Skeletal Muscle Mass Is Associated With Erythropoietin Response in Hemodialysis Patients

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Abstract

Background: Hyporesponsiveness to erythropoietin stimulating agent (ESA) is associated with poor outcome in patients with chronic kidney disease. Although ESA hyporesponsiveness and sarcopenia have common pathophysiological background, clinical evidence linking them are scarce. The purpose of the study was to investigate the relationship between ESA responsiveness and skeletal muscle mass in hemodialysis patient.

Methods: This cross-sectional study analyzed 70 patients on maintenance hemodialysis who were treated with ESA. ESA responsiveness was evaluated by erythropoietin resistance index (ERI), calculated as weekly dose of ESA divided by body weight and hemoglobin (IU/kg/week/dL), and weekly dose of ESA/hemoglobin (IU/week/dL). Dose of ESA was equivalated with epoetin β. Correlations between ESA responsiveness and clinical parameters including skeletal muscle mass were analyzed.

Results: Among the 70 patients, ERI was positively correlated to age ($p < 0.002$), whereas negatively correlated to height ($p < 0.001$), body weight ($p < 0.001$), BMI ($p < 0.001$), skeletal muscle mass ($p < 0.001$), transferrin saturation (TSAT) ($p = 0.049$), and zinc ($p = 0.006$). In the multiple linear regression analysis, TSAT, zinc and skeletal muscle mass were associated with ERI and weekly ESA dose/hemoglobin.

Conclusions: Skeletal muscle mass was the independent predictor for ESA responsiveness as well as TSAT and zinc. Sarcopenia is another target for the management of anemia in patients with hemodialysis.

Background

Anemia is one of the major complications in patients with chronic kidney disease (CKD) or receiving hemodialysis (HD), and is related to poor outcome [1][2]. Erythropoietin stimulating agents (ESAs) has been used in patients with HD for many years [3][4]. ESAs have enabled to archive recommended hemoglobin level and are the most established agents for renal anemia [5][6]. On the other hand, approximately 15% of the patient are hyporesponsive to ESA [7][8]. ESA hyporesponsiveness is associated with mortality and cardiovascular events in patients with CKD [9][10]. Several conditions such as iron deficiency and zinc deficiency, which are easily treated, can cause ESA hyporesponsiveness. Therefore, it is necessary to look into relevant cause when the patients show no or little response to ESA.

Among many factors that are associated with ESA hyporesponsiveness, malnutrition is one of the causes [11]. Deficiency of nutrients required for hematopoiesis, such as iron, zinc, vitamin B12, and cupper, leads to ESA hyporesponsiveness. Infection and inflammation are the other causes for ESA hyporesponsiveness via disturbance of iron utilization. Since each of these conditions require different treatments, it is important to appropriately identify relevant cause when the patients show no or little response to ESA.

Sarcopenia is characterized by loss of skeletal muscle mass that progresses with aging. It is being recognized as a great health issue in the elderly population [12][13]. A growing number of evidences have revealed that sarcopenia is related to cardiovascular disease [14], cognitive function [15], physical performance [16], and mortality [17]. The pathogenesis of sarcopenia involves various conditions such as malnutrition and inflammation. These conditions are associated with erythropoietin resistance. In addition, recent studies demonstrated the associations between muscle mass and erythropoiesis [18][19]. Although there is potentially an association between sarcopenia and ESA hyporesponsiveness, clinical evidence linking them are lacking. We hypothesized that muscle wasting is associated with ESA response, by reflecting nutritional and inflammation status. In the present study, we aimed to investigate the relationship between muscle mass and ESA hyporesponsiveness in hemodialysis patients receiving ESA.

Methods

Study population

This cross-sectional study included 96 patients who had been on maintenance hemodialysis at least 3 months at our hospital between April to June 2018. Patients with a past history of amputation of extremities, with hemorrhagic lesions, and who could not reach the dry weight during the investigation were excluded from the study. Patients who were not treated with ESA were also excluded. All the patients were receiving three times-weekly hemodialysis/hemodiafiltration. Patient's characteristics including the cause of end-stage renal disease, duration of hemodialysis, height, body weight were collected from their medical records. Blood samples were collected at the beginning and the end of the dialysis session following a 2-day interval. Laboratory results at the day for more than one month of stable ESA dose were used for the analysis. Erythropoietin resistance index (ERI) was calculated as weekly dose of epoetin β divided by body weight and hemoglobin level (IU/kg/week/g/dL). Since the patients were treated with different ESAs, a dose conversion ratio of 1:200 for darbepoetin α and 1:225 for CERA were used with respect to epoetin β [20][21]. The normalized protein catabolic rate (nPCR) and the dialysate dosage, the clearance of urea (K; mL/min) multiplied by the time on dialysis (t; min) divided by the volume of distribution (V; mL), were calculated as previously described [22]. This study was approved by the ethical committee of the Tottori University Hospital (approval number: 19A222) and conducted in accordance with the Declaration of Helsinki.

