Elevated Urinary Titin and its Associated Clinical Outcomes after Acute Stroke

Manabu Ishihara, MD,* Nobuto Nakanishi, MD,† Rie Tsutsumi, PhD,‡
Kanako Hara, BS,‡ Kyoka Machida, BS,‡ Nobuaki Yamamoto, MD, PhD,§
Yasuhisa Kanematsu, MD, PhD,* Hiroshi Sakaue, MD, PhD,‡ Jun Oto, MD, PhD,† and
Yasushi Takagi, MD, PhD*

Introduction: Urinary titin is a biomarker of muscle atrophy, which is a serious complication after stroke. However, there are currently no clinical data regarding urinary titin in stroke patients. *Methods:* Consecutive stroke patients admitted to the stroke care unit were included. Spot urine samples were collected immediately after admission, and on days 3, 5, and 7. The primary outcome was the trend of urinary titin in patients after acute stroke. The secondary outcomes included the association between the peak urinary titin level and the modified Rankin Scale (mRS) score, the National Institutes of Health Stroke Scale (NIHSS) score, and the Barthel index (BI) upon hospital discharge. Multivariate analysis was adjusted for age, sex, NIHSS at admission, and the peak urinary titin to predict poor outcome (mRS 3−6). *Results:* Forty-one patients were included (29 male; age, 68 ± 15 years), 29 had ischemic stroke, 8 had intracerebral hemorrhage, and 4 had subarachnoid hemorrhage. The levels of urinary titin on days 1, 3, 5, and 7 were 9.9 (4.7−21.1), 16.2 (8.6−22.0), 8.9 (4.8−15.2), and 8.7 (3.6−16.2) pmol/mg Cr, respectively. The peak urinary titin level was associated with the mRS score ($r = 0.55$, $p < 0.01$), the NIHSS score ($r = 0.72$, $p < 0.01$), and the BI ($r = -0.59$, $p < 0.01$) upon hospital discharge. In multivariate analysis, the peak urinary titin was associated with poor outcome ($p = 0.03$). *Conclusions:* Urinary titin rapidly increased after stroke and was associated with impaired functional outcomes at hospital discharge.
Key Words: Stroke—Titin—Muscle atrophy—Biomarker—Prognosis
© 2020 Elsevier Inc. All rights reserved.

Introduction

Stroke induces a progressive loss of muscle mass,[1] and muscle atrophy is a serious complication in patients after stroke.[2] Muscle atrophy can reach 15.0%−20.8% within the 2 weeks following stroke,[3] and persists for many years after hospital discharge[4]. Muscle atrophy can lead to impaired physical functions and impaired clinical outcomes.[5,6] This prolonged muscle loss, termed stroke-related sarcopenia, is observed in 42% of stroke survivors.[7]

Muscle atrophy is evaluated by anthropometric measurements, computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound.[8] However,

anthropometric measurements are often unreliable,[9] and CT and MRI cannot be used for bedside muscle atrophy monitoring. Although ultrasound is frequently used for the measurement of muscle mass assessment, considerable skill and experience are required for accurate measurement.[10] The identification of a suitable biomarker is urgently needed for better monitoring of muscle atrophy in patients after stroke, as well as improved management of nutrition and rehabilitation.

Recently, urinary titin has been reported to be a biomarker of catabolism and muscle atrophy in critically ill patients.[11] Titin is a spring-like protein that connects actin and myosin, and works to contract muscles. Although titin can be measured in urine samples and can be used as a biomarker of muscle breakdown, it remains poorly understood in many fields including stroke. Since stroke induces muscle atrophy, we hypothesized that the level of urinary titin is increased in patients after stroke, and can be used to assess the prognosis of the disease. Therefore, we conducted a prospective observational study to observe the time course of urinary titin and its associated clinical outcomes in patients after stroke.

