VITAMIN D STATUS AND THE RISK OF RECURRENT STROKE AND MORTALITY IN ISCHEMIC STROKE PATIENTS: DATA FROM A 24-MONTH FOLLOW-UP STUDY IN CHINA

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Abstract: *Purpose:* In this study, we determined serum 25-hydroxyvitamin D [25(OH) D] levels in serum, and investigated their associations with risk of recurrent stroke and mortality in a 24-month follow up study in Chinese patients with first-ever ischemic stroke. *Methods:* In this preplanned post hoc analysis, serum levels of 25(OH) D and NIH stroke scale (NIHSS) were measured at the time of admission in a cohort of patients with ischemic stroke. We used logistic regression model to assess the relationship between 25(OH) D levels and risk recurrent stroke or mortality. *Results:* The follow-up rate was 98.2% in 220 stroke patients. Of 216 patients, 18.5% (95%CI: 13.3%-23.7%) patients had a stroke recurrence, and 30.1% (95% CI: 24.0%-36.2%) died. After adjustment for traditional risk factors, serum 25(OH) D levels were negatively associated with the risk of stroke recurrence (odds ratio [OR], 0.77; 95% confidence interval [CI], 0.70–0.85; P < 0.001) and negatively associated with mortality during 24 months of follow-up (OR, 0.72; 95% CI, 0.64–0.80; P< 0.001). Compared with the first quartile of serum 25(OH) D levels, the ORs for stroke recurrence and mortality were as follows: second quartile, 0.80 (95% CI, 0.63–0.93) and 0.77 (95% CI, 0.65–0.89); third quartile, 0.42 (95% CI, 0.31–0.55) and 0.39 (95% CI, 0.30–0.52); fourth quartile, 0.12 (95% CI, 0.07–0.19) and 0.10 (95% CI, 0.06–0.15), respectively. *Conclusions:* Lower serum levels of 25(OH) D are independently associated with the stroke recurrence and mortality at 24 months in ischemic stroke patients.

Key words: : 25-hydroxyvitamin D, vitamin D, ischemic stroke, recurrent stroke, mortality.

Introduction

Stroke is a leading cause of death and disability in China and each year, 2.5 million people suffer a stroke (1). Survivors often require long-term care, and are at high risk of recurrent stroke. Vitamin D deficiency has been proposed as a risk factor for mild cognitive impairment (2), obesity (3), dementia (4) and cardiovascular disease (5). A study found that the majority of adult Italian population has an important deficiency in vitamin D (6). In our previous study, we found that vitamin D deficiency was common (78.2%) in ischemic stroke patients (7). Similarly, Rolland et al. (8) found that most residents in nursing homes have vitamin D insufficiency, and would benefit from vitamin D supplement. However, only few residents are actually treated. In addition, an increase in yearly trend of calcium and vitamin D supplements was observed in US population (9).

Previous studies indicate that vitamin D deficiency is a risk factor for strokes [10-11]. Brøndum-Jacobsen et al. (12) observed stepwise increasing risk of symptomatic ischemic stroke with decreasing plasma 25-hydroxyvitamin D [25(OH) D] concentrations. The association between serum vitamin D status and functional outcomes, all-cause mortality and recurrent vascular events were observed in ischemic stroke patients (13-16). In a previous study, we demonstrated that 25(OH) D was an independent prognostic marker for functional outcome within 90 days in ischemic stroke patients (7). Thus, in this study, we determined 25(OH) D levels in serum, and investigated their associations with risk of recurrent stroke and mortality in a 24-month follow up study in Chinese patients with first-ever ischemic stroke.

Subjects and Methods

Patients

The study methods have been described elsewhere (7). Briefly, all patients were registered in two hospitals in Beijing, China. From February 1, 2010 to September 30, 2012, all consecutive patients with a first-ever AIS event were included. Patients were eligible for inclusion if they were admitted with an acute ischemic stroke and with symptom onset within 24 hours. During the inclusion period, 364 patients were registered. Acute ischemic stroke was diagnosed in 231 patients and 220 completed 3-month follow-up. However, these 220 patients were similar in terms of baseline characteristics [age (P=0.42), gender (P=0.77), NIHSS (P = 0.72), weight (P = 0.39) and live in rural areas (P=0.51)] compared to the overall cohort.

