Changes in physical activity and risk of all-cause mortality in patients on maintenance hemodialysis: a retrospective cohort study

Takahiro Shimoda 1, Ryota Matsuzawa 2*, Kei Yoneki 1, Manae Harada 1, Takaaki Watanabe 1, Mika Matsumoto 1, Atsushi Yoshida 3, Yasuo Takeuchi 4 and Atsuhiko Matsunaga 1

Abstract

Background: A previous cohort study indicated a significant association of lower baseline level of physical activity in hemodialysis patients with elevated risks of mortality. However, there have been no reports regarding the association between changes in physical activity over time and mortality in hemodialysis patients. This study was performed to examine the prognostic significance of physical activity changes in hemodialysis patients.

Methods: This retrospective cohort study was performed in 192 hemodialysis patients with a 7-year follow-up. The average number of steps taken per non-dialysis day was used as a measure of physical activity. Forty (20.8%) patients had died during the follow-up period. The percentage change in physical activity between baseline and 12 months was determined, and patients were divided into three categories according to changes in physical activity. A decrease or increase in physical activity > 30% was defined as becoming less or more active, respectively, while decrease or increase in physical activity < 30% were classified as stable.

Results: Forty seven (24.5%), 51 (26.6%), and 94 (49.0%) patients were classified as becoming less active, becoming more active, and stable, respectively. The hazard ratio on multivariate analysis in patients with decreased physical activity was 3.68 (95% confidence interval, 1.55–8.78; P < 0.01) compared to those with increased physical activity.

Conclusions: Reductions in physical activity were significantly associated with poor prognosis independent of not only patient characteristics but also baseline physical activity. Therefore, improved prognosis in hemodialysis patients requires means of preventing a decline in physical activity over time.

Keywords: Chronic renal failure, Dialysis, Exercise, Survival analysis

Background

Despite continuous progress in dialysis technology and disease management, mortality rate remains high in patients on hemodialysis. Physically inactive could contribute to mortality excess of hemodialysis patient, as hemodialysis patients are inactive compared to individuals with normal kidney function [1–4] and approximately 60% of hemodialysis patients were reported to exercise less than once a week [5]. However, routine care for hemodialysis patients does not include interventions to address this sedentary behavior.

A previous systematic review indicated a significant association between lower baseline level of physical activity and elevated risks of chronic diseases, such as cancer, diabetes, and vascular disease [6]. In addition to baseline physical activity, an inverse association was observed between decrease in physical activity over time and the risks of adverse events in subjects with impaired glucose tolerance and chronic heart failure [7, 8]. Hemodialysis patients showed an association between lower initial levels of physical activity and elevated mortality risk [9–13]. As the levels of physical activity...
in hemodialysis patients are affected by not only time constraints during dialysis treatment but also by aging, physical function decline, exacerbation of dialysis-related symptoms or comorbidities, depression, and other social factors, these patients would show further reductions in physical activity over time. Therefore, the effects of yearly changes in physical activity on prognosis in hemodialysis patients should be evaluated.

To our knowledge, there have been no previously reports regarding the influence of yearly changes in physical activity on prognosis in hemodialysis patients, and it is unclear whether changes in physical activity over time are associated with mortality risk. The present study was performed to examine whether changes in physical activity affected all-cause mortality in hemodialysis patients.

**Methods**

**Study population**

A total of 550 patients from the Hemodialysis Center, Sagami Circulatory Organ Clinic, were retrospectively enrolled in this study between October 2002 and March 2014. All patients were undergoing maintenance hemodialysis therapy three times a week, which is the most common schedule in Japan according to the Japanese Society for Dialysis Therapy. The exclusion criteria were as follows: hospitalization within 3 months prior to the study and at 12 months; recent myocardial infarction or angina pectoris; uncontrolled cardiac arrhythmia, hemodynamic instability, uncontrolled hypertension, or renal osteodystrophy with severe arthralgia; or the requirement for assistance in walking from another person. The study was approved by the Kitasato University Allied Health Sciences Research Ethics Committee.

**Demographic and clinical factors**

Data on demographic factors (age, sex, and time on hemodialysis), physical constitution (body mass index), primary causes of end-stage renal disease, and comorbidities (atherosclerotic heart disease, congestive heart failure, cerebrovascular accident/transient ischemic attack (TIA), peripheral vascular disease, dysrhythmia, and other cardiovascular diseases, chronic obstructive pulmonary disease, gastrointestinal bleeding, liver disease, cancer, and diabetes) were collected at the time of entry into the study. Serum albumin levels were obtained from hospital charts. Comorbid illnesses were quantified using a comorbidity index developed for dialysis patients consisting of primary cause of end-stage renal disease and 11 comorbidities, and was calculated using the method described previously to analyze survival of hemodialysis patients [14].

