Body weight (kg) (IQR)	60.00 (53.13, 68.75)	58.00 (51.00, 67.00)	0.162
BMI (kg/m <sup>2</sup> ) (IQR)	21.88 (20.32, 24.48)	20.76 (19.17, 23.60)	0.067
Hypertension [n, (%)]	119 (87.50)	63 (92.65)	0.264
RAAS inhibitor therapy [n, (%)]	11 (8.09)	7 (10.29)	0.601
Diabetes [n, (%)]	3 (2.21)	1 (1.47)	0.721
Smoking history [n, (%)]	49 (36.03)	21 (30.88)	0.465
<i>Preoperative laboratory data</i>			
Hemoglobin (g/L)	104.48 ± 15.93	103.50 ± 19.48	0.702
Serum $K_{base}^+$ (mmol/L) (IQR)	5.00 (4.70, 5.40)	4.10 (3.90, 4.30)	<0.001
Serum calcium (mmol/L)	2.52 ± 0.18	2.53 ± 0.18	0.827
Serum phosphorus (mmol/L) (IQR)	2.30 (2.03, 2.62)	2.13 (1.80, 2.46)	0.007
Serum albumin (g/L)	38.62 ± 4.16	37.74 ± 4.61	0.169
Serum ALP (IU/L) (IQR)	322.75 (180.60, 616.93)	269.05 (160.00, 499.58)	0.131
Serum iPTH (pg/mL) (IQR)	1622.95 (1201.68, 2159.58)	1358.45 (1038.43, 1906.33)	0.030
Baseline level of serum urea (mmol/L)	24.73 ± 3.56	25.21 ± 3.23	0.867
Baseline KT/V	1.52 ± 0.11	1.57 ± 0.09	0.658
Total volume of parathyroid glands at ultrasounds (cm <sup>3</sup> ) (IQR)	5.35 (0.54, 10.73)	5.54 (0.61, 11.05)	0.894
<i>Intra- and postoperative laboratory data</i>			
Serum $K_{d0}^+$ (mmol/L) (IQR)	5.94 (5.70, 6.40)	4.81 (4.62, 5.22)	<0.001
Serum $Ca_{0d}^{2+}$ (mmol/L) (IQR)	2.42 (2.30, 2.56)	2.49 (2.34, 2.57)	0.186
Operative duration (min) (IQR)	65.00 (60.00, 80.00)	61.50 (56.00, 73.25)	0.120
Total mass of parathyroid glands surgically removed (g) (IQR)	3.65 (2.11, 5.32)	3.77 (2.09, 5.56)	0.674
<i>Postoperative calcium supplement</i>			
Method (intravenous injection:oral administration)	122:14	59:9	0.531
Intravenous injection duration (h) (IQR)	60.00 (44.25, 100.13) (N=122)	48.00 (35.00, 93.00) (N=59)	0.054
Intravenous calcium supplement dosage (mmol) (IQR)	94.70 (67.50, 211.63) (N=122)	90.70 (60.47, 151.16) (N=59)	0.091
Total calcium supplement dosage during intravenous calcium supplement (IQR)	585.47 (338.03, 1564.54)	479.02 (307.79, 826.05)	0.060

IQR interquartile range, BMI body mass index, RAAS renin–angiotensin–aldosterone system,  $K_{base}^+$  preoperative baseline level of serum potassium, ALP alkaline phosphatase, iPTH intact parathyroid hormone,  $K_{d0}^+$  The higher levels between serum potassium during and immediately after surgery,  $Ca_{0d}^{2+}$  Serum calcium levels at the same time to  $K_{d0}^+$

**Table 2** Multivariate linear stepwise regression analysis for influence factor of serum potassium level during and immediately after surgery ( $K_{d0}^+$ )

Variables	Unstandardized $\beta$ coefficients (95% CI)	Standardized $\beta$ coefficients	P value
Serum $K_{base}^+$ level	0.868 (0.754–0.981)	0.727	< 0.001
Constant	1.103 (0.559–1.646)	–	< 0.001

( $F=226.794$ ;  $R=0.727$ ;  $R^2=0.529$ ;  $P<0.001$ )

$K_{base}^+$  preoperative baseline level of serum potassium,  $K_{d0}^+$  the higher levels between serum potassium during and immediately after surgery, CI confidence interval

### Influence factors for serum potassium level 3 days after surgery ( $K_{d3}^+$ )

Multivariate regression analysis finds that serum  $K_{base}^+$  level, preoperative serum ALP level, and total calcium supplement dosage during intravenous calcium supplement are the independent influencing factors for  $K_{d3}^+$  (Table 3).