Methods

Study design and setting

This was a single-center prospective observational study at the stroke care unit of Tokushima University Hospital from May 2020 to October 2020. The study was approved by the Clinical Research Ethics Committee at Tokushima University Hospital (approval number: 2593), and was registered as a clinical trial (UMIN-Clinical Trials Registry: 000047776). We obtained written informed consent from patients or their family at the time of admission.

Study population

Consecutive stroke patients who were admitted in the stroke care unit of Tokushima University Hospital were included. In our facility, patients are directly transported to the stroke care unit without the need for an emergency department visit. The stroke subtypes included ischemia, intracranial hemorrhage, and subarachnoid hemorrhage. Since the aim of this study was to evaluate the acute change in urinary titin in adults living independently, we excluded patients based on following criteria: (1) in-hospital onset; (2) modified Rankin Scale (mRS) score ≥ 2 before admission; (3) > 24 h from the onset or the last known well time; (4) dialysis, due to the possible influence on urinary titin; (5) unable to collect urine due to urinary incontinence or no urethral catheter; (6) craniotomy or other invasive surgery (not including catheter treatment, percutaneous hematoma drainage, and insertion of external ventricular drainage); (7) seizure; and (8) age < 18 years old. Patients were withdrawn if they met any of these exclusion criteria.

Urinary titin measurement

Urinary titin was measured by ELISA (27900 Titin N-Fragment Assay Kit; Immuno-Biological Laboratories, Gunma, Japan),[12] which measured the N-terminal fragment of titin excreted in urine. The urinary titin was corrected by urinary creatinine to attenuate renal function. Urinary creatinine was measured at LSI medience corporation (Tokyo, Japan). The reference range in healthy adults was approximately $1-3$ pmol/mg Cr.[12] Spot urine was used because titin does not fluctuate throughout the day.[13] The first urine sample was collected immediately after admission to the stroke care unit, and the subsequent urine samples were collected from 8 am to noon on days 3, 5, and 7. We avoided collecting urine immediately after rehabilitation and other invasive procedures. Urine samples were stored in a freezer (-20 °C) until required for measurements. The urinary titin level on day 1, day 3, and at the peak were used for comparison.

Physical assessment

The severity of stroke was assessed at admission using the National Institutes of Health Stroke Scale (NIHSS) score, which ranges from 0 to 42, with higher scores indicating increasing severity.[14] Upon hospital discharge, the clinical outcomes were assessed using the NIHSS score, the mRS score,[15] and the Barthel index (BI). The mRS score ranges from 0 (no disability) to 6 (death) with $0-2$ or $3-6$ defined as good or poor outcomes, respectively, while the BI is used to evaluate activities of daily living, and ranged from 0 to 100, with higher scores indicating better functional status.[16] The NIHSS score and the mRS score were assessed by neurologists, and the BI was assessed by physical therapists.

Outcomes

The primary outcome was the time course of urinary titin in patients after stroke. In secondary analysis, we investigated the relationship between the urinary titin level and clinical outcomes, including the mRS score, the NIHSS score, and the BI at hospital discharge.

Statistics

Continuous data are presented as the mean (standard deviation [SD]) or median (interquartile range [IQR]), and categorical data are presented as counts (%). Comparisons were conducted using the t-test or the Mann−Whitney U test. The three stroke subtypes were compared using the Kruskal−Wallis test with a post hoc Steel−Dwass test. The Pearson correlation coefficient was used to investigate the relationship between urinary titin and clinical outcomes. In the sensitivity analysis, these variables were compared between ischemic and hemorrhage stroke (intracerebral and subarachnoid hemorrhage). Multivariate analysis was conducted to assess if urinary titin was

independently associated with good or poor outcomes, defined by modified Rankin Scale score. In the multivariate analysis, in addition to age and sex, NIHSS at admission was included as the severity of stroke. Since urinary titin has not been previously investigated in stroke patients, 40 patients were planned for enrollment based on feasibility. All clinical data on urinary titin and clinical characteristics are presented in a supplemental file (Table I in the supplemental file). All p-values were two tailed, and p-values < 0.05 were considered statistically significant. JMP version 13.1.0 (SAS Institute Inc., NC, USA) and R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analysis.