**Physical activity**

An accelerometer (Lifecorder; Suzuken Co. Ltd., Nagoya, Japan) that can continuously measure the intensity, duration, and frequency of activities was used for objective assessment of physical activity. The accuracy and reliability of this instrument were reported previously [15, 16]. In this study, the accelerometer was worn around the waist, and translated body acceleration as motion recorded as number of steps taken. The patients were instructed to wear the device continuously during waking hours for 7 days and to avoid allowing it to come into contact with water. The patients were also asked to maintain their typical weekly schedules. To ensure that measurement periods were typical of their weekly activity patterns, data were excluded when patients traveled or had an acute illness. Prior to analysis, the accelerometer data were inspected to ensure that there were no obvious errors, such as failure to acquire data or wear the device. Measurements from a period of 4 consecutive non-dialysis days were analyzed. Physical activity was reassessed using the same criteria 1 year later. The effects of changes in physical activity on survival were assessed using Cox proportional hazard models.

**Statistical analysis**

The average number of steps taken per non-dialysis day was used as a measure of physical activity. The difference in physical activity between baseline and 12 months was calculated, and the percentage change in physical activity was obtained by dividing the difference between the two time points by the baseline physical activity using the following formula: \( \frac{(\text{physical activity at 12 months} - \text{physical activity at baseline})}{\text{physical activity at baseline}} \times 100 \).

A decrease or increase in physical activity > 30% was defined as becoming less or more active, respectively [7, 17], while subjects with an decrease or increase in physical activity < 30% were classified as stable. Hemodialysis patients were divided into three categories according to change in physical activity: (a) becoming less active group, (b) stable group, and (c) becoming more active group.

Demographic and clinical factors are reported by category of change in physical activity, and are presented as means ± standard deviation (SD) or number (percent-age) and were tested for significance by analysis of variance (ANOVA). The \( \chi^2 \) test for trends was used to test for dose–response relations of variables between the three groups. For Kaplan–Meier estimates of survival curves, the data for the 7-year follow-up period were truncated to avoid an insufficient number of patients at risk, and the differences between the three groups were examined using the log-rank test. The independent prognostic effect of change in physical activity on survival was estimated by Cox proportional hazard regression analysis after adjustment for confounders, including age, sex, time on hemodialysis, body mass index, primary kidney disease, comorbidities, serum albumin, and
baseline physical activity. We used the restricted cubic spline procedure to model the non-linear relation between change in physical activity as a continuous variable and mortality. We considered five knots for the model at the 5, 27.5, 50, 72.5, and 95th centiles. In addition, we investigated the prognostic significance of baseline physical activity. Patients were categorized into two physical activity groups by a physical activity cutoff value of 5000 steps per day [18], and the difference between groups was tested using a log-rank test. Statistical analyses were performed using R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria) [19]. In all analyses, \( P < 0.05 \) was taken to indicate statistical significance.

Results
Baseline characteristics and changes in physical activity
The eligibility of 439 Japanese outpatients on hemodialysis for inclusion in the present study was assessed. Of these, 157 patients did not agree to participate in the study, and 90 patients dropped out between baseline and follow-up surveys. Therefore, 192 hemodialysis patients were finally included in the present study (Fig. 1). The baseline characteristics of the study population are shown in Table 1. The patients consisted of 96 (50%) men, mean age was 64.3 ± 10.3 years, mean time on dialysis was 6.5 ± 7.2 years, and the most common cause of end-stage renal disease was diabetic nephropathy (35.4%) followed by glomerulonephritis (32.8%). Based on the change in physical activity, 47 (24.5%) patients were classed as becoming less active group, 51 (26.6%) patients were classed as becoming more active group, 94 (49.0%) patients were classed as stable group. Age and physical activity at baseline and 12 months were different between groups, but no significant differences were observed in other baseline characteristics between groups.