Measurement Of Skeletal Muscle

The skeletal muscle mass of each patients was measured by bioimpedance analysis (BIA) using InBody (InBody Japan, Tokyo, Japan). The measurement was performed after a session of hemodialysis to eliminate the influence of excess body fluid. The dry weight was determined according to their physical findings, chest radiograph, and serum brain natriuretic peptide or human atrial natriuretic peptide. Skeletal muscle index (SMI) was calculated as skeletal muscle mass divided by their height (kg/m^2).

Statistical analysis

The distribution of the continuous variables was evaluated by Kolmogorov–Smirnov test. The variables were expressed as mean ± SD or median (range). Correlations between skeletal muscle mass or SMI and the patient's characteristics were analyzed by Pearson's correlation coefficient for normally distributed variables and Spearman's correlation coefficient for non-normally distributed variables. Multiple linear regression analysis, in which sex, age, and laboratory findings were selected with stepwise forward selection method, was performed to investigate the influencing factor for skeletal muscle or SMI. A two-tailed p value of less than 0.05 was considered as statistically significant. Statistical analyses were performed using StatFlex (ver7.0 for Windows, Artec, Osaka, Japan) or GraphPad Prism (ver7.0 for Windows, GraphPad Software, San Diego, CA, USA).

Results

Patient characteristics

Excluding 26 (20 without ESA, 5 could not reach the dry weight, and 1 with amputation) patients from the cohort, 70 patients (45 male and 25 female) were included in the analysis (Figure 1). The characteristics of the study population was summarized in Table 1. The mean age of the participants was 67.2 ± 13.0 years, the mean ERI was 7.2 ± 5.4, and the mean skeletal muscle mass was 21.8 ± 5.4 kg.

Correlations between ERI and clinical parameters

We first investigated the correlations between ERI and clinical parameters. ERI positively correlated to age ($p < 0.002$), whereas negatively correlated to height ($p < 0.001$), body weight ($p < 0.001$), BMI ($p < 0.001$), skeletal muscle mass ($p < 0.001$), transferrin saturation (TSAT) ($p = 0.049$), and zinc ($p = 0.006$). Since there were significant correlations between ERI and body size, we further investigated the correlations between weekly ESA dose/hemoglobin and clinical parameters. As a result, we observed positive correlations to age ($p = 0.020$) and negative correlations to height ($p = 0.017$), body weight ($p = 0.029$), skeletal muscle mass ($p = 0.011$), TSAT ($p = 0.009$), and zinc ($p = 0.013$). These correlations were summarized in Table 2.

Correlations between skeletal muscle mass and clinical parameters

Correlations between skeletal muscle mass and clinical parameters were also investigated. Skeletal muscle mass was positively correlated with height ($p < 0.001$), body weight ($p < 0.001$), BMI ($p < 0.001$), albumin ($p = 0.001$), and zinc ($p = 0.029$), whereas negative correlation was observed in age ($p < 0.001$), nPCR ($p = 0.028$), and Kt/V ($p < 0.001$) (Table 3).

Determinants of skeletal muscle mass

Multiple linear regression analysis was performed to investigate the influencing factor for ERI. Age, sex, skeletal muscle mass, albumin, TSAT, intact PTH, Zinc, CRP, and Kt/V were selected as explanation

variable with stepwise forward selection method. TSAT, zinc, skeletal muscle mass, and Kt/V were determined to be the independent predictors for ERI (Table 3). Multiple linear regression analysis was also performed for weekly ESA dose/hemoglobin. As a result, TSAT, zinc, and skeletal muscle mass showed independently association between weekly ESA dose/hemoglobin (Table 4).

Discussion

In the present study, we observed that ESA responsiveness was associated with skeletal muscle mass. TSAT, zinc, and skeletal muscle mass are the independent predictor for ESA responsiveness.