Written informed consent was obtained from all patients; and, this study conformed to the principles of the Declaration of Helsinki was approved by the investigational review board of the China Rehabilitation Research Center. At baseline, demographic data (age and sex), admission season and history

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of conventional vascular risk factors were obtained. Brain computed tomography/magnetic resonance imaging (CT/MRI), or both were performed on the same days. Stroke severity was assessed on admission using the National Institutes of Health Stroke Scale (NIHSS) by a neurologist. The information about stroke subtype and clinical stroke syndrome were obtained. Serum biomarkers were also evaluated at the first morning after admission.

End Points and follow-up

We followed the participants for a median of 24 months (range, 23-25 months) using a standard questionnaire, and telephone or household contact by physician investigators. Two trained research collected study data at discharge and at 3, 12 and 24 months after stroke onset blinded to vitamin D levels. Those works were finished by Qiu and Tu, who had undergone a standard pre-job training. Reliability analysis of the diagnosis (internal consistency) yielded Cronbach's α of 0.95 in the pilot study. The follow-up rate was 98.2% in 220 stroke patients. The primary endpoint was recurrence of stroke. Secondary endpoints were onset of all-cause death (including fatal stroke, other cardiovascular death, or death by any causes). Stroke recurrence was defined as a sudden functional deterioration in neurological status with a decrease of the NIHSS score of 4 or more, or a new focal neurological deficit of vascular origin lasting >24 hours (17). In patients who had a recurrent stroke, medical records from the stroke admission were reviewed by the investigators.

Statistical Analysis

The results are expressed as percentages for categorical variables and as mean (standard deviation, S.D.) or median (interquartile range, IQR) for the continuous variables depending on their normal distribution. The relation of serum 25(OH) D with the two end points was investigated with the use of logistic regression models. We used crude models and multivariate models adjusted for all significant outcome predictors and report odds ratios (ORs). ORs were also calculated according to equal quartiles of the distributions of serum 25(OH) D, and trends across these quartiles were tested by using conditional logistic regression models. For multivariate analysis, we included significantly predictors as assessed in univariate analysis ($P<0.05$). Further, receiver operating characteristic curves (ROC) was used to test the overall prognostic accuracy of the 25(OH) D and other markers and results were reported as area under the curve (AUC). Lastly, in order to study the ability of 25(OH) D for recurrence and mortality prediction, we calculated Kaplan–Meier curves method and stratified patients by 25(OH) D quartiles. All statistical analysis was performed with SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $P< 0.05$.

Table 1
Baseline characteristics of stroke patients

Demographic characteristics	N=216
Male sex (%)	133(61.6)
Age (years), median(IQR)	65(55-76)
Stroke severity, median NIHSS score (IQR)	8(4-12)
Admission to hospital(hours), median(IQR)	5.5(2.2-10.5)
Lesion volumes(mL), n=146(median, IQR)	16(8-34)
Hospital stay(days), median(IQR)	32(21-56)
Vascular risk factors no. (%)	
Hypertension	173(80.1)
Diabetes mellitus	93(43.1)
Atrial fibrillation	54(25.0)
Hypercholesterolemia	99 (45.8)
Coronary heart disease	54(25.0)
Family history for stroke	60(27.8)
Active smokers	56(25.9)
Clinical findings (median, IQR)	
Systolic blood pressure(mmHg)	150(141-165)
Diastolic blood pressure(mmHg)	85(80-90)
Temperature (°C)	37.1(36.6-37.4)
BMI (kg m-2)	25.0(23.0-27.5)
Heart rate (beats min-1)	84(72-94)
Laboratory findings(IQR)	
25(OH) D (ng mL-1)	14.2(10.3-18.9)
Total cholesterol(mmol L-1)	4.1(3.3-5.1)
Triglycerides (mmol L-1)	1.4(1.1-1.8)
High-density lipoproteins (mmol L-1)	1.4(1.1-1.7)
Low-density lipoproteins(mmol L-1)	2.1(1.3-2.8)
Glucose(mmol L-1)	5.40(4.88-6.49)
C-reactive protein (mgL-1)	4.8(2.2-10.2)
Stroke syndrome no. (%)	
TACS	31(14.4)
PACS	88(40.7)
LACS	41(19.0)
POCS	56(25.9)
Stroke etiology no. (%)	
Small-vessel occlusive	48(22.2)
Large-vessel occlusive	45(20.8)
Cardioembolic	78(36.1)
Other	11(5.1)
Unknown	34(15.8)

IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; LACS, lacunar syndrome; PACS, partial anterior circulation syndrome; POCS, posterior circulation syndrome; TACS, total anterior circulation syndrome; BMI, body mass index

Results

Patients

Of the original 220 ischemic stroke patients, 216 (98.2%) completed the 24-month (Range, 23-25months) follow-up.