Survival probability and baseline physical activity
Forty patients died during follow-up over a period of up to 7 years (infection, \( n = 6 \); cardiovascular disease, \( n = 18 \); cancer, \( n = 2 \); cerebral vascular disease, \( n = 2 \); other causes, \( n = 3 \); and unknown causes, \( n = 9 \)). The overall follow-up period ranged from 1 to 84 months. The 7-year cumulative survival rate were 83.7% in the \( \geq 5000 \) steps of physical activity and 65.2% in the <5000 steps (\( P = 0.04 \)) (Fig. 2). This finding indicates superior survival in patients with greater baseline physical activity.

Effects of changes in physical activity on survival
The median follow-up periods in becoming less active group, stable group, and becoming more active group were 44, 63, and 65 months, respectively (Fig. 3). There were 16 (34.0%) deaths among becoming less active group, 14 (14.9%) deaths among stable group, and 10 (19.6%) deaths among becoming more active group during 7 years. Two in the becoming less active group had died, 1 in the stable group had died during the first year. On the other hand, all participants in being more active group were alive after the first year. The cumulative

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**Fig. 1 Flow diagram of patient selection and exclusion process**

550 Japanese outpatients undergoing hemodialysis 3 times per week at the Department of Hemodialysis Center, Sagami Circulatory Organ Clinic, from October 2002 to March 2014

111 patients were excluded because they did not meet inclusion criteria
•38 patients had severe cardiovascular disease
•20 patients were hospitalized or changed clinic within 3 months prior to the study
•11 patients needed assistance in walking or daily activities
•5 patients underwent leg or toe amputation
•37 patients had other reasons

439 patients were eligible for the study

157 patients did not agree to participate in the study

282 patients participated baseline survey

90 patients dropped out during baseline and follow-up survey

192 patients agreed to participation in the study and were followed up for up to 7 years
survival rate in the becoming less active group was significantly lower than those in the other groups. Table 2 presents the results of univariate and multivariate Cox proportional hazard analyses for all-cause mortality in the present study population. The crude hazard ratios for all-cause mortality in the becoming less active group and stable group were 2.21 (95% confidence interval (CI): 1.00–4.89; \( P = 0.05 \)) and 0.70 (95% CI: 0.34–1.74; \( P = 0.54 \)), respectively, compared to the becoming more active group. Following adjustment for age, sex, time on hemodialysis, body mass index, primary kidney disease, atherosclerotic heart disease, congestive heart failure, diabetes, cerebrovascular accident/TIA, transient ischemic attack, other comorbidities, and serum albumin, hypertension, and physical activity, the hazard ratios for all-cause mortality in the becoming less active group and stable group remained significant, whereas the hazard ratio for the becoming more active group was not significant.

Table 1: Demographic and clinical characteristics of hemodialysis patients according to change in physical activity

|                          | All \((n = 192)\) | Becoming more active group \((n = 51)\) | Stable group \((n = 94)\) | Becoming less active group \((n = 47)\) | \( P \) value |
|--------------------------|-----------------|---------------------------------------|--------------------------|---------------------------------------|---------------|
| Age, years               | 64.3 ± 10.3     | 65.6 ± 10.7                           | 63.0 ± 9.6               | 67.6 ± 10.2                           | < 0.01        |
| Male, %                  | 96 (50.0%)      | 23 (45.1%)                            | 55 (58.5%)               | 18 (38.3%)                            | 0.06          |
| Time on hemodialysis, years | 6.5 ± 7.2     | 5.9 ± 6.7                             | 6.4 ± 6.9                | 7.4 ± 8.3                             | 0.59          |
| Body mass index, kg/m²   | 21.3 ± 3.3      | 21.5 ± 3.3                            | 21.4 ± 3.2               | 20.9 ± 3.5                            | 0.62          |
| Primary kidney disease, %|                 |                                       |                          |                                       | 0.30          |
| Glomerulonephritis       | 63 (32.8%)      | 14 (27.5%)                            | 28 (29.8%)               | 21 (44.7%)                            |               |
| Diabetes                 | 68 (35.4%)      | 23 (45.1%)                            | 29 (30.9%)               | 16 (34.0%)                            |               |
| Other                    | 24 (12.5%)      | 7 (13.7%)                             | 12 (12.8%)               | 4 (8.5%)                              |               |
| Unknown                  | 20 (10.4%)      | 3 (5.9%)                              | 14 (14.9%)               | 3 (6.4%)                              |               |
| Hypertension             | 17 (8.8%)       | 4 (7.8%)                              | 10 (10.6%)               | 3 (6.4%)                              |               |
| Comorbidities, %         |                 |                                       |                          |                                       |               |
| Diabetes                 | 84 (43.8%)      | 25 (49.0%)                            | 36 (38.3%)               | 23 (48.9%)                            | 0.39          |
| Cerebrovascular accident/TIA | 66 (34.4%)     | 17 (33.3%)                            | 13 (13.8%)               | 36 (76.6%)                            | 0.02          |
| Atherosclerotic heart disease | 50 (26.0%)   | 15 (29.4%)                            | 17 (18.1%)               | 18 (38.3%)                            | 0.04          |
| Congestive heart failure | 24 (12.5%)      | 10 (19.6%)                            | 10 (10.6%)               | 4 (8.5%)                              | 0.20          |
| Comorbidity index, score | 5.0 ± 3.2       | 5.7 ± 3.4                             | 4.6 ± 3.0                | 5.0 ± 3.3                             | 0.13          |
| Serum albumin, g/dL      | 4.0 ± 0.3       | 4.0 ± 0.4                             | 4.0 ± 0.3                | 3.9 ± 0.4                             | 0.62          |
| Physical activity, steps |                 |                                       |                          |                                       |               |
| Baseline                 | 4421 ± 3048     | 2951 ± 2300                           | 5261 ± 3274              | 4335 ± 2703                           | <0.01         |
| 12 months                | 4291 ± 3211     | 5028 ± 3817                           | 4929 ± 3003              | 2214 ± 1701                           | <0.01         |