ESA hyporesponsiveness is caused by various conditions. Iron deficiency is one of the major conditions leading to ESA hyporesponsiveness. It is recommended to measure TSAT and ferritin to assess the status of iron deficiency or overload. Serum ferritin is affected by inflammation, and TSAT is the most commonly used marker for the availability of iron [23]; thus, we included TSAT in the multivariate analysis in the study. Our finding that TSAT was the independent predictor for ERI is in line with the widely accepted recognition that iron deficiency causes ESA hyporesponsiveness. Inflammation can lead to ESA hyporesponsiveness [24]. Pro-inflammatory cytokines such as interleukin-6 increases the expression of hepcidin, which is the regulator of iron homeostasis [25][26]. HD patients with inflammation showed increased hepcidin levels together with decreased intestinal absorption of iron [27]. Although we did not find associations between CRP levels and ERI in the multivariate analysis, inflammatory conditions in our cohort might be, in some part, reflected to the iron status.

In the present study, we observed that skeletal muscle mass was associated with ERI. Since both skeletal muscle mass and ERI, calculated as weekly dose of epoetin β divided by body weight and hemoglobin level, are closely related to body weight, we further analyzed the association between skeletal muscle mass and weekly ESA dose/hemoglobin to eliminate. As a result, there was still an association between skeletal muscle mass and ESA responsiveness. Previous investigation in murine myoblast cells have revealed that erythropoietin receptor was expressed in myoblasts, and erythropoietin promoted the proliferation of myoblasts [18]. Erythropoietin receptor was expressed in human skeletal muscle [19][28]. In addition, muscle fibers have an ability to release erythropoietin after exercise, and erythropoietin-induced JAK2 phosphorylation, which is necessary to induce downstream signaling pathways of erythropoietin, increased after acute exercise [19]. On the other hand, long-term recombinant erythropoietin had no significant affect to muscle fiber hypertrophy [28]. These in vitro and human study indicates that muscle mass is associated with ESA responsiveness and that muscle wasting is potentially a new target for managing anemia in patients with CKD.

We observed that zinc was also the independent predictor for ERI. Zinc deficiency is another cause for ESA hyporesponsiveness. It has been reported that the prevalence of zinc deficiency is extremely high in HD patients and zinc supplementation reduced the dosage of erythropoietin [29]. Since most of zinc distributes to skeletal muscle and bone [30], this might influence the correlation of skeletal muscle and ERI. However, we still observe that skeletal muscle mass was associated to ERI independently to zinc.

There are some limitations in this study. ESA hyporesponsiveness is caused by various conditions that was not included in our study. Carnitine, vitamin or folic acid are involved in ESA response. This is a retrospective study; thus, further investigation is required whether exercise or intervention to skeletal muscle improve ESA responsiveness.

Conclusions

In conclusion, we revealed that skeletal muscle mass was the independent predictor for ESA responsiveness as well as TSAT and zinc. Sarcopenia is another target for the management of anemia in patients with HD.

List Of Abbreviations

CKD chronic kidney disease

HD hemodialysis

ESA erythropoietin stimulating agent

ERI erythropoietin resistance index

BIA bioimpedance analysis

SMI skeletal muscle index

TSAT transferrin saturation

CRP C-reactive protein

Declarations

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the ethical committee of the Tottori University Hospital (approval number 19A222). Informed consent was obtained from all individual participants included in the study.

Consent for publication: N/A

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

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

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Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by TT. The first draft of the manuscript was written by TT and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

1. Kovesdy CP, Trivedi BK, Kalantar-Zadeh K, Anderson JE. Association of anemia with outcomes in men with moderate and severe chronic kidney disease. Kidney Int. 2006;69:560–4.
2. Keane WF, Brenner BM, de Zeeuw D, Grunfeld J-P, McGill J, Mitch WE, et al. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. Kidney Int. 2003;63:1499–507.
3. Gouva C, Nikolopoulos P, Ioannidis JPA, Siamopoulos KC. Treating anemia early in renal failure patients slows the decline of renal function: a randomized controlled trial. Kidney Int. 2004;66:753–60.
4. Kuriyama S, Tomonari H, Yoshida H, Hashimoto T, Kawaguchi Y, Sakai O. Reversal of anemia by erythropoietin therapy retards the progression of chronic renal failure, especially in nondiabetic patients. Nephron.
5. Tsubakihara Y, Gejyo F, Nishi S, Iino Y, Watanabe Y, Suzuki M, et al. High target hemoglobin with erythropoiesis-stimulating agents has advantages in the renal function of non-dialysis chronic kidney disease patients. Ther Apher Dial. 2012;16:529–40.
6. Akizawa T, Pisoni RL, Akiba T, Saito A, Fukuhara S, Asano Y, et al. Japanese haemodialysis anaemia management practices and outcomes (1999–2006): results from the DOPPS. Nephrol Dial Transplant. 2008;23:3643–53.
7. Hayashi T, Kato H, Tanabe K, Nangaku M, Hirakata H, Wada T, et al. Initial responsiveness to darbepoetin alfa and its contributing factors in non-dialysis chronic kidney disease patients in Japan. Clin Exp Nephrol. 2020.
8. Besarab A, Yee J. Candidate biomarkers for erythropoietin response in end-stage renal disease. Kidney Int. 2011;79:488–90.
9. Szczech LA, Barnhart HX, Inrig JK, Reddan DN, Sapp S, Califf RM, et al. Secondary analysis of the CHOIR trial epoetin-alpha dose and achieved hemoglobin outcomes. Kidney Int. 2008;74:791–8.
10. Minutolo R, Conte G, Cianciaruso B, Bellizzi V, Camocardi A, De Paola L, et al. Hyporesponsiveness to erythropoiesis-stimulating agents and renal survival in non-dialysis CKD patients. Nephrol Dial Transplant. 2012;27:2880–6.