Results

One hundred and five patients were admitted in the stroke care unit, and 90 were diagnosed with stroke during the study period (Fig. 1). We excluded 43 patients, and recruited 47 patients. Six patients were withdrawn and 41 patients were included in the final analysis, with a total of 159 urinary titin measurements. Forty-one patients remained in the study on day 5, and 36 patients remained in the study on day 7. The patient characteristics are summarized in Table 1. The age of the included patients was 68 ± 15 years, and 29 patients were male. The median NIHSS score was 6 (IQR, 2−21), and the clinical subtypes of stroke were ischemic stroke ($n = 29$, 71%), intracranial hemorrhage ($n = 8$, 20%), and subarachnoid hemorrhage ($n = 4$, 10%).

Trend of urinary titin

The levels of urinary titin on days 1, 3, 5, and 7 were 9.9 (4.7−21.1), 16.2 (8.6−22.0), 8.9 (4.8−15.2), and 8.7
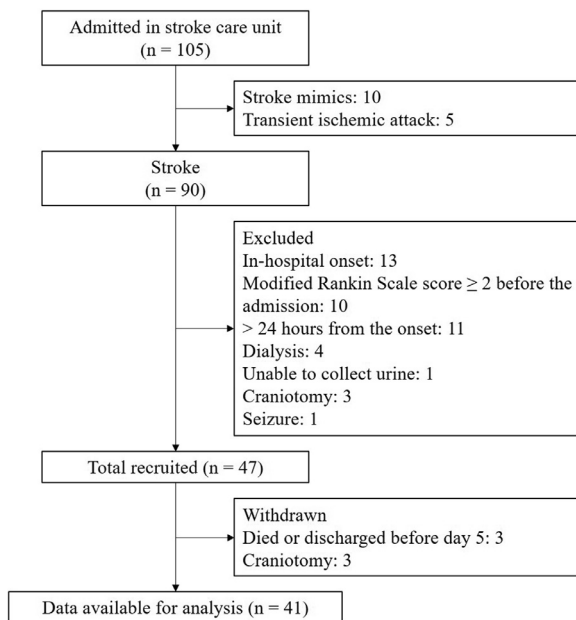
Table 1. Patient Characteristics.

Variables	Overall (n = 41)
Age, years (mean [SD])	68 ± 15
Sex (Male), n (%)	29 (71)
Body mass index, kg/m^2	24 (22−27)
Comorbidities	
High blood pressure	30 (73)
Diabetes mellitus	12 (29)
Chronic kidney disease	2 (5)
Modified Rankin Scale at admission	
0	36 (88)
1	5 (12)
NIHSS	6 (2−21)
Length of SCU stay, days	11 (8−15)
Length of hospital stay, days	15 (13−20)
Stroke type, n (%)	
Ischemic stroke	29 (71)
Intracranial hemorrhage	8 (20)
Subarachnoid hemorrhage	4 (10)

SD = standard deviation, NIHSS = National Institutes of Health Stroke Scale, SCU = stroke care unit

Data were presented as median (interquartile range) unless otherwise indicated.

(3.6−16.2) pmol/mg Cr, respectively (Fig. 2). The peak urinary titin was 18.8 (9.7−44.2) pmol/mg Cr; in stroke subtypes, the peak urinary titin was 18.3 (7.4−31.6) at ischemic stroke, 60.5 (15.7−112.2) at intracranial hemorrhage, and 15.7 (10.8−43.9) pmol/mg Cr at subarachnoid hemorrhage. There was no statistically significant difference in peak urinary titin among the stroke subtypes (Kruskal−Wallis test, $p = 0.10$; Steel−Dwass test, $p = 0.10−1.00$). The titin measurement on day 1 was obtained 7 (3−16) h after the onset

