Urinary titin is a novel biomarker for muscle atrophy in nonsurgical critically ill patients: a two-center, prospective observational study

Short running title: Urinary titin as a biomarker for muscle atrophy

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Reprints: None

Conflict of interest: None

Funding: None

Abstract

Objective: Although skeletal muscle atrophy is common in critically ill patients, biomarkers associated with muscle atrophy have not been identified reliably. Titin is a spring-like protein found in muscles and has become a measurable biomarker for muscle breakdown. We hypothesized that urinary titin is useful for monitoring muscle atrophy in critically ill patients. Therefore, we investigated urinary titin level and its association with muscle atrophy in critically ill patients.

Design: Two-center, prospective observational study

Setting: Mixed medical/surgical intensive care unit (ICU) in Japan

Patients: Nonsurgical adult patients who were expected to remain in ICU for >5 days

Interventions: None

Methods: Urine samples were collected on days 1, 2, 3, 5, and 7 of ICU admission. To assess muscle atrophy, rectus femoris cross-sectional area and diaphragm thickness were measured with ultrasound on days 1, 3, 5, and 7. Secondary outcomes included its relationship with ICU-acquired weakness (ICU-AW), ICU Mobility Scale (IMS), and ICU mortality.

Measurements and Main Results: Fifty-six patients and 232 urinary titin measurements were included. Urinary titin (normal range: 1–3 pmol/mg Cr) was 27.9 (16.8–59.6), 47.6 (23.5–82.4), 46.6 (24.4–97.6), 38.4 (23.6–83.0), and 49.3 (27.4–92.6) pmol/mg Cr on days 1, 2, 3, 5, and 7, respectively. Cumulative urinary titin level was significantly associated with rectus femoris muscle atrophy on days 3–7 ($p < 0.03$), although urinary titin level was not associated with change in diaphragm thickness ($p = 0.31$–0.45). Furthermore, cumulative urinary titin level was associated with incidence of ICU-AW ($p = 0.01$) and ICU mortality ($p = 0.02$) but not with IMS ($p = 0.18$).

Conclusions: In nonsurgical critically ill patients, urinary titin level increased 10–30 times compared with the normal level. The increased urinary titin level was associated with lower limb muscle atrophy, incidence of ICU-AW, and ICU mortality. (300 words)

Keywords: urinary titin, ultrasound, muscle atrophy, rectus femoris muscle, diaphragm, intensive care unit-acquired weakness

INTRODUCTION

Skeletal muscle atrophy is common in critically ill patients (1, 2). After intensive care unit (ICU) admission, muscle atrophy develops within 3 days and progressively worsens thereafter (3). In our previous study, upper and lower limb muscle mass of critically ill patients decreased by 13%–21% within 7 days of admission (4). Muscle atrophy in ICU is associated with long-term functional impairment and weakness among ICU survivors (5).

Although monitoring of muscle mass is important, reliable biomarkers for muscle atrophy are unavailable for critically ill patients. However, recently, an enzyme-linked immunosorbent assay (ELISA) kit was developed for detecting urinary N-terminal fragment of titin (6). Titin is a giant sarcomeric protein, which functions as a spring for muscle extension and viscoelasticity. Because urinary titin reflects muscle breakdown, it is used as a biomarker for diagnosing muscular dystrophy (7). In patients with muscular dystrophy, urinary titin level increases 100 times compared with that in healthy volunteers (normal range: 1–3 pmol/mg Cr). To the best of our knowledge, no study has investigated urinary titin level in critically ill patients and its association with muscle atrophy.

We hypothesized that urinary titin will increase in critically ill patients and can be used to assess muscle atrophy. According to a study, surgical insult increased urinary titin level (8). Therefore, we investigated urinary titin level in nonsurgical critically ill patients. The aim of this exploratory study was to investigate the relationship between urinary titin level and limb muscle atrophy in nonsurgical critically ill patients. Secondary objectives were to assess its relationship with changes in diaphragm thickness, incidence of ICU-acquired weakness (ICU-AW), ICU

Mobility Scale (IMS), and ICU mortality.

MATERIALS AND METHODS

Study Design

From May 2019 to February 2020, we conducted a two-center, prospective observational study at the mixed medical/surgical ICU of Tokushima University Hospital and Tokushima Prefectural Central Hospital. This study was approved both clinical research ethics committees of Tokushima University Hospital (approval number 2593) and Tokushima Prefectural Central Hospital (approval number 1739). This trial was registered as a clinical trial (UMIN-Clinical Trials Registry: 000031316). At the time of enrollment, written informed consent was obtained from patients or their authorized surrogate decision makers.

Consecutive adult patients who were expected to remain in ICU for >5 days were enrolled. Patients were prospectively recruited within 12 h of ICU admission. Nonsurgical critically ill patients were included because surgical insult reportedly increased urinary titin level (Table S1) (8). We excluded patients based on the following exclusion criteria: surgery not including percutaneous abscess drainage, chest tube insertion, and tracheostomy in ICU; age of <18 years; current pregnancy; diagnosis of primary neuromuscular disease; trauma at the measurement point, and unclear ultrasound image.

Urinary Titin Measurement

The first urine sample was collected using a urethral catheter within 12 h of ICU admission and 24-hour urine samples on days 2, 3, 5, and 7. Urinary titin was measured using an ELISA kit (27900 Titin N-Fragment Assay Kit, Immuno-Biological Laboratories Co. Ltd., Japan) (6). This kit is capable of measuring urinary N-terminal fragment of titin cleaved by calpain-3. This is a highly sensitive kit used to measure a whole range of biologically relevant protein levels in urine samples (9). The reference range was 1–3 pmol/mg Cr in healthy volunteers, which was

creatinine (Cr) corrected to adjust for various physiological conditions (6). We used cumulative urinary titin level (area under the curve of urinary titin level for individual patients) for comparison.

Urine volume and concentration are influenced by kidney function, dehydration, age, and sex. Therefore, Cr correction is necessary in the measurement of titin level (6). We analyzed the influence of acute kidney injury (AKI) and renal replacement therapy because renal function is often impaired in critically ill patients. AKI was defined as Kidney Disease Improving Global Outcomes stages 1–3 without renal replacement therapy (RRT). Twenty-six and six patients were classified into AKI and RRT, respectively. Cr correction seems to attenuate the influence of AKI and RRT (**Fig. 1**).