Table 2
Univariate and multivariate logistic regression analysis for stroke recurrence and mortality

Parameter	Univariate Analysis			Multivariate Analysis		
	OR [a]	95% CI [a]	P	OR [a]	95% CI [a]	P
Predictor: stroke recurrence						
25 (OH) D	0.62	0.55-0.74	< 0.0001	0.77	0.70-0.85	<0.0001
Age	1.06	1.02-1.09	<0.001	1.05	1.02-1.08	<0.001
Male sex	0.90	0.67-1.22	0.456	—		
BMI	1.20	0.75–1.95	0.464	—		
Seasons of blood sampling(Winter)	1.50	1.04-2.15	0.032	1.06	0.73-2.77	0.472
TPA-T(yes vs. no)	0.73	0.65-0.79	<0.001	0.77	0.69-0.84	<0.001
Risk factors						
Hypertension	1.68	1.12-2.98	0.148	—		
Diabetes mellitus	1.34	1.08-2.16	0.082	—		
Atrial fibrillation	1.65	1.34–1.99	<0.001	1.45	1.24-1.68	<0.001
Hypercholesterolemia	1.22	0.62-2.43	0.526	—		
Coronary heart disease	1.20	1.05-1.87	0.004	1.07	1.01-1.24	0.012
Family history for stroke	1.37	1.05-1.74	0.021	1.26	1.03-1.58	0.039
Active smokers	1.04	0.89-1.35	0.723	—		
Infarct volume [b]	1.20	1.11-1.33	0.006	1.15	1.05-1.27	0.009
NIHSS	1.52	1.25-1.77	< 0.0001	1.30	1.10-1.49	< 0.0001
Stroke syndrome(TACS vs. other)	3.02	1.55- 6.62	0.009	2.44	0.98-9.17	0.626
Stroke etiology (LAA vs. other)	3.10	2.08-4.55	<0.0001	2.68	1.77-4.12	<0.0001
Blood biomarkers						
Glucose	1.12	1.03-1.30	0.018	1.06	1.01-1.24	0.024
Hs-CRP	2.22	1.43-2.99	0.006	1.84	1.24-2.56	0.011
Triglyceride	0.61	0.21–1.80	0.376	—		
Cholesterol	1.05	0.68–1.62	0.845	—		
Predictor: mortality						
25 (OH) D	0.62	0.55-0.74	< 0.0001	0.77	0.70-0.85	<0.0001
Age	1.10	1.04-1.18	< 0.001	1.08	1.02-1.15	< 0.001
Male sex	0.90	0.67-1.22	0.456	—		
BMI	1.81	1.41–3.54	0.532	—		
Seasons of blood sampling(Winter)	1.66	1.10-2.24	0.024	1.10	0.89-2.55	0.396
TPA-T(yes vs. no)	0.70	0.62-0.77	<0.001	0.74	0.65-0.80	<0.001
Risk factors						
Hypertension	1.06	0.48–2.31	0.894	—		
Diabetes mellitus	1.75	0.75–4.09	0.196	—		
Atrial fibrillation	1.30	1.10–1.65	0.047	1.25	0.98-1.87	0.312
Hypercholesterolemia	0.96	0.47–1.97	0.901	—		
Coronary heart disease	1.22	0.92-1.99	0.212	—		
Family history for stroke	1.54	0.91-3.16	0.297	—		
Active smokers	0.66	0.29–1.48	0.302	—		
Infarct volume [b]	1.25	1.13-1.38	0.005	1.18	1.06-1.28	0.011
NIHSS	1.62	1.20-1.89	< 0.0001	1.34	1.12-1.55	0.002
Stroke syndrome(TACS vs. other)	2.72	1.24- 4.77	0.136	—		
Stroke etiology (LAA vs. other)	1.96	1.02–3.82	0.059	—		
Blood biomarkers						
Glucose	1.10	1.03-1.66	0.011	1.05	0.92-1.76	0.181
Hs-CRP	2.45	1.56-3.12	0.004	1.77	1.20-2.47	0.009
Triglyceride	1.14	0.77–1.68	0.502	—		
Cholesterol	1.21	0.45–3.21	0.705	—		