Fig. 1. Flowchart of patients During the study period, 47 patients were recruited and 41 patients were included in the final analysis.
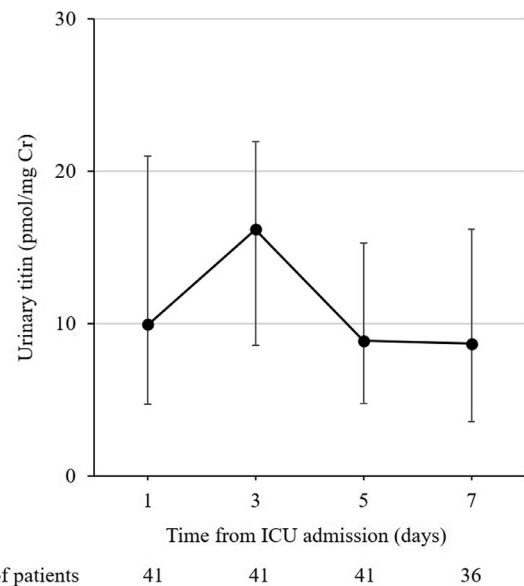

Fig. 2. Trend of urinary titin after stroke Urinary titin was increased compared to the normal level (1−3 pmol/mg Cr). Data are presented as median (interquartile range), and the number of patients is shown below the graph.
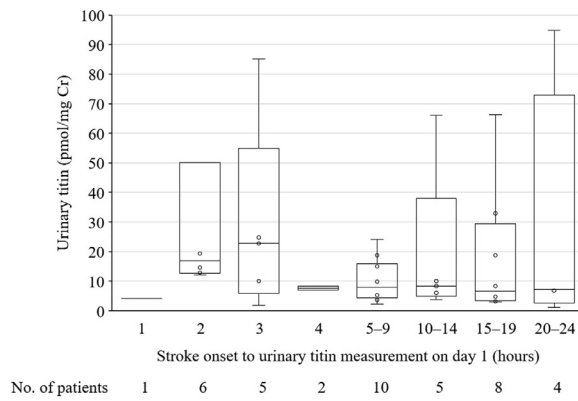
Fig. 3. *Level of urinary titin from stroke onset to the measurement on day 1 Compared to the normal level (1−3 pmol/mg Cr), urinary titin increased within the 24 h following stroke onset. Points within a box are shown in the figure, and the number of patients is shown below the graph.*

of stroke or the last known well time. The urine sample was obtained at 2 or 3 h from the onset in 6 or 5 patients respectively; the urinary titin levels of these patients were already elevated at 17.0 (12.7−50.1) or 22.8 (5.9−54.9) pmol/mg Cr, respectively (Fig. 3). There was no correlation between the time of stroke onset and the urinary titin on day 1 ($r = 0.01$, $p = 0.94$). The urinary titin on day 1 was weakly associated with the NIHSS score at admission ($r = 0.34$, $p = 0.03$) (Table 2).

Relationship with clinical outcomes

The peak urinary titin was associated with the mRS score ($r = 0.55$, $p < 0.01$), the NIHSS score ($r = 0.72$, $p < 0.01$), and the BI ($r = −0.59$, $p < 0.01$) upon hospital discharge. On the other hand, urinary titin on day 1 and 3 was weakly associated with these outcomes, compared to the peak urinary titin. In the sensitivity analysis of ischemic stroke, the peak urinary titin was associated with the mRS score ($r = 0.57$, $p < 0.01$) (Table II in the supplemental file), the NIHSS score ($r = 0.71$, $p < 0.01$), and the BI upon hospital discharge ($r = −0.59$, $p < 0.01$), while in intracranial and subarachnoid hemorrhage, the association was only observed in the NIHSS score ($r = 0.67$, $p = 0.02$) (Table III in the supplemental file).