Values are shown as the mean ± SD or \( n (\%) \)

TIA transient ischemic attack, SD standard deviation

Fig. 2 Kaplan-Meier survival analysis of all-cause mortality by baseline physical activity in hemodialysis patients

Fig. 3 Kaplan-Meier survival analysis of all-cause mortality by changes in physical activity in hemodialysis patients
heart failure, cerebrovascular accident/TIA, diabetes, serum albumin, and baseline physical activity, the hazard ratios for all-cause mortality in the becoming less active group and stable group were 2.73 (95% CI: 1.12–6.62; P = 0.03) and 1.41 (95% CI: 0.53–3.67; P = 0.49), respectively, compared to the becoming more active group (Model 1). In the Model 2, we adjusted the effect of co-morbidity index, which was consisted of cause of end-stage renal disease and 11 comorbidities. Following adjustment for the effects of these covariates, the hazard ratios for all-cause mortality in the becoming less active group and stable group were 3.68 (95% CI: 1.55–8.78; P < 0.01) and 1.93 (95% CI: 0.75–4.99; P = 0.17), respectively, compared to the becoming more active group. These results indicated a significant association between the yearly change in physical activity and poor prognosis of hemodialysis patients independent of not only patient characteristics but also baseline physical activity. Graphical visualization of the Cox proportional hazard model using the restricted cubic spline procedure indicated a significant relation between change in physical activity and all-cause mortality risk in the present study population (Fig. 4).

**Discussion**
The present study was performed to examine all-cause mortality rate in a cohort of 192 hemodialysis patients. Forty (20.8%) patients died during the observation period lasting up to 7 years, with cardiovascular disease as the leading cause of death. Almost one quarter of the patients showed a decline in physical activity over time, which was significantly related to elevated mortality risk independent of patient characteristics and baseline physical activity. To our knowledge, this is the first report of an association between change in physical activity and mortality in hemodialysis patients. Our findings suggest that it is important to prevent a decline in physical activity over time in hemodialysis patients to improve their prognosis.

Only a few studies have examined changes in physical activity evaluated with an accelerometer or pedometer and mortality. Yates et al. reported that a decrease and an increase of approximately 25% in steps per day from baseline were noted in 25.2 and 24.9% of people with impaired glucose tolerance, respectively, at 1 year follow-up [7]. In the present study, 24.5% and 26.6% of hemodialysis patients experienced a decrease and an increase of 30%, respectively, in physical activity level from baseline. Our findings are consistent with those of the previous study targeting people with chronic illness. In addition, 90 patients dropped out during baseline and follow-up surveys in the present study. If an assessment of yearly changes in physical activity levels of these patients was possible, the rate of patients who experienced a > 30% decrease in physical activity might have been higher.