Ultrasonographic Measurement

Rectus femoris muscle area and diaphragm thickness were evaluated with serial ultrasound measurements on days 1, 3, 5, and 7 of ICU admission. Recordings were discontinued at death or ICU discharge. Cross-sectional area of the rectus femoris muscle was evaluated at the midway between the anterior superior iliac spine and the proximal end of the patella. A transducer was placed perpendicularly to the long axis of the rectus femoris muscle with patients in the supine position under passive knee extension. Patients were divided into a high- or low-atrophy group on days 3, 5, and 7 of ICU admission based on the extent of muscle atrophy. The cutoff value was decided based on the median atrophy rate each day.

The diaphragm was evaluated at the zone of apposition on the right chest wall. Its thickness was measured during the end-expiration phase. Beds were adjusted at a 30° angle. We excluded patients whose diaphragm was unclear or difficult to measure. Patients were divided into three groups according to the changes in diaphragm thickness: atrophy, unchanged, and increased. A 10% change in diaphragm thickness was regarded as the cutoff value in the three groups. Atrophy was first classified with >10% decrease in diaphragm thickness from day 1 to

the lowest value over the measurement period. Thereafter, increased thickness group was classified when >10% increase was observed. The rest of the patients were classified into the unchanged group, as previously reported (10). In the analysis, the unchanged group was compared with atrophy and increased groups and their combination because both increased and decreased diaphragm thickness significantly influence clinical outcomes (11).

Rectus femoris cross-sectional area and diaphragm thickness were measured thrice, and the median value was used for evaluation. All measurements were conducted by two examiners. Intraclass and interclass correlation coefficients were 0.99 and 0.99 for rectus femoris cross-sectional area and 0.95 and 0.95 for diaphragm thickness, respectively (Table S2).

Physical Assessment and Mobilization

When patients were awake and attentive, physical therapists evaluated the Medical Research Council (MRC) score and incidence of ICU-AW on days 1, 3, 5, and 7 of ICU admission. Intact level of consciousness and awareness was defined by patient's response to at least three of five orders (12). ICU-AW was defined as an MRC score of <48 on two separate occasions, and patients with expected prehospital functional status of <48 were excluded (13). We used the incidence of ICU-AW following the last measurement for comparison. IMS is a measure of mobilization capabilities from 0 (lying in bed) to 10 (walking independently) (14). We evaluated maximum IMS score during the study period because the maximum level of mobility is an important prognostic factor (15). Two facilities in this study used the same progressive mobilization protocol (16).

Outcomes

Our primary outcome was the relationship between cumulative urinary titin level and rate of rectus femoris muscle atrophy on days 3, 5, and 7 of ICU admission. In secondary analysis, we evaluated the relationship between urinary titin level and change in diaphragm thickness

(atrophy, unchanged, and increased groups), incidence of ICU-AW, IMS, and ICU mortality.

Statistical Analysis

Continuous data are presented as mean ± standard deviation or median [interquartile range (IQR)], as appropriate, whereas categorical data are presented as number (%). Variables were compared using *t*-test the Mann–Whitney *U*-test for two-group comparison, and one-way analysis of variance or the Kruskal-Wallis test for three-group comparison. Post hoc correction for multiple comparisons was performed with Tukey's or Steel–Dwass test. Changes in muscle mass were assessed by mixed-effect model for repeated measures (MMRM). MMRM was also used to evaluate the association between urinary titin level on day 1 and change in diaphragm thickness or rectus femoris cross-sectional area over time. We constructed receiver operating characteristic (ROC) curves to determine whether urinary titin level can discriminate ICU-AW. Because titin has not been investigated in critically ill patients, 60 patients were planned for enrollment based on a previous study (17). Data analyses were conducted using JMP version 13.1.0 (SAS Institute Inc., Cary, NC). All statistical tests were two tailed, and the chosen type 1 error rate was *p*-value of <0.05.

RESULTS

In total, 62 patients were recruited, among whom 56 remained in the study till day 3, 40 till day 5, and 24 till day 7 of ICU admission (**Fig. S1**). Six patients were excluded due to only one measurement, with 56 patients and 232 urinary titin level measurements in the final analysis. Patient characteristics are summarized in **Table 1**. The mean age of the patients was 72 ± 13 years, and 33 patients were male. Acute Physiology and Chronic Health Evaluation II score was 25 (IQR, 19–29) and length of ICU stay was 6 days (IQR, 4–9 days). Thirty-seven (66%) patients were mechanically ventilated, with additional 13 (23%) patients under noninvasive positive pressure ventilation or high-flow nasal cannula. The main reasons for admission were

respiratory failure (n = 23, 41%), heart failure (n = 12, 21%), non-respiratory sepsis (n = 6, 11%), and cardiac arrest (n = 6, 11%), with 23 (41%) patients meeting the sepsis III criteria.

Trend of Urinary Titin Level

Urinary titin level was 27.9 (16.8–59.6), 47.6 (23.5–82.4), 46.6 (24.4–97.6), 38.4 (23.6–83.0), and 49.3 (27.4–92.6) pmol/mg Cr on days 1, 2, 3, 5, and 7 of ICU admission, respectively (**Fig. 1a**). In 19 (34%) patients, the highest urinary titin level was >100 pmol/mg Cr, whereas 4 patients had a level of >300 pmol/mg Cr. Urinary titin level significantly increased from day 1 to days 2–7 of ICU admission ($p \leq 0.02$). The trend of urinary titin level is shown for each admission in Table S1, and patients who met the sepsis III criteria had a higher urinary titin level than non-sepsis patients (sepsis vs non-sepsis, 93.0 vs 57.9 pmol/mg Cr, $p = 0.02$).

Relationship between Urinary Titin Level and Rectus Femoris Cross-Sectional Area

A total of 176 images of 56 patients were analyzed. Rectus femoris cross-sectional area changed progressively by −8.6% ± 4.9%, −13.8% ± 5.9%, and −18.2% ± 5.6% on days 3, 5, and 7 of ICU admission, respectively ($p < 0.01$; MMRM, **Fig. 1b**). Regarding high and low-atrophy groups, the median cutoff value was set at −8.6%, −12.0%, and −17.5% for days 3, 5, and 7 of ICU admission, respectively. There was a significant difference in cumulative urinary titin level between these groups: 138.2 (78.9–247.3) vs 63.9 (29.2–104.3) pmol/mg Cr ($p < 0.01$) on day 3, 289.9 (166.2–411.2) vs 111.8 (72.2–229.6) pmol/mg Cr ($p < 0.01$) on day 5, and 515.4 (241.1–799.1) vs 192.4 (135.9–326.2) pmol/mg Cr ($p = 0.03$) on day 7 of ICU admission (high vs low-atrophy group; **Fig. 2**). The change in rectus femoris cross-sectional area was associated with urinary titin level on day 1 (Titin $p = 0.01$, Time $p < 0.01$; MMRM). The correlation is presented in **Fig. S3**.

