Impact of extracellular-to-intracellular fluid volume ratio on albuminuria in patients with type 2 diabetes: A cross-sectional and retrospective cohort study

CURRENT STATUS: POSTED

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DOI:
10.21203/rs.2.24737/v1

SUBJECT AREAS
Cardiac & Cardiovascular Systems  Endocrinology & Metabolism

KEYWORDS
Bioelectrical impedance analysis, Body fluid volume, Diabetic kidney disease, Fluid overload, Nephropathy, Volume overload
Abstract
Background Body fluid volume imbalance is common in patients with kidney failure and is associated with all-cause mortality. We hypothesized that fluid volume imbalance occurs in diabetic patients without kidney failure and is associated with change in albuminuria. This study aimed to investigate the association between fluid volume imbalance and albuminuria in patients with type 2 diabetic mellitus without kidney failure.

Methods We included 432 and 368 participants in the cross-sectional and retrospective studies. Body fluid imbalance was determined by measuring the extracellular water (ECW) to intracellular water (ICW) ratio (ECW/ICW) using bioelectrical impedance analysis. Change in urinary albumin to creatinine ratio (ACR) was defined as the ratio of urinary ACR at follow-up to that at baseline. ECW/ICW ratio was compared to level of albuminuria.

Results In this cross-sectional study, ECW/ICW ratio increased with level of albuminuria (0.634 ± 0.002 in normo-, 0.645 ± 0.003 in micro- and 0.656 ± 0.004 in macroalbuminuria, all p <0.001 by Tukey’s HSD test). In addition, there was an association between ECW/ICW ratio and logarithms urinary ACR after adjusting for covariates (β = 0.217, p <0.001). Moreover, ECW/ICW ratio was associated with change in the urinary ACR after adjusting for covariates (β = 0.171, p = 0.005) in this retrospective study.

Conclusions ECW/ICW ratio is independently associated with level of albuminuria in patients with type 2 diabetes mellitus without kidney failure. This reinforces the importance of monitoring fluid balance in patients with type 2 diabetes mellitus.

Background
The incidence of diabetic kidney disease (DKD), which is the leading cause of end stage renal disease (ESRD), have been increasing worldwide (1). DKD is also related with cardiovascular disease (CVD) and all-cause mortality (2,3,4). Lifestyle modifications and medications for diabetes, hypertension, and dyslipidemia are effective for preventing ESRD for patients with DKD (5,6,7).

Albuminuria, which indicates microvascular endothelial injury, is an independent risk factor for progression to ESRD, particularly in patients with DKD (3,8). Thus, early detection of DKD, especially
worsening of albuminuria, is important in management of patients with diabetes mellitus. In healthy adults, fluid distribution between the intracellular water (ICW) and extracellular water (ECW) compartments is tightly regulated (9). This regulation is however impaired in patients with ESRD and hemodialysis, resulting in fluid overload (10,11). One of the mechanism of fluid overload is thought to be inflammation, which causes hypoalbuminemia and increased vascular permeability (12). The result is extravascular fluid shift which leads to ECF volume overload (13). We hypothesized that subtle fluid imbalance occurs in patients with diabetes mellitus without kidney failure, and that there is an association between fluid imbalance and albuminuria. No studies have reported on this association. In this cross-sectional and retrospective cohort studies, we therefore investigated the association between ECW/ICW ratio and albuminuria in patients with type 2 diabetes mellitus without kidney failure, and the effect of ECW/ICW ratio on the level of albuminuria, using bioelectrical impedance analysis (BIA) which is used to evaluate body composition and fluid compartments (14,15).

Materials And Methods

Study population
The present study was a sub-analysis of the KAMOGAWA-DM cohort study, the details of which have been described elsewhere (16). Briefly, the KAMOGAWA-DM cohort Study is an ongoing cohort study of subjects at Kyoto Prefectural University of Medicine (Kyoto, Japan) and Kameoka Municipal Hospital (Kameoka, Japan). The purpose of this cohort study is to clarify the natural history of people with diabetes. In the cross-sectional study, we investigated the relationship between ECW/ICW ratio and the prevalence of diabetic nephropathy, and in the retrospective study, we investigated the association between ECW/ICW ratio and change in ACR.

We extracted data of patients with type 2 diabetes mellitus who had body composition analysis, and measured urinary albumin to creatinine ratio (ACR) during the years from 2014 to 2017 from the KAMOGAWA-DM cohort study. The exclusion criteria of the cross-sectional study were as follows: missing data of covariates (serum creatinine level and duration of diabetes) and patients with estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m² (17); and the exclusion criteria of the
retrospective study were no follow-up data (including treatment interruption, transfer to another hospital, death and less than three times measurement of urinary ACR).

**Variables**

Body mass index (BMI), intracellular water (ICW), extracellular water (ECW), total body water (TBW), body fat mass, and skeletal muscle mass were measured with fasting state by BIA (18). ECW/ICW ratio and skeletal muscle index (SMI, kg/m$^2$) was calculated from the obtained data (19,20).