| Parameter          | Univariate analysis* | Multivariate analysis: Model 1b | Multivariate analysis: Model 2c |
|--------------------|----------------------|-------------------------------|-------------------------------|
|                    | Hazard ratio (95% CI) | P value                       | Hazard ratio (95% CI)           | P value                       |
| Change in physical activity |                     |                               |                               |                               |
| Becoming more active group | 1.00 | 1.00 | 1.00 |                     |
| Stable group       | 0.70 (0.34–1.74)     | 0.54                          | 1.41 (0.53–3.67)               | 0.49                          |
| Becoming less active group | 2.21 (1.00–4.89) | 0.05                          | 2.73 (1.12–6.62)               | 0.03                          |
|                    |                     |                               | 3.68 (1.55–8.78)               | < 0.01                       |

Analyses were performed using Cox proportional hazards regression
Confidence interval
*Unadjusted by characteristic factors on survival
bAdjusted by age, sex, time on hemodialysis, body mass index, primary kidney disease, atherosclerotic heart disease, congestive heart failure, cerebrovascular accident/transient ischemic attack, diabetes, serum albumin, and baseline physical activity
cAdjusted by age, sex, time on hemodialysis, body mass index, primary kidney disease, comorbidity index, serum albumin, and baseline physical activity

**Table 2 Cox proportional hazards model for all-cause mortality**

**Fig. 4** Cubic spline survival analyses exhibiting the association between change in physical activity and mortality. * Adjusted by age, sex, time on hemodialysis, body mass index, primary kidney disease, comorbidity index, serum albumin, and baseline physical activity
There are several possible explanations for the association between decline in physical activity over time and poor prognosis in hemodialysis patients observed in the present study. First, arteriosclerotic disease is the main cause of death in patients on hemodialysis, as also indicated in this study. Previous reports indicated that engaging in physical activity or low-intensity walking exercise improved risk factors for arteriosclerotic disease in hemodialysis patients, i.e., hypertension, arterial stiffness, plasma triglyceride and cholesterol levels, cardiac autonomic system dysfunction, and reduced maximal oxygen consumption [20–28]. Therefore, reductions in level of physical activity over time in such patients could lead to elevated risk of arteriosclerosis. The results of the present study suggested that changes in physical activity may be related to risk factors for cardiovascular disease. However, the associations could have been confounded by lifestyle-related factors that were either not measured or were measured inaccurately. In addition, the observational design of this study did not allow us to establish a causal link between change in physical activity and all-cause mortality rate. Second, patients whose physical activity at 12 months could not be determined due to severe condition were excluded from the analyses in the present study. However, we may have failed to exclude some patients with slight deterioration of overall condition that did not reach a level requiring medical treatment. Regardless of these possibilities, this study indicated that it is important to evaluate changes in physical activity among hemodialysis patients to predict their prognosis and to allow better disease management.

Tudor-Locke et al. reported that 5000 steps per day at baseline are a “borderline for mortality” in older adults [18]. In this study, participants indicates superior survival in patients with \( \text{steps per day} \geq 5000 \) steps per day. Previous studies indicated an association between baseline physical activity and survival in hemodialysis patients [10, 12, 13]. Therefore, the results of the present observational study extend previous research by suggesting that baseline physical activity and changes in physical activity are both important and independent determinants of all-cause mortality in hemodialysis patients.

Byberg et al. examined the effects of changes in physical activity on prognosis in a large community dwelling population-based cohort [29], and reported a higher mortality risk in people with a decline in physical activity over time from a high to low or moderate level, even if their baseline levels of physical activity was high. In addition, Yates et al. reported that each increase of 2000 steps per day from baseline to 12 months was significantly associated with an additional 8% difference in the cardiovascular event rate in people with impaired glucose tolerance [7]. These previous reports indicating relations between decline in physical activity over time and poor prognosis in myocardial infarction patients, diabetes, and community dwelling populations were similar to the results of the present study [17, 29–31]. As a more representative measure of physical activity, especially in hemodialysis patients, not only baseline physical activity but also change in physical activity should be evaluated. In addition, we measured physical activity with an accelerometer rather than by interview or questionnaire; determination of physical activity by interview or questionnaire has been used frequently due to its ease, but use of an accelerometer during routine daily activities is recommended in hemodialysis patients [32], as previous studies determined physical activity of hemodialysis patients using accelerometers or pedometers [1, 3]. The present study indicated the association between changes in physical activity measured objectively using an accelerometer and mortality in hemodialysis patients.