Relationship between Urinary Titin Level and Diaphragm Thickness

A total of 156 diaphragm images of 50 patients were analyzed. Six patients were excluded due to unsuccessful measurement. Diaphragm thickness decreased by >10% in 32 (64%) patients, remained unchanged in 12 (24%), and increased by >10% in 6 (12%). In total, diaphragm thickness changed by −4.9% ± 15.8%, −8.0% ± 16.9%, and −15.4% ± 10.2% on days 3, 5, and 7 of ICU admission, respectively ($p < 0.01$; MMRM). There was no significant difference in cumulative urinary titin level: 147.9 (79.0–257.8) vs 192.4 (104.0–367.5) pmol/mg Cr ($p = 0.33$); 426.1 (140.8–578.2) pmol/mg Cr ($p = 0.45$); 206.5 (99.3–440.8) pmol/mg Cr, ($p = 0.31$), respectively (unchanged vs atrophy, increased, and combination groups, **Fig. 3a**). The median difference was 44.9 [95% confidence interval (CI), −63.4–186.2], 230.0 (95% CI, −144.5–527.3), and 52.0 (95% CI, −57.5–223.9) pmol/mg Cr in atrophy, increased, and combination groups, respectively. The change in diaphragm thickness was not associated with urinary titin level on day 1 (Titin $p = 0.51$, Time $p = 0.01$; MMRM).

Relationship between Urinary Titin Level and Other Outcomes

We assessed ICU-AW in 35 of the 56 included patients; subsequently, 9 patients that met the diagnostic criteria of ICU-AW. Cumulative urinary titin level was significantly higher in patients with ICU-AW: 314.1 (181.5–464.7) in patients with ICU-AW vs 86.6 (66.3–171.1) pmol/mg Cr in patients without ICU-AW ($p = 0.01$; **Fig. 3b**). The area under the curve of ROC curves of ICU-AW were 0.78 (95% CI, 0.61–0.95) for cumulative urinary titin level and 0.75 (95% CI, 0.56–0.94) cumulative urinary titin level on day 2 of ICU admission (**Fig. S2**). The maximum IMS was not associated with cumulative urinary titin level: 191.8 (96.9–366.9) in IMS 0–1 vs 116.7 (60.8–368.4) pmol/mg Cr in IMS 2–10 ($p = 0.18$). On the other hand, cumulative urinary titin level was associated with ICU mortality: 466.5 (206.7–1055.9) for dead patients vs 152.9 (75.6–312.5) pmol/mg Cr for alive patients ($p = 0.02$).

DISCUSSION

In this two-center prospective observational study, we found that urinary titin level increased in nonsurgical critically ill patients, and cumulative urinary titin level reflected the extent of rectus femoris muscle atrophy. In addition, cumulative urinary titin level was associated with the incidence of ICU-AW and ICU mortality. Our study indicates urinary titin level can be used to evaluate catabolism and muscle atrophy in critically ill patients. To the best of our knowledge, no study has investigated urinary titin level in critically ill patients.

Urinary titin level was 10–30 times higher in critically ill patients than in healthy volunteers (1–3 pmol/mg Cr), and 34% of patients had >100 pmol/mg Cr, which is the equivalent level in muscular dystrophy (7). Catabolism was clearly ongoing in critically ill patients. As a biomarker, urinary titin level may rapidly respond to the patient's status and can reflect ongoing catabolism due to the high urinary titin level on admission day and significant increase from day 2 of admission. Oshida et al. suggested urinary titin level may reflect skeletal muscle atrophy in nonalcoholic fatty liver disease because this level was higher in low-muscle mass group (18). In our study, we clearly observed that urinary titin level is a good biomarker for ongoing catabolism and subsequent muscle atrophy in critically ill patients. As a result, it is reasonable that higher cumulative urinary titin level is associated with ICU-AW and ICU mortality.

Urinary titin level can be a good biomarker to diagnose ICU-AW, which is closely related to muscle atrophy (19). Because ICU-AW is established by evaluating volitional measurement (20), its use as a biomarker may help in identifying high-risk patients of ICU-AW including unconscious patients at an early phase. In a study, neurofilament level was higher in ICU-AW, but its peak was observed on day 7 (5–14) of ICU admission (21). On the other hand, urinary titin level significantly increased from day 2 of ICU admission, and the level on day 2 showed good discriminative power. This is because muscle damage occurs more rapidly than neural damage in critically ill patients (22). Creatine phosphokinase (CK) might also be one of the candidate biomarkers for ICU-AW. However, it was not useful in identifying ICU-AW (23) because CK leaks from various tissues such as the brain as well as mitochondria (24). In the

diagnosis of muscular dystrophy, CK showed high false-positive rates (25) and urinary titin level was a better biomarker (9). Titin is a functional protein as actin and myosin, unlike CK. Therefore, urinary titin level represented deteriorated muscle function in critically ill patients.

In this study, we obtained evidence that links elevated urinary titin level to rectus femoris muscle atrophy but not to diaphragm thickness. Because the quadriceps femoris muscle is the most voluminous muscle of the body and is a good indicator of whole-body muscle mass (26), it is understandable that elevated urinary titin level was correlated with rectus femoris muscle atrophy. On the other hand, the amount of muscle in the diaphragm is lesser than that in the quadriceps femoris muscle, with different physiologies (27). Moreover, the diaphragm is not the only respiratory muscle to be atrophied (10). Thus, we could not observe a significant association between change in diaphragm thickness and cumulative urinary titin level. However, a study suggested that titin is involved in diaphragm atrophy (28). Therefore, we need to create a diaphragm-specific titin antibody akin to the cardiac-specific antibody in use (29).

The current standard for diagnosing muscle atrophy is ultrasound, which can be used noninvasively at bedside (30). Ultrasound is the most reliable method for monitoring muscle mass because it is not influenced by fluid balance in critically ill patients (31). However, it requires measurement equipment, time, and skills. On the other hand, because measuring urinary titin level is simple, noninvasive, and needs no equipment, it may be more helpful for critically ill patients. Based on the urine test, we corrected kidney function by dividing urinary creatine (pmol/mg Cr). The influence of AKI seems small, although corrected urinary titin differed on day 7 of ICU admission due to limited number of patients (**Fig. 1**). Urinary titin level seemed to be corrected in patients requiring RRT because the levels of urinary titin and Cr were higher without correction. However, this result should be carefully interpreted because the removal ratio may depend on the dialysis membrane used for RRT.