11. Tsubakihara Y, Nishi S, Akiba T, Hirakata H, Iseki K, Kubota M, et al. 2008 Japanese Society for Dialysis Therapy: Guidelines for Renal Anemia in Chronic Kidney Disease. Ther Apher Dial. 2010;14:240–75.
12. Chen L-K, Woo J, Assantachai P, Auyeung T-W, Chou M-Y, Iijima K, et al. Asian Working Group for Sarcopenia: 2019 Consensus Update on Sarcopenia Diagnosis and Treatment. J Am Med Dir Assoc. 2020;21:300–307.e2.
13. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48:16–31.
14. Kang DO, Park SY, Choi BG, Na JO, Choi CU, Kim EJ, et al. Prognostic Impact of Low Skeletal Muscle Mass on Major Adverse Cardiovascular Events in Coronary Artery Disease: A Propensity Score-Matched Analysis of a Single Center All-Comer Cohort. J Clin Med. 2019;8:712.
15. Kim J, Choi K-H, Cho S-G, Kang S-R, Yoo SW, Kwon SY, et al. Association of muscle and visceral adipose tissues with the probability of Alzheimer's disease in healthy subjects. Sci Rep. 2019;9:949.
16. Tanimoto Y, Watanabe M, Sun W, Sugiura Y, Tsuda Y, Kimura M, et al. Association between sarcopenia and higher-level functional capacity in daily living in community-dwelling elderly subjects in Japan. Arch Gerontol Geriatr. 2012;55:e9-13.
17. Takata T, Motoe A, Tanida K, Taniguchi S, Ida A, Yamada K, et al. Feasibility of computed tomography-based assessment of skeletal muscle mass in hemodialysis patients. J Nephrol. 2020 Sep 29.
18. Ogilvie M, Yu X, Nicolas-Metral V, Pulido SM, Liu C, Ruegg UT, et al. Erythropoietin stimulates proliferation and interferes with differentiation of myoblasts. J Biol Chem. 2000;275:39754–61.
19. Rundqvist H, Rullman E, Sundberg CJ, Fischer H, Eisleitner K, Ståhlberg M, et al. Activation of the erythropoietin receptor in human skeletal muscle. Eur J Endocrinol. 2009;161:427–34.
20. Vega A, Abad S, Verdalles U, Aragoncillo I, Velazquez K, Quiroga B, et al. Dose equivalence between continuous erythropoietin receptor activator (CERA), Darbepoetin and Epoetin in patients with advanced chronic kidney disease. Hippokratia. 2014;18:315–8.
21. Koibuchi K, Miyagi M, Arai T, Aoki T, Aikawa A, Sakai K. Comparing the efficacy of continuous erythropoietin receptor activator and darbepoetin Alfa treatments in Japanese patients with chronic kidney disease during the predialysis period: A propensity-matched analysis. Nephrology (Carlton). 2015;20 Suppl 4:22–8.
22. Shinzato T, Nakai S, Fujita Y, Takai I, Morita E, Nakane K, et al. Determination of Kt/V and Protein Catabolic Rate Using Pre- and Postdialysis Blood Urea Nitrogen Concentrations. Nephron. 1994;67:280–90.
23. Kidney Disease: Improving Global Outcomes (KDIGO) Anemia Work Group. KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease. Kidney Int Suppl. 2012;2:279–335.
24. Gluba-Brzózka A, Franczyk B, Olszewski R, Rysz J. The Influence of Inflammation on Anemia in CKD Patients. Int J Mol Sci. 2020;21.

25. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood. 2006;108:3204–9.
26. Tsuchiya K, Nitta K. Hepcidin is a potential regulator of iron status in chronic kidney disease. Ther Apher Dial. 2013;17:1–8.
27. Nakanishi T, Kuragano T, Kaibe S, Nagasawa Y, Hasuike Y. Should we reconsider iron administration based on prevailing ferritin and hepcidin concentrations? Clin Exp Nephrol. 2012;16:819–26.
28. Lundby C, Hellsten Y, Jensen MBF, Munch AS, Pilegaard H. Erythropoietin receptor in human skeletal muscle and the effects of acute and long-term injections with recombinant human erythropoietin on the skeletal muscle. J Appl Physiol. 2008;104:1154–60.
29. Fukushima T, Horike H, Fujiki S, Kitada S, Sasaki T, Kashihara N. Zinc deficiency anemia and effects of zinc therapy in maintenance hemodialysis patients. Ther Apher Dial. 2009;13:213–9.
30. Hara T, Takeda T-A, Takagishi T, Fukue K, Kambe T, Fukada T. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J Physiol Sci. 2017;67:283–301.

Tables

Table 1. Patient's characteristics	
	N = 70
Age, years	67.2 ± 13.0
Sex (male/female)	45 / 25
Duration of hemodialysis, months	216 (5-1219)
Height, m	1.60 ± 0.10
Body weight, kg	57.9 ± 13
BMI, kg/m^2	22.3 ± 3.5
ERI, IU/kg/week/g/dL	7.2 ± 5.4
Skeletal muscle mass, kg	21.8 ± 5.4
Hemoglobin, g/dL	10.9 ± 0.9
Albumin, g/dL	5.6 ± 0.4
CRP, mg/dL	0.19 (0.05-3.25)
Calcium, mg/dL	8.6 ± 0.6
Phosphate, mg/dL	5.4 ± 1.3
Intact PTH, pg/mL	94 (5-887)
Magnesium, mg/dL	2.6 ± 0.3
TSAT, %	25.3 ± 12.1
Ferritin, ng/mL	79 (11-662)
Zinc, mg/dL	54.7 ± 9.0
Copper, mg/dL	98,3 ± 17.7
nPCR, g/kg/ideal body weight/day	0.82 ± 0.17
Kt/V urea	1.76 (0.98-2.99)
BMI, body mass index; ERI, erythropoietin resistance index; CRP, C-reactive protein; PTH, parathyroid hormone; TSAT, transferrin saturation; nPCR, normalized protein catabolic rate.	

Table 2. Correlations between ESA resnponse and clinical parameters				
	ESA dose / Hb		ERI	
	r	p value	r	p value
Age	0.282	0.020	0.373	0.002
Duration of hemodialysis	0.057	0.65	0.116	0.35
Height	-0.288	0.017	-0.531	< 0.001
Body weight	-0.265	0.029	-0.565	< 0.001
BMI	-0.192	0.12	-0.455	< 0.001
Muscle mass	-0.307	0.011	-0.541	< 0.001
Albumin	-0.119	0.33	-0.173	0.16
CRP	-0.003	0.98	-0.027	0.83
Calcium	0.003	0.98	0.056	0.65
Phosphate	-0.096	0.43	-0.122	0.32
Intact PTH	-0.127	0.30	-0.147	0.23
Magnesium	0.077	0.58	0.018	0.90
TSAT	-0.317	0.009	-0.239	0.049
Ferritin	-0.135	0.27	-0.063	0.61
Zinc	-0.303	0.013	-0.331	0.006
Copper	0.040	0.75	0.016	0.89
nPCR	0.019	0.88	0.175	0.16
Kt/V	0.016	0.90	0.159	0.20

SMI, skeletal muscle index; BMI, body mass index; PTH, parathyroid hormone; CRP, C-reactive protein, TSAT, transferrin saturation; nPCR, normalized protein catabolic rate.