Despite evidence regarding the health benefits of physical activity and exercise, most hemodialysis patients are not sufficiently physically active. There are several means of increasing physical activity, including the use of a pedometer that reports the change in number of steps walked per day. Adults were reported to show an increase in physical activity by 26.9% over baseline over 18 weeks of pedometer use [33]. Early studies also indicated that use of a pedometer motivated hemodialysis patients to increase their level of physical activity [34, 35]. The level of physical activity can also be increased by exercise training. Frailty is a primary pathway to disability, defined as a pathological condition that results in a constellation of signs and symptoms and is characterized by high susceptibility to adverse health outcomes, impending decline in physical function, and high risk of death [36], which is common in hemodialysis patients [37–41]. Frailty in such patients is a major factor preventing the adoption of an active lifestyle. However, there is a great deal of evidence that exercise training is beneficial for older adults at high risk of frailty [42]. Hence, it is possible that the levels of physical activity can be increased by ameliorating frailty. In fact, Chen et al. reported increases in physical activity among hemodialysis patients that participated in a low-intensity intradialytic exercise program accompanied by improvement in physical performance [43]. Therefore, most hemodialysis patients should change from a sedentary to a non-sedentary lifestyle to improve their long-term prognosis.

This study had a number of limitations. First, in this study, the patients in becoming less active group were more likely older, and had a greater prevalence of cerebrovascular accident/TIA and atherosclerotic heart disease, and had higher physical activity level at baseline than the others. Although we should perform subgroup analyses according to age, the prevalence of these comorbidities...
and baseline physical activity level in order to adjust the effects of differences in baseline characteristics between 3 groups, we abandon the analyses because of the small sample size. Instead of this, we analyzed the association between change in physical activity and mortality risks using Cox proportional hazards regression models adjusting for the differences in baseline characteristics of the study participants. And, this is one of only a few studies reported to date involving examination of objectively measured physical activity using an accelerometer or pedometer in hemodialysis patients. Further large-scale observational studies are needed. Second, we excluded patients that required assistance with walking and did not complete the evaluation of physical activity at 12 months because of adverse events or lower adherence. Therefore, the comorbidities in the participants were mild, which should be taken into consideration when generalizing our results to patients with more severe limitations. Third, we measured participant physical activity over the course of 4-day monitoring. Although previous studies recommended the use of data from at least 3- to 4-day monitoring to reliably predict total physical activity behavior over long term periods [44, 45], our survey period was short. In addition, the lack of data that could interfere with the measurement of true physical activity (e.g., season, social factors) might have introduced bias in our assessment. Forth, one of the five participants was classified as “becoming less active” (24.5%). We could not provide an explanation as to why physical activity levels decreased in many patients after 12 months, as we did not collect information relating to factors that could limit physical activities in hemodialysis patients, such as higher age, comorbidities, physical function, body pain, exacerbation of dialysis-related symptoms, depression symptoms, adherence to exercise, and cohabiting family. Accordingly, an observational study will be necessary to further assess the causes of sedentary lifestyle among hemodialysis patients, including factors associated with changes in physical activity over time. Finally, although we showed that mortality risk was higher in patients with a decline in physical activity over time compared with the other groups, the mechanisms underlying these observations remain to be elucidated.

Conclusions
Reductions in physical activity were significantly associated with poor prognosis independent of not only patient characteristics but also baseline physical activity. Therefore, improved prognosis in hemodialysis patients requires means of preventing a decline in physical activity over time.

Abbreviations
ANOVA: Analysis of variance; CI: Confidence interval; SD: Standard deviation; TIA: Transient ischemic attack

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Availability of data and materials
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors’ contributions
This manuscript has been seen and approved by all authors. Conception or design, or analysis and interpretation of data, or both: TS, KY, MH, TW, MM, AY; drafting the article: TS; been involved in drafting the manuscript or revising it critically for important intellectual content: RM, AM; providing intellectual content of critical importance to the work described: YT; final approval of the version to be published: AM.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Kitasato University School of Allied Health Sciences and was conducted in accordance with the standards set forth by the latest revision of the Declaration of Helsinki. All patients received a detailed explanation of the study protocol and provided informed consent.

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Author details
1Department of Rehabilitation Sciences, Kitasato University Graduate School of Medical Sciences, Sagamihara, Japan. 2Department of Rehabilitation, Kitasato University Hospital, 1-15-1 Kitasato, Sagamihara, Kanagawa 252-0375, Japan. 3Department of Hemodialysis Center, Sagami Circulatory Organ Clinic, Sagamihara, Japan. 4Department of Nephrology in Internal Medicine, Kitasato University School of Medicine, Sagamihara, Japan.

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