Urinary titin level is an emerging biomarker gaining attention in many fields (9). Its use may bring a paradigm shift in the muscle mass monitoring system, an approach that attenuates

muscle atrophy in critically ill patients. Despite the potential role of titin, it is not routinely available now. It is possible to create a spot urine test because urinary titin level does not fluctuate during the day (9). It is important to be aware about the catabolic state and identify muscle atrophy and ICU-AW to initiate early rehabilitation and nutritional support, which may improve long-term outcomes.

Limitations

First, this study had a small sample size, particularly in the analysis of diaphragm thickness. Second, the primary urinary titin level depends on the time of urine collection from the onset of the disease and does not reflect the normal state of patients. Lastly, although urinary titin level was associated with rectus femoris muscle atrophy, it showed a wide range of values. We cannot exclude the possible influence of various physiological conditions on Cr correction (pmol/mg Cr).

CONCLUSIONS

In nonsurgical critically ill patients, urinary titin level increased 10–30 times compared with the normal level. The increased urinary titin level reflected lower limb muscle atrophy. Furthermore, urinary titin level was associated with incidence of ICU-AW and ICU mortality. Urinary titin level could be used as a biomarker for catabolism and subsequent muscle atrophy.

ACKNOWLEDGEMENT

The authors thank ICU nurses and physical therapists at Tokushima University Hospital and Tokushima Prefectural Central Hospital. The authors thank Yoshihiro Okayama (Clinical Trial Center for Development Therapeutics, Tokushima University Hospital) for his statistical support. The authors thank people who supported the muscle atrophy zero project, which aims to prevent muscle atrophy in critically ill patients.

Conflicts of Interest

The authors have no conflict of interest to declare.

Funding

This research received no specific grant from any funding agency in the public, commercial, or nonprofit sectors.

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patients: comparison of ultrasound and two bioelectrical impedance analysis devices. J Intensive Care 2019; 7:61

Figure Legends

Figure 1. Influence of acute kidney injury or renal replacement therapy. A, Urinary titin (pmol/L). B, Urinary Creatinine (mg/dL). C, Urinary titin (pmol/mg Cr). AKI = acute kidney injury, RRT = renal replacement therapy, KDIGO = Kidney Disease Improving Global Outcomes. AKI was defined as KDIGO stages 1–3 without RRT. Number of patients on days 3, 5, and 7 of ICU admission was 24, 16, and 10 for normal kidney function; 26, 18, and 8 for AKI; and 6, 6, and 6 for RRT, respectively.

Figure 2. Time course of urinary titin level and muscle atrophy. A, Urinary titin level was higher than the normal level (1–3 pmol/mg Cr), with significant elevation from day 1 to days 2–7 of ICU admission ($p \leq 0.02$). B, Rectus femoris CSA and diaphragm thickness significantly decreased. ICU = intensive care unit, CSA = cross-sectional area. Data are expressed as median (interquartile range). The number of patients on each studied day is shown below the graph.

Figure 3. Relationship between cumulative urinary titin level and rectus femoris muscle atrophy on days 3, 5, and 7 of ICU admission. We evaluated the relationship between cumulative urinary titin level and the extent of muscle atrophy on days 3, 5, and 7 of ICU admission. Patients were divided into two groups (high and low atrophy) based on the median cutoff value of each day. Cumulative urinary titin level was associated with rectus femoris muscle atrophy ($p < 0.01$, $p < 0.01$, $p = 0.03$ on days 3, 5, and 7 of ICU admission, respectively).

Figure 4. Relationship between cumulative urinary titin level and diaphragm atrophy or incidence of ICU-AW. ICU-AW, intensive care unit-acquired weakness. Change in diaphragm thickness and incidence of ICU-AW was assessed in 50 and 35 patients, respectively. Cumulative urinary titin level was not associated with change in diaphragm thickness ($p = 0.31–0.45$), but it was associated with incidence of ICU-AW ($p = 0.01$).

Supplemental File Legends

Figure S1. Flow chart of patients included in this study. Sixty-two patients were recruited, and 56 were included in the analysis.

Figure S2. Titin-relayed ROC curves for incidence of ICU-AW. We show ROC curves for ICU-AW: A. cumulative urinary titin level B. urinary titin level on day 2 of ICU admission. ICU-AW = intensive care unit-acquired weakness, ROC = receiver operating characteristic, AUC = area under the curve, CI = confidence interval. ICU-AW was identified in 9 of 35 patients. AUC was 0.78 (95% CI, 0.61–0.95) for cumulative urinary titin level and 0.75 (95% CI, 0.56–0.94) for urinary titin level on day 2 of ICU admission.

Figure S3. Relationship between cumulative urinary titin level and rectus femoris muscle or diaphragm atrophy. A–C. Rectus femoris muscle atrophy on days 3, 5, and 7 of ICU admission. D–F. Diaphragm atrophy on days 3, 5, and 7 of ICU admission. Cumulative urinary titin level was associated with rectus femoris muscle atrophy ($p \leq 0.03$) but not with diaphragm atrophy ($p = 0.20$–0.64).