**Table 3** Multivariate linear stepwise regression analysis for influence factors of serum potassium level 3 days after surgery ( $K_{d3}^+$ )

Variables	Unstandardized $\beta$ coefficients (95% CI)	Standardized $\beta$ coefficients	<i>P</i> value
Serum $K_{base}^+$ level	0.231 (0.159–0.303)	0.214	< 0.001
Serum ALP level	$1.553 \times 10^{-4}$ ( $0.275 \times 10^{-4}$ – $2.811 \times 10^{-4}$ )	0.092	0.029
Total calcium supplement dosage during intravenous calcium supplement	$3.131 \times 10^{-4}$ ( $1.965 \times 10^{-4}$ – $4.103 \times 10^{-4}$ )	0.600	< 0.001
Constant	3.349 (3.008–3.689)	–	< 0.001

( $F = 39.839$ ;  $R = 0.718$ ;  $R^2 = 0.510$ ;  $P < 0.001$ )

$K_{base}^+$  preoperative baseline level of serum potassium,  $K_{d3}^+$  serum potassium levels 3 days after surgery, *CI* confidence interval

## Discussion

The current study reports a 66.7% rate of occurrence of hyperkalemia during or immediately after tPTX + AT, which is consistent with 25–80% incidence reported before [7–10, 19]. As all enrolled patients were treated with hemodialysis 12–18 h before surgery and the planned surgery would be canceled if the serum potassium levels immediately before surgery were  $\geq 5.5$  mmol/L, we think that  $K_{d0}^+$  levels  $\geq 5.5$  mmol/L should be a certain impact by surgical procedure, while they should not be considered hyperkalemia before the start of dialysis session. Multivariate analysis further finds that the preoperative predialysis serum potassium level (baseline level of serum potassium,  $K_{base}^+$ ) constitutes the independent influencing factors for  $K_{d0}^+$ . This trend is similar to that in the literature. A few previous studies have reported that when hemodialysis patients undergoing PTX have a high normal or elevated level of baseline serum potassium, intraoperative hyperkalemia is rather a common phenomenon [7, 9, 10]. Another recent study reports that the preoperative serum potassium level could preferably predict postoperative hyperkalemia immediately after PTX [19].

Despite regular hemodialysis, a 31.9% occurrence rate of prehemodialysis hyperkalemia 3 days post-surgery was recorded in the current study. Multivariate analysis reveals that  $K_{base}^+$ , preoperative serum ALP level, and total calcium supplement dosage during intravenous calcium supplement are the independent influencing factors for  $K_{d3}^+$ . Although hyperkalemia is recognized as a complication within the initial 24 h postoperatively after PTX [8], a prolonged follow-up duration for continued evaluation is of less concern. Koshkelashvili et al. report on a male hemodialysis patient, who had undergone PTX and presented to the emergency department with hyperkalemia of 9.2 mmol/L 1-month post-surgery [6]. However, to the best of our knowledge, there is no other report on > 24-h follow-up for the evaluation of postoperative hyperkalemia after PTX.