a. Note that the odds ratio corresponds to a unit increase in the explanatory variable; b. N=146 ; OR, odds ratio; CI, confidence interval; BMI, body mass index; Hs-CRP, High-sensitivity-C-reactive protein; NIHSS, National Institutes of Health Stroke Scale; TACS, total anterior circulation syndrome; LAA, large-artery atherosclerosis; TPA-T:Tissue plasminogen activator-treated

Baseline characteristics have been reported previously (5). In the study population, 133 (61.6%) were male and median age was 65 years (IQR 55–76). At admission, the median NIHSS score was 8.0 (IQR, 4-12). The median serum 25 (OH) D levels in AIS patients was 14.2 (IQR, 10.3-18.9)ng/ml. The principal baseline characteristics of all patients were provided in Table 1.

Table 3

Logistic regression model for serum levels of 25 (OH) D using stroke recurrence and mortality as the dependent variables, after adjustment by age, gender, BMI, seasons of blood sampling, risk factors, NIHSS, infarct volume, stroke etiology, TPA-T, stroke syndrome and serum levels of Hs-CRP, glucose, total cholesterol and triglyceride

Dependent variables	OR (95% CI)	P value
Stroke recurrence [a]		
Serum 25 (OH) D (1°quartile)	reference	—
Serum 25 (OH) D (2°quartile)	0.80(0.63-0.93)	0.015
Serum 25 (OH) D (3°quartile)	0.42(0.31-0.55)	<0.001
Serum 25 (OH) D (4°quartile)	0.12(0.07-0.19)	<0.0001
Mortality [a]		
Serum 25 (OH) D (1°quartile)	reference	—
Serum 25 (OH) D (2°quartile)	0.77(0.65-0.89)	0.012
Serum 25 (OH) D (3°quartile)	0.39(0.30-0.52)	<0.001
Serum 25 (OH) D (4°quartile)	0.10(0.06-0.15)	<0.0001

a. serum 25(OH) D levels in Quartile 1 (<10.3ng/ml), Quartile 2 (10.3–14.2 ng/ml), Quartile 3 (14.2–18.9 ng/ml), and Quartile 4 (>18.9ng/ml); OR, odds ratio; CI, confidence interval; BMI, body mass index; NIHSS, National Institutes of Health Stroke Scale; TPA-T: Tissue plasminogen activator-treated

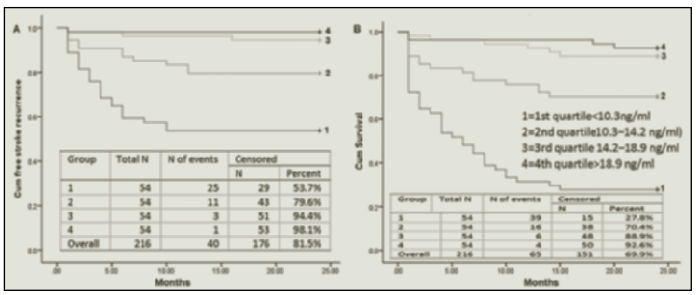
Primary End Point

In the 40 patients [18.5%, 95%CI: 13.3%-23.7%] with recurrent stroke, 25 (OH) D serum levels were lower compared with those free of recurrent strokes [9.2(IQR, 8.2 -11.7)ng/mL vs. 15.2(IQR, 11.9 to 20.0 ng/mL); P<0.0001]. Logistic regression analysis considering traditional risk factors showed an inverse relationship between serum 25 (OH) D levels and risk of recurrent stroke when serum 25 (OH) D was used as a continuous variable (OR, 0.77; 95% CI, 0.70–0.85; P < 0.001; Table 2). In the subgroup of patients (n=146) in whom MRI evaluations were performed, 25(OH) D was an independent recurrent stroke predictor with an OR of (OR, 0.72; 95% CI, 0.66–0.80; P< 0.001) after adjustment for both lesion size and the NIHSS score. Compared with the first quartile of serum 25 (OH) D level, the second quartile OR for recurrent stroke was 0.80 (95% CI, 0.63–0.94, P=0.015). For the third and fourth quartiles, it was 0.42 (95% CI, 0.31–0.55, P<0.001) and 0.12 (95% CI, 0.07–0.19; P<0.001), respectively; Table 3.