Figure 1

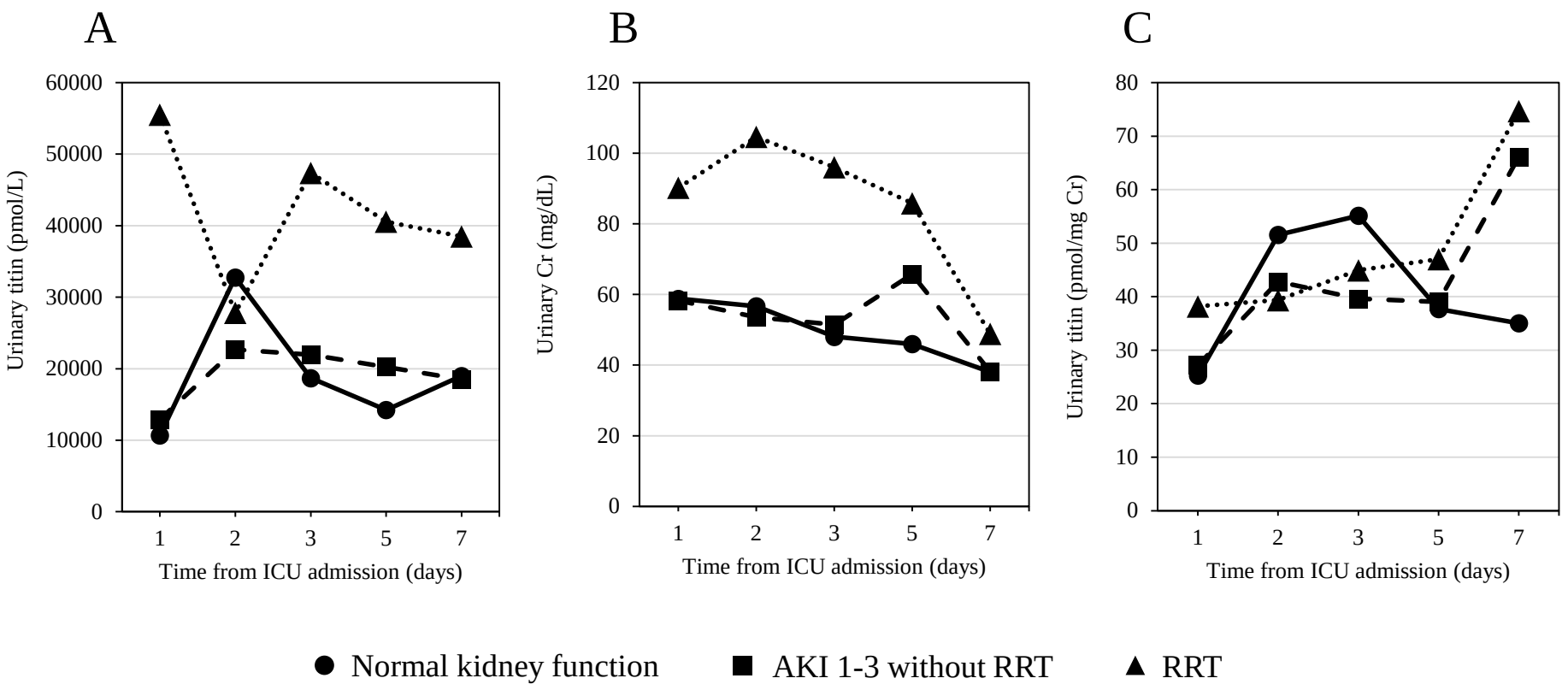
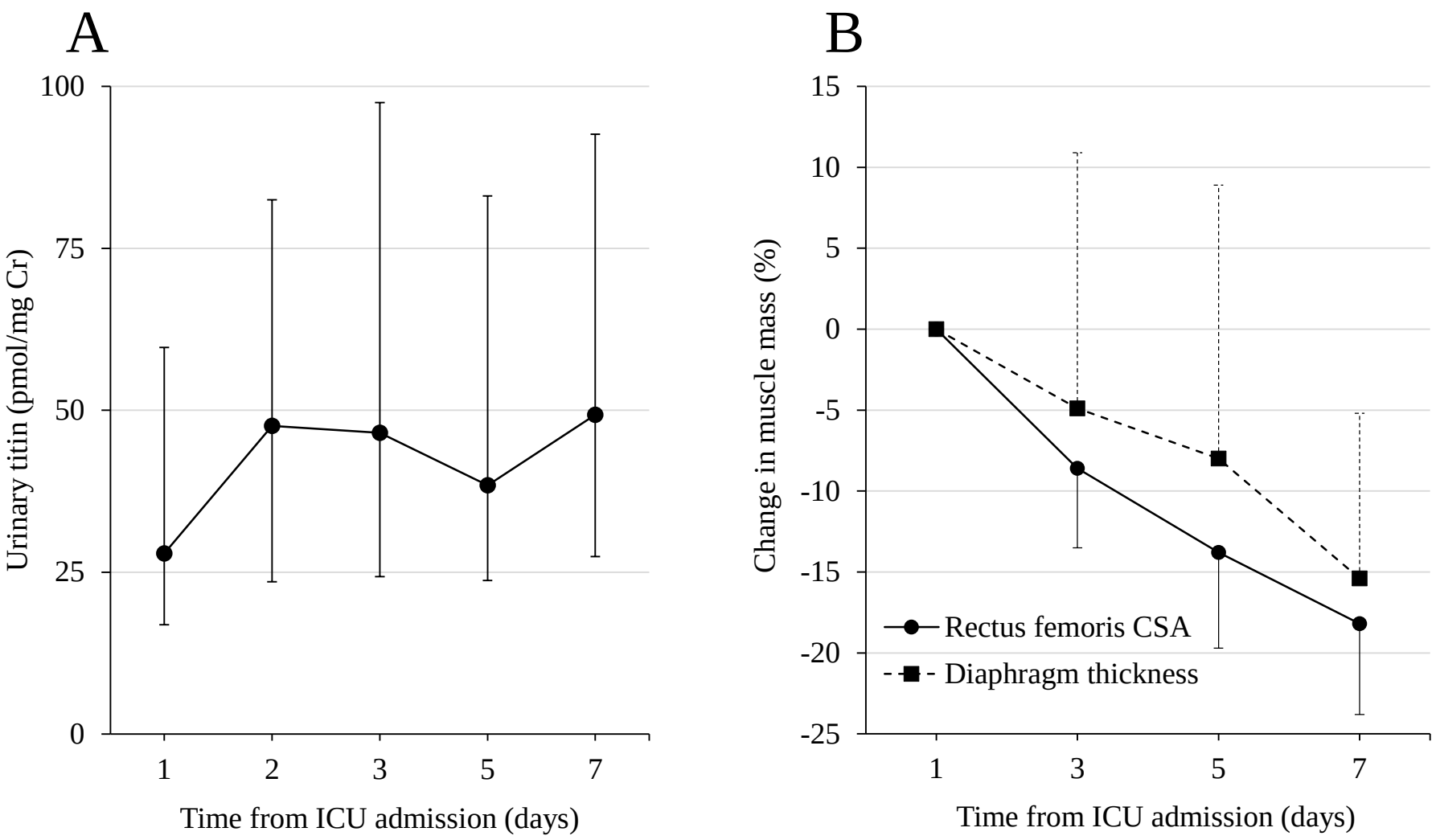

A
B
C
Urinary titin (pmol/L)
60000
50000
40000
30000
20000
10000
0
1
2
3
5
7
Time from ICU admission (days)
Urinary Cr (mg/dL)
120
100
80
60
40
20
0
Time from ICU admission (days)
Urinary titin (pmol/mg Cr)
80
70
60
50
40
30
20
10
0
Time from ICU admission (days)
Normal kidney function
AKI 1-3 without RRT
RRT