The exact causes of elevated levels of serum potassium are unclear. In the present study, none of the patients received depolarizing neuromuscular blockers or

potassium-containing intravenous fluid during surgery, no acidemia was recorded, and hyperkalemic patients did not require a prolonged surgical duration. In the setting of uremia, homeostatic matching of renal excretion to potassium is disrupted and patients rely upon dialysis as a surrogate provider of electrolyte clearance. A dynamic maintenance of serum potassium levels relies upon flux from the extracellular to the intracellular compartment, which is primarily regulated by the energy-dependent sodium–potassium transmembrane channels in skeletal muscle affected by multiple metabolic inputs, including acid–base and endocrine systems [20, 21]. Therefore, the efflux of potassium from the intracellular compartment is an attractive potential mechanism. One possible explanation is as follows: for rHPT patients undergoing PTX, withdrawal of prolonged elevation in serum PTH level leads to calcium influx into bone, a decreased level of calcium ions in extracellular fluid (ECF), and hypocalcemia [8, 22–24]. A decreased level of calcium ions in extracellular fluid (ECF) can promote the influx of sodium ions into skeletal muscle cells (SMC) under the mechanism of membrane barrier action of sodium–calcium exchanger (NCX), which counteracts sodium ion efflux and potassium ion influx powered by the activation of Na/K ATPase pump, and can also reduce the resting potential of SMC and increase cell excitability [20, 25], thereby explaining the increased serum potassium level during and after PTX.

So, it is easy to understand in the current study that the patients with higher  $K_{base}^+$  would suffer from higher  $K_{d0}^+$ . Potential preventive management for preoperative hyperkalemia, such as avoiding high-potassium foods, increasing the dialysis frequency, improving vascular access dysfunction and dialysis adequacy, and reducing renin–angiotensin–aldosterone system inhibitor applications for intractable hyperkalemia patients [26], is important to reduce the risk of perioperative hyperkalemia to some extent. Interestingly, serum calcium level is not an accurate representation of the extracellular fluid calcium level of SMC within a short surgery duration. Thus, no significant difference might be detected in serum  $Ca_{0d}^{2+}$  of

patients with and without hyperkalemia during and immediately after surgery.

Several studies report that higher serum ALP level is a predictor for severe postoperative hypocalcemia [4, 27, 28]. Therefore, serum ALP level, intravenous injection duration for calcium supplement, and total calcium supplement dosage during intravenous calcium supplement, which can indicate the severity of postoperative hypocalcemia, are shown to be influencing factors for  $K_{d3}^+$  in our study. Serum calcium does not appear in the multivariate analysis as an independent factor, mainly because postoperative serum calcium levels have been corrected by calcium supplement and cannot indicate the severity of postoperative hypocalcemia.

It has been reported previously that age was inversely related to serum potassium levels during parathyroidectomy and may be associated with higher muscle mass in patients developing hyperkalemia [9, 10]. However, there was no significant age difference in the patients developing compared to those not developing hyperkalemia in the current study. This phenomenon may be because middle-aged people were in the majority in our patients.

Although an ECG is not a sensitive indicator of hyperkalemia, and 50% of patients with potassium levels greater than 6.5 mmol/L will not manifest any ECG changes [29, 30], the patients with hyperkalemia in the current study showed high incidence of ECG changes due to hyperkalemia. This phenomenon may be attributed to the combination of hyperkalemia and hypocalcemia, which has a cumulative effect on the atrioventricular and intraventricular conduction delay and facilitates the development of ventricular fibrillation [29]. However, ECG changes due to hypocalcemia such as prolongation of QTc interval were undetected in the current study.

Nevertheless, the present study has several limitations. The number of enrolled patients within a single center was small, thereby constraining the conclusions. Due to the retrospective nature of the analysis, stringent perioperative diet for potassium intake was not standardized, which might result in different defects in the study. We did not formally assess recirculation of vascular access in the patients, which might somewhat influence the conclusion. In addition, monitoring of ion concentration in the extracellular fluid and endocrine measures might provide in-depth insights into the underlying mechanisms.

## Conclusion

This study highlights the need to raise awareness of perioperative hyperkalemia for hemodialysis patients undergoing PTX. Based on these observations, there is a strong indication for perioperative electrolyte surveillance and preoperative serum potassium control. In the case of patients with

high serum  $K_{base}^+$  and severe postoperative hypocalcemia who require a prolonged calcium supplement, extending the monitoring of serum potassium level and ECG is also essential.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The current study has been approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Nanjing, China) and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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