The area under the curve (AUC) of 25 (OH) D to predict recurrent stroke was 0.82 (95% CI, 0.75to 0.88) and significantly higher than for glucose (AUC 0.52 (0.46–0.63), P<0.0001), C-reactive protein (AUC 0.64 (0.55–0.73), P<0.001), infarct volume AUC 0.68 (0.63–0.79), p<0.001) and the NIHSS score (AUC 0.72 (0.64-0.80, P<0.01)). In addition, patients in the upper two quartiles (25(OH) D >18.9ng/ml and 25(OH) D between 14.2 and 18.9ng/ml) had a minor risk of recurrent stroke compared to patients with 25(OH) D levels in the lower two quartile (25(OH) D <10.3ng/ml and 25(OH) D between 10.3 and 14.2ng/ml, p<0.001), Figure 1A.

Figure 1

Kaplan–Meier curves analysis based on 25(OH) D quartiles. (A) Time to stroke recurrence was analysed by Kaplan–Meier curves based on 25(OH) D quartiles. Patients in the upper two quartile (25(OH) D >18.9ng/ml and 25(OH) D between 14.2 and 18.9ng/ml) had a minor risk of stroke recurrence compared to patients with 25(OH) D levels in the lower two quartile (25(OH) D <10.2ng/ml and 25(OH) D between 10.2 and 14.2ng/ml, P<0.001); (B) Time to death was analysed by Kaplan–Meier curves based on 25(OH) D quartiles. Patients in the upper two quartile (25(OH) D >18.9ng/ml and 25(OH) D between 14.2 and 18.9ng/ml) had a minor risk of death compared to patients with 25(OH) D levels in the lower two quartile (25(OH) D <10.2ng/ml and 25(OH) D between 10.2 and 14.2ng/ml, P<0.001)



Secondary End Point

After 24 months, 65 patients had died, thus the mortality rate was 30.1% (95% CI: 24.0%-36.2%). Serum 25(OH) D levels in patients who survived were significantly greater as compared with patients who died (15.8 [IQR, 12.9-20.3] vs 9.2 [IQR, 7.6–12.7]ng/ml; P<0.001). Logistic regression analysis considering traditional risk factors showed an inverse relationship between serum 25 (OH) D levels and risk of mortality when serum 25 (OH) D was used as a continuous variable (OR, 0.72; 95% CI, 0.64–0.80; P < 0.001; Table 2). Similarly, in the subgroup of patients (n=146) in whom MRI evaluations were performed, 25(OH) D was an independent mortality predictor with an OR of (OR, 0.69; 95% CI, 0.62–0.77; P< 0.001) after adjustment for both lesion size and the NIHSS score. Compared with the first quartile of serum 25 (OH) D level, the second quartile OR for mortality was 0.77 (95% CI, 0.65–0.89, P=0.012). For the third and fourth quartiles, it was 0.39 (95% CI, 0.30–0.52, P<0.001) and 0.10 (95% CI, 0.06–0.15; P<0.001), respectively; Table 3.

The area under the curve (AUC) of 25 (OH) D to predict

mortality was 0.83 (95% CI, 0.76 to 0.89) and significantly higher than for glucose (AUC 0.60 (0.52–0.68), P<0.0001), C-reactive protein (AUC 0.66 (0.58–0.74), p<0.001), infarct volume AUC 0.70 (0.63–0.80), p<0.001) and the NIHSS score (AUC 0.70 (0.62-0.80, P<0.01)). In addition, patients in the upper two quartiles (25(OH) D >18.9ng/ml and 25(OH) D between 14.2 and 18.9ng/ml) had a minor risk of mortality compared to patients with 25(OH) D levels in the lower two quartile (25(OH) D <10.3ng/ml and 25(OH) D between 10.3 and 14.2ng/ml, p<0.001), Figure 1b.