Figure 2



Number of patients					
Titin	56	56	56	40	24

Number of patients				
Rectus femoris	56	56	40	24
Diaphragm	50	50	36	20

Figure 3

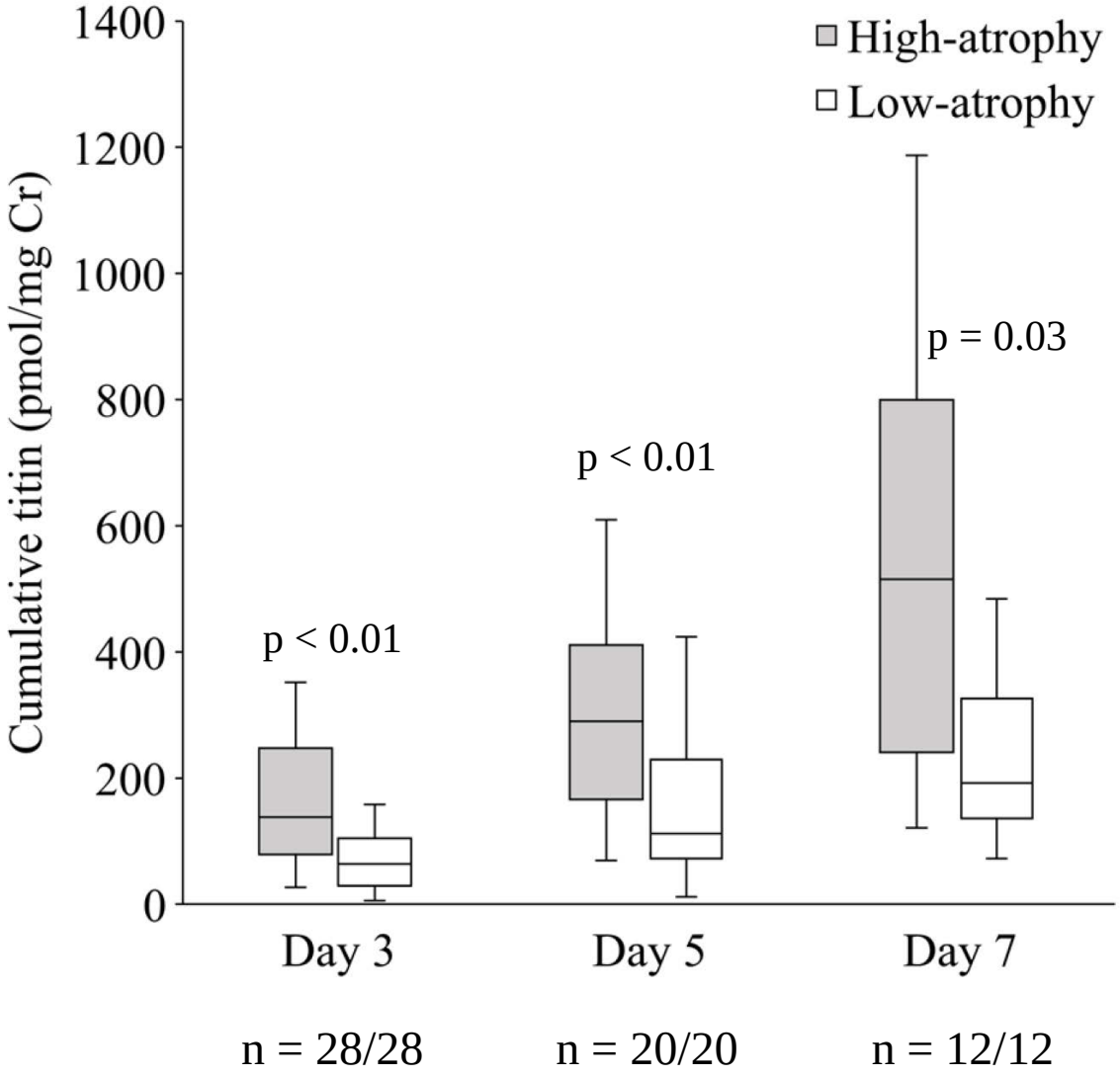

1400
1200
1000
800
600
400
200
0
Cumulative titin (pmol/mg Cr)
High-atrophy
Low-atrophy
p < 0.01
p < 0.01
p = 0.03
Day 3
Day 5
Day 7
n = 28/28
n = 20/20
n = 12/12