Medication data were also collected; medication for diabetes, including sodium-glucose cotransporter 2 (SGLT-2) inhibitors; and medication for hypertension, including renin angiotensin aldosterone (RAS) inhibitor and diuretics. The smoking status was categorized into three groups: never-, ex-, and current smoker. 'Exercise habit' was defined as regularly performing any type of sport ≥ 1 /week (21).

**Data collection**

HbA1c, creatinine, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured using the subjects' venous blood after an overnight fast. The eGFR was determined using the Japanese Society of Nephrology equation: eGFR = 194 × Cre$^{-1.094}$ × age$^{-0.287}$ (ml/min/1.73 m$^2$) (× 0.739 for women) (22).

Urinary albumin and creatinine concentrations were measured using early morning spot urine samples. In this study, a mean value for urinary ACR, which was determined from three urine collections, were used for analyses. According to the Joint Committee on Diabetic Nephropathy, we divided the subjects into three groups; normo- micro- and macro-albuminuria (17). Follow-up examinations were performed one year later, we also collected urine samples for calculation of urinary ACR three times a year. Change in urinary ACR was calculated as follows: dividing the follow up urinary ACR by the baseline urinary ACR (23).

**Ethical considerations**

This study was approved by the ethics committee of Kyoto Prefectural University of Medicine (Approval number RBMR-E-466-5), and undertaken in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants. To protect the confidentiality of participants, personal identifiable information was removed and medical data stored in a database which was password protected.
Statistical analysis

Statistical analyses were performed using JMP ver. 13.2 software (SAS, Cary, NC). A p-value < 0.05 was considered significant. For normally distributed continuous variables, data was summarized using mean and standard deviation. Continuous variables with a skewed distribution were summarized using median and inter-quartile range. Categorical variables were described using proportions. Differences between the groups were analyzed as follows: the baseline clinical characteristics of the groups were compared using Pearson’s chi-squared test or Fisher's Exact test as appropriate. For normally distributed continuous variables, we compared mean difference between groups using the one-way Analysis of Variance (ANOVA) and Tukey's honestly significant difference (HSD) test. Because ACR had a skewed distribution, logarithmic transformation was undertaken before correlation and multiple logistic regression analyses. Variables found to be statistically different in bivariate analysis were controlled for in multiple regression analysis. We investigated the relationships between ECW/ICW ratio and logarithmic of ACR or other factors using Pearson's correlation coefficient. Multiple regression analysis for logarithmic of urinary ACR was undertaken. Furthermore, we also investigated the effect of ECW/ICW ratio on change of urinary ACR by multiple regression analysis. We considered several potential confounders as co-variants: age, sex, BMI, HbA1c, creatine, triglycerides, duration of diabetes, smoking status, exercise, and usage of RAS inhibitor and SGLT-2 inhibitor, diuretics, ECW/ICW and logarithmic of urinary ACR at baseline examination.

Results

The inclusion of participants is summarized in Fig. 1. Out of the 481 (261 men and 220 women) participants eligible for the study, 49 (30 men and 19 women) were excluded due to missing data on serum creatinine and duration of diabetes (Fig. 1).

The baseline characteristics of study subjects is summarized in Table 1. The mean age, BMI, skeletal muscle mass, and SMI was 66.7 ± 11.1 year, 24.3 ± 3.98 kg/m², 24.1 ± 5.34 kg, and 6.93 ± 1.07 kg/m² respectively. The mean ECW/TBW ratio was 0.390 ± 0.01, and mean urinary ACR was 134.5 ± 397.6 mg/g Cr. Mean ICW and ECW were 20.3 ± 0.25 kg and 12.8 ± 0.15 kg in patients with normo-
albuminuria, 19.1 ± 0.37 kg, and 12.3 ± 0.22 kg in patients with micro-albuminuria, and 20.6 ± 0.59 kg and 13.5 ± 0.35 kg in patients with macro-albuminuria. ECW/ICW ratio increased with albuminuria stage.

The differences of ECW/ICW ratio according to sex was 0.632 ± 0.002 in men and 0.647 ± 0.002 in female (p < 0.001). The associations between the ECW/ICW ratio and other covariates is shown in Table 2. The log-transformed urinary ACR was associated with elevated ECW/ICW ratio (r = 0.313, 95%CI (0.226–0.396), p < 0.001). Moreover, ECW/ICW ratio was negatively associated with SMI levels.
(r = -0.273, 95% CI (-0.358 - -0.184), p < 0.001).

Table 2
Univariate Analysis: co-relation between ECW/ICW ratio and covariates.

| Variables                        | r (95%CI)              | p      |
|----------------------------------|------------------------|--------|
| Age, yrs                         | 0.420 (0.339, 0.494)   | < 0.001|
| Body mass index, kg/m²           | 0.027 (-0.121, 0.067)  | 0.572  |
| Skeletal muscle index, kg/m²     | -0.273 (-0.358, -0.184)| < 0.001|
| HbA1c, mmol/mol                  | 0.014 (-0.081, 0.108)  | 0.778  |
| Creatinine, μmol/L               | 0.088 (-0.006, 0.181)  | 0.067  |
| logarithmic of urinary ACR       | 0.313 (0.226, 0.396)   | < 0.001|
| Duration of diabetes             | 0.208 (0.116, 0.296)   | < 0.001|

ICW: intracellular water, ECW: extracellular water, ACR: albumin to creatinine ratio.