Discussion

Vitamin D deficiency has been proposed as a risk factor for cardiovascular disease, including stroke (18). Durup et al. (19) reported that low and high levels of 25(OH) D were associated with cardiovascular disease, stroke, and acute myocardial mortality in a nonlinear, reverse J-shaped manner, with the highest risk at lower levels. This study show that lower serum levels of 25(OH) D are independently associated with the stroke recurrence and mortality at 24 months in ischemic stroke patients. These data suggest that 25(OH) D may be a strong and independent protective factor against ischemic stroke, in the Chinese population. Similarly, the inverse association between serum 25 (OH) D levels and functional outcome in patients with acute ischemic stroke had been suggested (13-14). Daubail et al. (15) reported that a low serum 25(OH) D level at stroke onset may be associated with higher mortality at 1 year in stroke patients <75 years old. Interestingly, Huang et al. (20) suggested that reduced serum levels of 25(OH) D could predict the risk of early stroke recurrence (3-month) in patients with ischemic stroke.

In this study, the risk of recurrent stroke in ischemic stroke patients was 18.5% (95%CI: 13.3%-23.7%) during the 24 months follow-up. Similarly, Wang et al. (21) reported that of 11 560 patients with ischemic stroke, 2050 (17.7%) experienced a recurrent stroke within 1 year. Feng et al. (22) suggested that the Kaplan-Meier estimate of cumulative risk at 2 years for recurrent stroke was 15.2% in hospitalized stroke patients. In another study from China population, thirty-seven out of the 349 patients (10.6%) had a stroke recurrence within 3 months (20). In this study, we found that serum levels of 25(OH) D within the first quartile had a significant risk of recurrent stroke when compared with second, third and fourth quartiles (P>0.05, respectively)

The reasons why a low 25(OH) D level may increase the risk of stroke recurrence and post-stroke mortality remain uncertain. It may result from an increase in vascular events in patients with 25 (OH) D deficiencies (23). Contributing mechanisms have been linked to the association of vitamin D deficiency with the presence of hypertension, diabetes mellitus (24) atherosclerosis (25) and small vessel disease (26). Second, there is experimental evidence demonstrating that vitamin D exerts neuroprotective effects (18). Third, hyperparathyroidism has been proposed as one mechanism by which vitamin D deficiency could mediate cardiovascular events, since it may promote cardiac hypertrophy, vascular remodeling and inflammation (27).

Our study did have some shortcomings. First, the relatively small population size (n=216) could have made the study underpowered. Because of the limited sample size (40/216 and 56/216, respectively), over-adjustment could occur in multivariable analyses. In addition, our data came from two hospital-based registries, which could have hospital selection bias. Second, the data on vitamin D supplementation, nutrition, health education, social level, and parathyroid hormone level were not available. Potential unmeasured confounding factors may act in the observed associations. In addition, functional status before stroke could affect the outdoor activity, disability after stroke and the level of 25(OH) D. However, the information about pre-stroke functional status is not available. Therefore, we could not adjust multivariable analysis for these variables. Third, this is an observational study; no data about the management of vitamin D deficiency during patients' follow-up are available. It is difficult to draw any definite conclusion from this study. We also could not determine whether vitamin D supplementation improve survival in stroke patients. Further studies are needed to confirm our findings and to determine whether vitamin D supplementation can reduce the risk of stroke recurrence in stroke patients. Lastly, the acute stroke may result in acute inflammation and reduce vitamin D which could cause reverse causality bias.

Conclusions

In summary, lower serum levels of 25(OH) D are independently associated with the stroke recurrence and mortality at 24 months in ischemic stroke patients. These data suggest that 25(OH) D may be a strong and independent protective factor against ischemic stroke, in the Chinese population.

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Conflict of Interest Disclosures: The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Author Contributions: Zhao had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Qiu, Wang, Mi, Zhao, Tu, Liu. *Acquisition of data:* Qiu, Wang, Mi, Tu. *Analysis and interpretation of data:* Qiu, Zhao, Liu. *Drafting of the manuscript:* Qiu, Wang, Zhao. *Critical revision of the manuscript for important intellectual content:* Tu, Liu. *Obtained funding:* Zhao. Administrative, technical, or material support: Qiu, Wang, Mi, Tu *Study supervision:* Zhao.

Ethical standard: Written informed consent was obtained from all patients; and, this study conformed to the principles of the Declaration of Helsinki was approved by the investigational review board of the China Rehabilitation Research Center.

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