Table 3. Correlations between skeletal muscle mass and clinical parameters

	r	p-value
Age, years	-0.548	< 0.001
Duration of hemodialysis, months	-0.116	0.35
Height, m	0.857	< 0.001
Body weight, kg	0.799	< 0.001
BMI, kg/m^2	0.514	< 0.001
Hemoglobin, g/dL	0.140	0.25
Albumin, g/dL	0.376	0.001
CRP, mg/dL	-0.116	0.35
Calcium, mg/dL	-0.211	0.079
Phosphate, mg/dL	0.106	0.38
Intact PTH, pg/mL	0.184	0.13
Magnesium, mg/dL	-0.103	0.45
TSAT, %	0.092	0.45
Ferritin, ng/mL	-0.113	0.35
Zinc, mg/dL	0.265	0.029
Copper, mg/dL	-0.151	0.22
nPCR, g/kg/ideal body weight/day	-0.273	0.028
Kt/V urea	-0.424	< 0.001

BMI, body mass index; ERI, erythropoietin resistance index; CRP, C-reactive protein; PTH, parathyroid hormone; TSAT, transferrin saturation; nPCR, normalized protein catabolic rate.

Table 4. Multiple linear regression analysis				
	Dependent variable			
	ESA dose/Hb		ERI	
	Stdβ	p value	Stdβ	p value
TSAT	-0.3422	0.00298	-0.288	0.00359
Zinc	-0.2665	0.02236	-0.301	0.01087
Muscle mass	-0.231	0.04454	-0.533	0.00016
CRP	-0.1807	0.11129	-0.172	0.08741
SMI, skeletal muscle index; BMI, body mass index. Stepforward selection method				

Figures

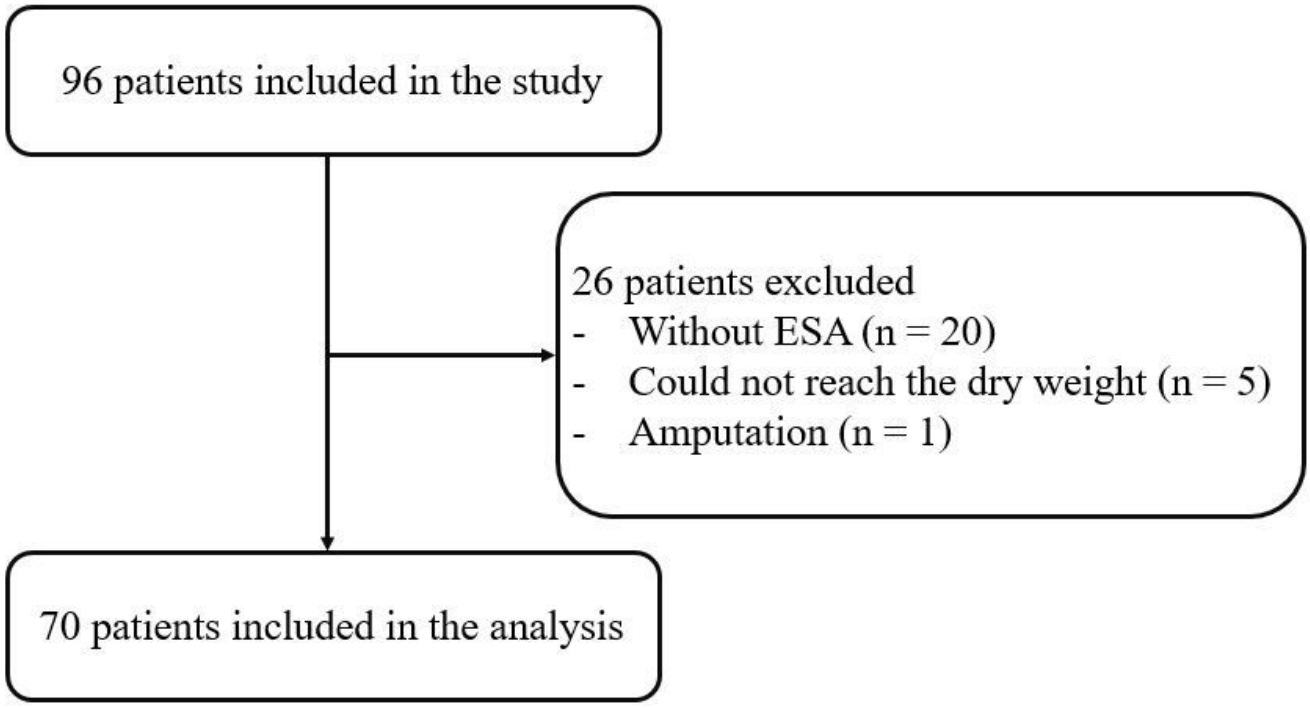

Figure 1

Study design. Excluding 26 patients, 70 patients were included in the analysis.