Figure 4

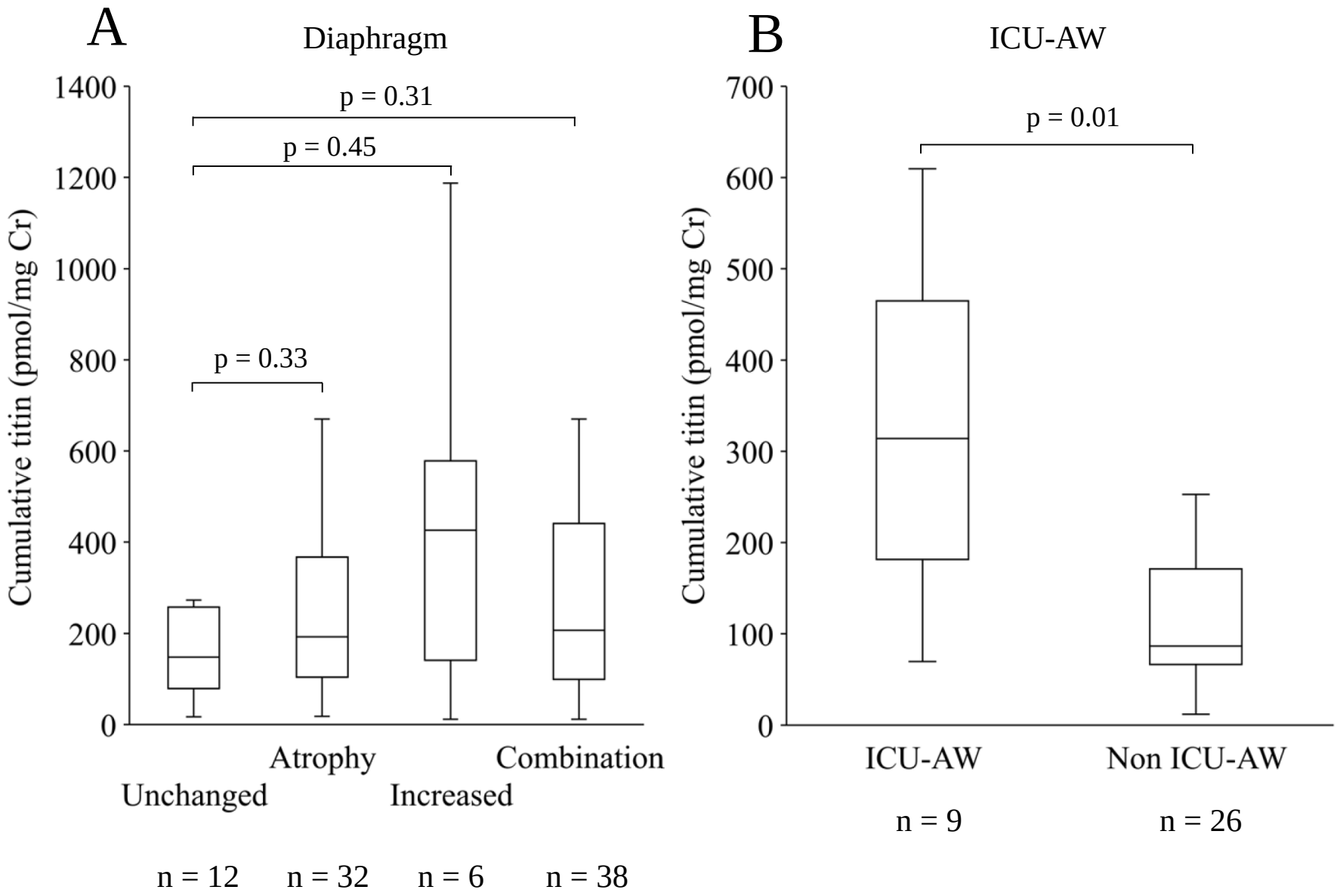

A
Diaphragm
Cumulative titin (pmol/mg Cr)
1400
1200
1000
800
600
400
200
0
p = 0.31
p = 0.45
p = 0.33
Unchanged
Atrophy
Increased
Combination
n = 12
n = 32
n = 6
n = 38
B
ICU-AW
Cumulative titin (pmol/mg Cr)
700
600
500
400
300
200
100
0
p = 0.01
ICU-AW
Non ICU-AW
n = 9
n = 26

TABLE 1 Patient Characteristics (n = 56)

Variables	Overall (n = 56)
Age, years (mean [SD])	72 ± 13
Gender (Men), n (%)	33 (59%)
Body mass index, kg/m^2	20.7 (18.1–24.1)
SOFA	6 (4–10)
APACHE II	25 (19–29)
Length of ICU stay, days	6 (4–9)
Mortality in the ICU, n (%)	8 (14%)
ICU admission reasons, n (%)	
Respiratory failure	23 (41%)
Heart failure	12 (21%)
Sepsis, non-respiratory	6 (11%)
Cardiac arrest	6 (11%)
Trauma	5 (9%)
Neurologic	4 (7%)

SD = standard deviation, SOFA = Sequential Organ Failure Assessment, APACHE = Acute Physiology and Chronic Health Evaluation, ICU = intensive care unit, IQR = interquartile range
Data were presented as median (IQR) unless otherwise indicated.

Supplemental File

Urinary titin is a novel biomarker for muscle atrophy in nonsurgical critically ill patients

TABLE S1 Urinary Titin Level by ICU Admission Reasons

ICU admission reasons	n (day 3, 5, 7)	Day 1	Day 2	Day 3	Day 5	Day 7
Respiratory failure	23, 17, 11	31.0 (16.8–48.9)	32.8 (18.2–60.2)	45.6 (27.7–93.0)	33.2 (15.7–91.3)	35.1 (19.6–159.8)
Heart failure	12, 8, 3	27.2 (8.6–52.7)	26.7 (17.2–66.7)	30.6 (17.8–96.7)	35.1 (18.1–57.4)	68.4 (60.2–73.1)
Sepsis, non-respiratory	6, 4, 2	89.2 (51.6–123.1)	94.2 (51.0–133.7)	97.8 (41.7–160.0)	140.6 (50.7–501.8)	277.9 (80.9–474.8)
Cardiac arrest	6, 5, 4	20.6 (13.2–29.4)	65.7 (30.8–94.6)	47.6 (25.2–73.3)	42.0 (26.0–92.8)	49.6 (20.3–108.3)
Trauma	5, 3, 2	32.9 (20.8–87.4)	44.9 (23.6–110.2)	50.6 (11.1–129.0)	24.3 (24.0–136.5)	64.4 (33.7–95.1)
Neurologic	4, 3, 2	15.6 (13.2–51.0)	68.8 (24.9–117.1)	57.6 (25.0–84.9)	42.2 (24.1–77.9)	35.0 (33.9–36.1)
Cardiothoracic surgery*	1, 1, 1	99.6	–	84.8	55.8	54.2

ICU = intensive care unit, IQR = interquartile range

*One cardiothoracic surgery was shown to explain urinary titin is increased after the surgery (aortic arch replacement).

Data were presented as median (IQR).

TABLE S2 Reproducibility of Measurements

	Correlation coefficient		Bland-Altman 95% CI	
Variables	**r**	**p**	**Bias**	**95% CI**
Intra-observer reproducibility				
Rectus femoris CSA	0.99	< 0.01	0.017 ± 0.029	-0.039 to 0.074
Diaphragm thickness	0.95	< 0.01	0.008 ± 0.013	-0.017 to 0.034
Inter-observer reproducibility				
Rectus femoris CSA	0.99	< 0.01	0.019 ± 0.024	-0.029 to 0.067
Diaphragm thickness	0.95	< 0.01	0.014 ± 0.013	-0.012 to 0.039

CI = confidence interval, CSA = cross-sectional area

Reproducibility was assessed for 56 patients at 176 measurements in rectus femoris muscle, and 50 patients at 156 measurements in diaphragm. The Pearson correlation coefficient and Bland-Altman plot were determined by using JMP statistical software version 13.1.0 (SAS Institute Inc., Cary, NC, USA).

Figure S1. Flow chart of patients included in this study. Sixty-two patients were recruited, and 56 were included in the analysis.