In multivariate analysis, the peak urinary titin was associated with poor outcome (odds ratio [OR], 1.06; 95% confidence interval [CI], 1.01−1.17 per unit increase, $p = 0.03$) as well as NIHSS (OR, 1.25; 95% CI, 1.08−1.55 per unit increase, $p < 0.01$, Table IV in the supplemental file). On the other hand, the urinary titin on day 1 was not associated with poor outcome (OR, 1.04; 95% CI, 0.99−1.13 per unit increase, $p = 0.13$), while urinary titin on day 3 was associated with poor outcome (OR, 1.11; 95% CI, 1.01−1.28 per unit increase, $p = 0.02$)

Discussion

In this observational study, we found that the levels of urinary titin were rapidly increased after stroke. Moreover, increased urinary titin was associated with functional outcomes at hospital discharge. To the best of our knowledge, this is the first study to investigate the time course of urinary titin and its associated outcomes in patients after stroke. Urinary titin may be used to evaluate the catabolism and clinical outcomes after stroke.

After stroke, the peak urinary titin was 19.2 (6.9−35.9) pmol/mg Cr, which was higher than values of healthy volunteers 2.1 (1.2−2.6 pmol/mg Cr)[12] and lower than the peak of critically ill patients 67.9 (35.7−116.2 pmol/mg Cr).[11] A previous study demonstrated that urinary titin is associated with catabolism and muscle atrophy.[11] Therefore, our results suggest that patients experience increased catabolism and muscle atrophy following stroke. Interestingly, urinary titin was already elevated in the 2 h following the onset of stroke. In this study, we excluded patients with impaired independent living and delayed admission after the stroke. Therefore, we considered urinary titin on day 1 rapidly increased after the stroke onset. The immediate increase in urinary titin cannot be explained by immobilization or impaired feeding, because these factors take days to cause muscle atrophy.[17] We consider that the rapid increase in urinary titin is due to the immediate breakdown of muscle from brain stimuli. Rapid crosstalk may occur from the brain to the muscle following acute stroke, with three potential pathways thought to cause the immediate

Table 2. *The association between urinary titin and functional scores.*

	Titin on day 1		Titin on day 3		Titin at the peak	
	r	p	r	p	r	p
At admission						
NIHSS	0.34	0.03	0.46	< 0.01	0.57	< 0.01
At hospital discharge						
modified Rankin Sale	0.45	< 0.01	0.46	< 0.01	0.55	< 0.01
NIHSS	0.36	0.02	0.58	< 0.01	0.72	< 0.01
Barthel index	−0.44	< 0.01	−0.49	< 0.01	−0.59	< 0.01

Pearson correlation coefficient was used to assess the correlations (n = 41).
NIHSS: National Institutes of Health Stroke Scale

catabolic signaling.[18] First, acute inflammatory cytokines produced as a result of brain stimulation, may cause immediate proteolysis due to their increase within 4 h of stroke.[19] Second, the hypothalamic-pituitary-adrenocortical axis may react immediately. Indeed, a previous animal study demonstrated that the plasma corticosterone level increased within 1 h in mice subjected to stroke.[20] Because steroid use represents a risk factor of muscle atrophy, this reaction may cause rapid muscle breakdown.[8] Third, stroke-induced brain damage may activate the sympathetic nervous system, resulting in accelerated catabolism and subsequent muscle breakdown. These immediate reactions are understandable because brain damages starts within minutes of stroke onset.[21]

The peak urinary titin level was independently associated with impaired physical functions. In a previous study, muscle atrophy after subarachnoid hemorrhage was associated with a decreased mRS score at hospital discharge.[22] Since urinary titin reflects muscle atrophy, the association between increased urinary titin and physical functions is understandable. Interestingly, in multivariate analysis adjusted for stroke severity, the urinary titin on day 3 was already associated with the clinical outcomes at hospital discharge; this indicates the possible clinical utility of urinary titin to predict patient outcomes. The correlation was also confirmed in the subanalysis including only ischemic stroke, although the sample size in the hemorrhage subanalysis was too small for a significant difference to be observed. Identification of biomarkers is important for effective patient management, since biomarkers can be measured in unconscious patients in whom physical assessment cannot be conducted. Moreover, reliable physical assessment requires skill and training, and there is often interobserver variability. Indeed, previous studies have also shown variability among the NIHSS score and mRS score with different observers.[23,24]

Targeting muscle metabolism in the acute phase will be essential for preventing muscle atrophy in stroke. This study indicates that urinary titin may be used to assess the catabolism, while immediate nutrition or rehabilitation might be needed to deal with the rapid muscle breakdown. Rehabilitation and protein administration can suppress proteolysis and prevent muscle atrophy.[25,26] However, a very early rehabilitation within 24 h is still controversial after acute stroke. In the AVERT III trial, although rehabilitation within 24 h was important, too early mobilization was associated with decreased favorable outcomes.[27] In another study, mobilization within 24 h was associated with poor outcomes.[28] The use of rehabilitation within 24 h is still debated, but the finding that muscle breakdown occurs within 24 h after stroke onset is important. Similarly, the efficacy of nutrition within 24 h is currently unknown,[29] although it is possible that early nutritional intervention prevents acute muscle breakdown. Further studies are required to determine whether early nutrition and rehabilitation intervention

decreases the rapid increase in urinary titin and impaired functional outcomes.

This study has several limitations. First, this study is based on a small sample size; in particular, the numbers of patients with intracranial and subarachnoid hemorrhage were fewer than those with ischemic stroke. Second, we did not use a 24 h urine sample; urinary titin does not have circadian fluctuance,[13] and we collected the spot urine in the same time frame. However, spot urine may still be affected by additional factors compared to a 24 h urine sample. Third, we did not measure the muscle loss in study subjects because our previous study has already reported that urinary titin reflects muscle atrophy.[11] Unlike creatinine kinase, titin exists only in sarcomere of muscles.[13] Thus, it is reasonable to understand the urinary titin reflects muscle breakdown, not brain damage.

Summary

Urinary titin was rapidly increased after stroke, and was associated with worse functional outcomes at hospital discharge. Urinary titin may be used as a biomarker of catabolism and prognosis after stroke.

Authors' contributions

MI and NN was involved in study design, analysis, interpretation of the data, and drafting of the manuscript. RT, KH, KM took part in laboratory matters and interpretation of the data. NY and YK were involved in the interpretation of the data. HS, JO, and YT took part in study concept, interpretation of the data, and critical revision of the manuscript. All authors read and approved the final manuscript.

Source of funding

Disclosures

None.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2020.105561.

References

1. Desgeorges MM, Devillard X, Toutain J, et al. Molecular mechanisms of skeletal muscle atrophy in a mouse model of cerebral ischemia. Stroke 2015;46:1673-1680.

2. English C, McLennan H, Thoirs K, et al. Loss of skeletal muscle mass after stroke: a systematic review. Int J Stroke 2010;5:395-402.

3. Nozoe M, Kanai M, Kubo H, et al. Changes in quadriceps muscle thickness, disease severity, nutritional status, and c-reactive protein after acute stroke. J Stroke Cerebrovasc Dis 2016;25:2470-2474.

4. Ryan AS, Ivey FM, Serra MC, et al. Sarcopenia and physical function in middle-aged and older stroke survivors. Arch Phys Med Rehabil 2017;98:495-499.

5. Maeda H, Imada K, Ishida K, et al. Quadriceps thickness and echo intensity predict gait independence in individuals with severe and mild hemiparetic stroke. Eur Neurol 2020;83:167-173.

6. Scherbakov N, Doehner W. Sarcopenia in stroke-facts and numbers on muscle loss accounting for disability after stroke. J Cachexia Sarcopenia Muscle 2011;2:5-8.

7. Su Y, Yuki M, Otsuki M. Prevalence of stroke-related sarcopenia: a systematic review and meta-analysis. J Stroke Cerebrovasc Dis 2020;29:105092.