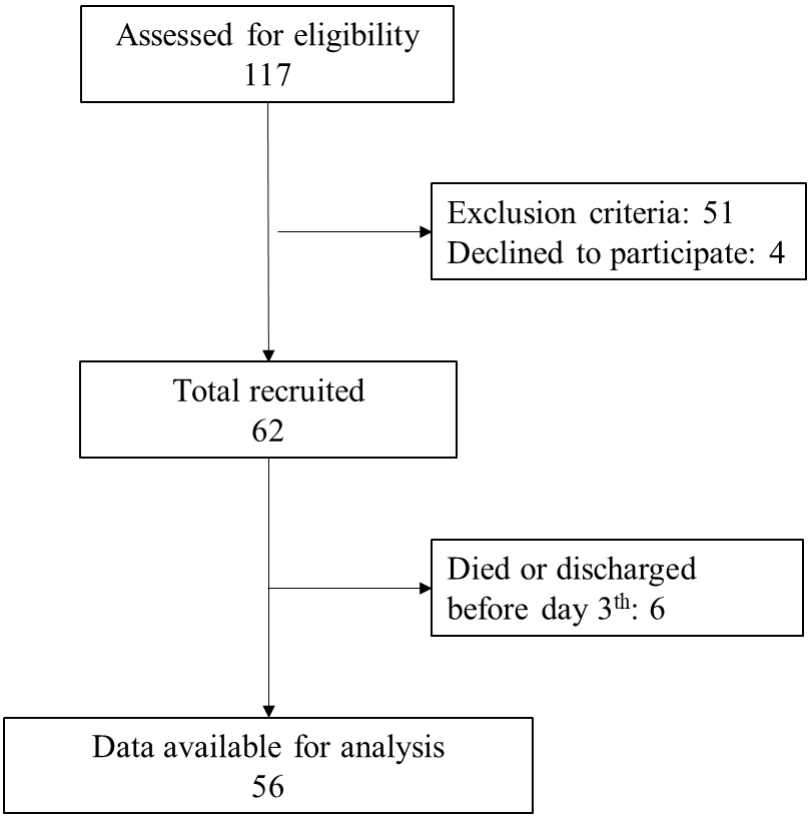


Figure S2. Relationship between cumulative urinary titin level and rectus femoris muscle or diaphragm atrophy. **A–C.** Rectus femoris muscle atrophy on days 3, 5, and 7 of ICU admission. **D–F.** Diaphragm atrophy on days 3, 5, and 7 of ICU admission. Cumulative urinary titin level was associated with rectus femoris muscle atrophy ($p \leq 0.03$) but not with diaphragm atrophy ($p = 0.20–0.64$).

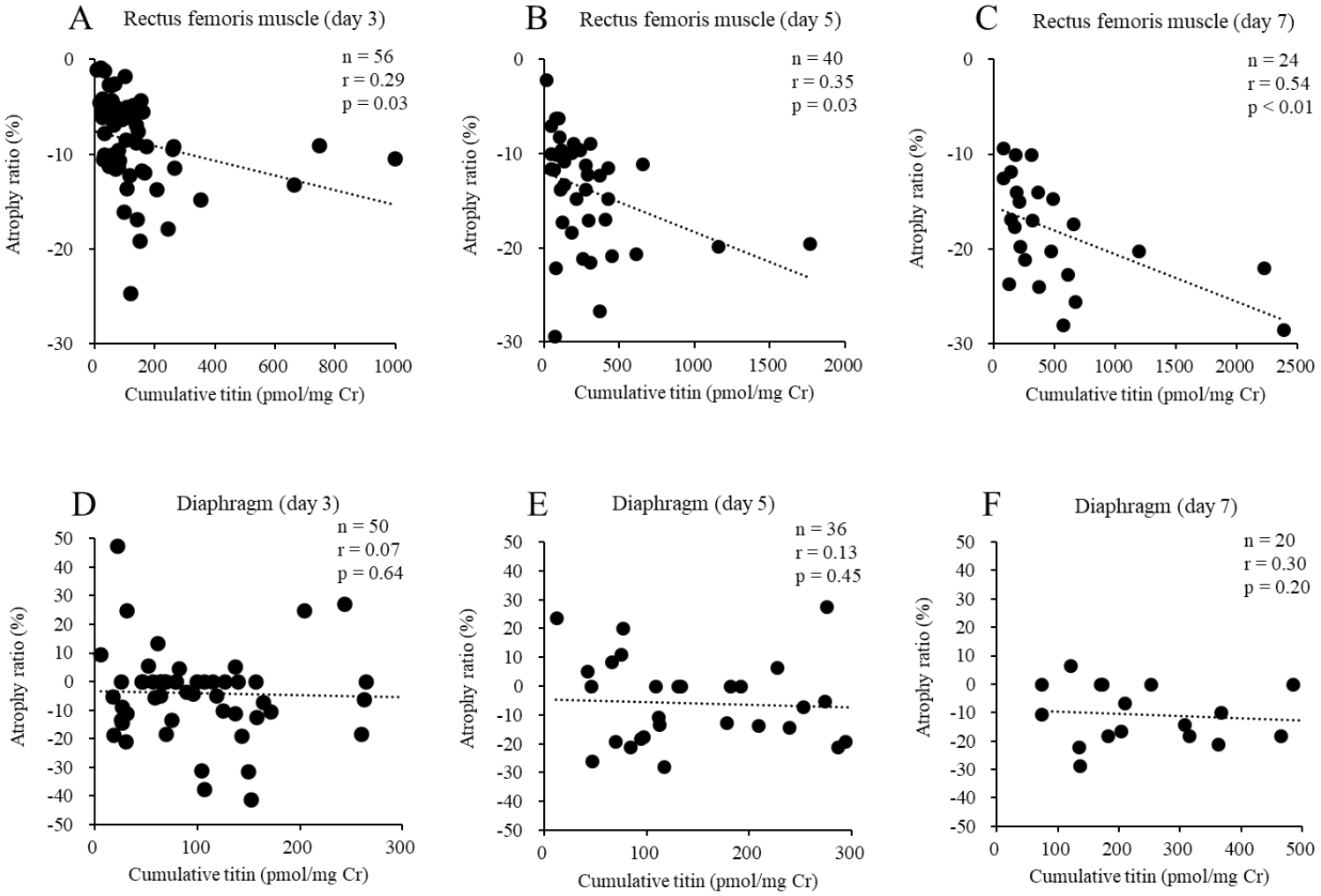


Figure S3. Titin-relayed ROC curves for occurrence of ICU-AW. **A.** Cumulative urinary titin level. **B.** Urinary titin level on day 2 of ICU admission. ICU-AW = intensive care unit-acquired weakness, ROC = receiver operating characteristic, AUC = area under the curve, CI = confidence interval. ICU-AW was identified in 9 of 35 patients. AUC was 0.78 (95% CI, 0.61–0.95) at the cumulative urinary titin level (Cut-off by Youden index: 181.5 pmol/mg Cr, sensitivity: 78%, specificity: 77%) and 0.75 (95% CI, 0.56–0.94) at the urinary titin level on day 2 of ICU admission (Cut-off by Youden index: 64.8 pmol/mg Cr, sensitivity: 78%, specificity: 81%).