8. Nakanishi N, Takashima T, Oto J. Muscle atrophy in critically ill patients: a review of its cause, evaluation, and prevention. J Med Invest 2020;67:1-10.

9. Chan KS, Mourtzakis M, Aronson Friedman L, et al. Upper arm anthropometrics versus DXA scan in survivors of acute respiratory distress syndrome. Eur J Clin Nutr 2018;72:613-617.

10. Nakanishi N, Tsutsumi R, Okayama Y, et al. Monitoring of muscle mass in critically ill patients: comparison of ultrasound and two bioelectrical impedance analysis devices. J Intensive Care 2019;7:61.

11. Nakanishi N, Tsutsumi R, Hara K, et al. Urinary titin is a novel biomarker for muscle atrophy in nonsurgical critically ill patients: a two-center, prospective observational study. Crit Care Med 2020;48:1327-1333.

12. Maruyama N, Asai T, Abe C, et al. Establishment of a highly sensitive sandwich ELISA for the N-terminal fragment of titin in urine. Sci Rep 2016;6:39375.

13. Matsuo M, Awano H, Maruyama N, et al. Titin fragment in urine: a noninvasive biomarker of muscle degradation. Adv Clin Chem 2019;90:1-23.

14. Kasner SE. Clinical interpretation and use of stroke scales. Lancet Neurol 2006;5:603-612.

15. van Swieten JC, Koudstaal PJ, Visser MC, et al. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604-607.

16. Mahoney FI, Barthel DW. Functional evaluation: the Barthel Index. Md State Med J 1965;14:61-65.

17. Springer J, Schust S, Peske K, et al. Catabolic signaling and muscle wasting after acute ischemic stroke in mice: indication for a stroke-specific sarcopenia. Stroke 2014;45:3675-3683.

18. Delezie J, Handschin C. Endocrine crosstalk between skeletal muscle and the brain. Front Neurol 2018;9:698.

19. Lambertsen KL, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke. J Cereb Blood Flow Metab 2012;32:1677-1698.

20. Yang J, Kim E, Beltran C, et al. Corticosterone-mediated body weight Loss Is an Important Catabolic Process for Poststroke Immunity and Survival. Stroke 2019;50: 2539-2546.

21. Hjort N, Christensen S, Sølling C, et al. Ischemic injury detected by diffusion imaging 11 minutes after stroke. Ann Neurol 2005;58:462-465.

22. Nozoe M, Kanai M, Kubo H, et al. Quadriceps muscle thickness changes in patients with aneurysmal subarachnoid hemorrhage during the acute phase. Top Stroke Rehabil 2018;25:209-213.

23. Josephson SA, Hills NK, Johnston SC. NIH stroke scale reliability in ratings from a large sample of clinicians. Cerebrovasc Dis 2006;22:389-395.

24. Quinn TJ, Dawson J, Walters MR, et al. Reliability of the modified Rankin Scale: a systematic review. Stroke 2009;40:3393-3395.

25. Kokura Y, Kato M, Taniguchi Y, et al. Energy intake during the acute phase and changes in femoral muscle thickness in older hemiplegic inpatients with stroke. Nutrition 2020;70:110582.

26. Nakanishi N, Oto J, Tsutsumi R, et al. Effect of electrical muscle stimulation on upper and lower limb muscles in critically ill patients: a two-center randomized controlled trial. Crit Care Med 2020;48:e997-e1003.

27. Efficacy and safety of very early mobilisation within 24 h of stroke onset (AVERT): a randomised controlled trial. Lancet 2015;386:46-55.

28. Sundseth A, Thommessen B, Rønning OM. Outcome after mobilization within 24 hours of acute stroke: a randomized controlled trial. Stroke 2012;43:2389-2394.

29. Burgos R, Bretón I, Cereda E, et al. ESPEN guideline clinical nutrition in neurology. Clin Nutr 2018;37:354-396.