To investigate the relationships between ECW/ICW ratio and logarithmic of ACR or other factors, Pearson's correlation coefficient was performed.

Table 3 shows the multiple regression analysis for logarithmic of urinary ACR and shows the strong relation between ECW/ICW ratio and urinary ACR (β = 0.217, p < 0.001).

Table 3
Multiple regression analysis of logarithms ACR.

| Variables                        | β (95%CI)              | p      |
|----------------------------------|------------------------|--------|
| Age, yrs                         | 0.035 (-0.009, 0.018)  | 0.521  |
| Male                             | 0.030 (-0.090, 0.180)  | 0.514  |
| Body mass index, kg/m²           | 0.067 (-0.009, 0.059)  | 0.149  |
| ECW/ICW ratio                    | 0.217 (6.458, 17.136)  | < 0.001|
| HbA1c, mmol/mol                  | 0.127 (0.005, 0.026)   | 0.003  |
| Creatinine, μmol/L               | 0.298 (0.008, 0.014)   | < 0.001|
| Duration of diabetes, years      | 0.047 (-0.006, 0.020)  | 0.302  |
| Current smoker                   | -0.059 (-0.054, 0.314) | 0.165  |
| Exercise habit                    | -0.048 (-0.195, 0.053) | 0.263  |
| Usage of RAS inhibitor           | 0.188 (0.150, 0.410)   | < 0.001|
| Usage of SGLT-2 inhibitor        | 0.056 (-0.059, 0.287)  | 0.197  |
| Usage of diuretics               | 0.061 (-0.063, 0.374)  | 0.164  |

ICW: intracellular water, ECW: extracellular water, ACR: albumin to creatinine ratio, RAS: renin-angiotensin-system, SGLT-2: sodium glucose cotransporter-2.

Current smoker was defined as never- and ex-smoker (0), or current smoker (1); exercise habit was defined as non-regular exerciser (0), regular exerciser (1); Usage of each medicine was defined as non-user (0) or user (1).

In the retrospective study, out of the 432 people (231 men and 201 women) eligible for the study, 64 (32 men and 32 women) were excluded, resulting in a study population of 368 people (199 men and 169 women) (Fig. 1).

Table 4 summarizes the characteristics of study subjects of retrospective study. Table 5 shows the results of the multiple regression analysis with change in the urinary ACR. ECW/ICW ratio was associated with change in the urinary ACR after adjusting for covariates (β = 0.171, p = 0.005).
Table 4
Characteristics of study subjects of retrospective study at the baseline examination.

| Variable                          | total      | n  |
|-----------------------------------|------------|----|
|                                 | 368        |    |
| Age, yrs                         | 65.5 ± 11.2|    |
| Male                             | 199 (54.1) |    |
| Height, cm                       | 161.1 ± 9.35|   |
| Weight, kg                       | 63.2 ± 12.7|    |
| Body mass index, kg/m²           | 24.3 ± 3.97|    |
| **Body composition**             |            |    |
| Body fat mass, kg                | 18.8 ± 7.81|    |
| Skeletal muscle mass, kg         | 24.1 ± 5.34|    |
| Skeletal muscle index (SMI; kg/m²)| 6.92 ± 1.09|    |
| ICW, kg                          | 19.9 ± 4.14|    |
| ECW, kg                          | 12.7 ± 2.45|    |
| ECW/ICW ratio                    | 0.64 ± 0.027|    |
| ECW/TBW ratio                    | 0.392 ± 0.01|    |
| HbA1c, mmol/mol (%)              | 54.5 ± 11.0 (7.14 ± 1.01)| |
| Creatinine, μmol/L               | 69.2 ± 20.0 |    |
| Urinary ACR, mg/gCr              | 109.0 ± 318.1|    |
| Duration of diabetes, years      | 13.4 ± 10.2 |    |
| Smoking: never-/ex-/current smoker | 220/86/62 (59.8/23.3/16.8) |    |
| Exercise habit                    | 184 (50.0) |    |
| Usage of RAS inhibitor           | 171 (46.5) |    |
| Usage of SGLT-2 inhibitor        | 63 (17.1)  |    |
| Usage of diuretics               | 31 (8.4)   |    |

Data are expressed as the number (percentage), mean ± standard deviation. ICW: intracellular water, ECW: extracellular water, TBW: total body water, ACR: albumin to creatinine ratio, RAS: renin-angiotensin-system, SGLT-2: sodium glucose cotransporter-2.

Table 5
Multiple regression analysis for the factors affecting change in the urinary ACR.

| Variables                      | β (95%CI)  | p  |
|--------------------------------|------------|----|
| Age, yrs                       | 0.008 (-0.016, 0.018) | 0.899 |
| Male                           | 0.125 (0.013, 0.778)  | 0.043 |
| BMI, kg/m²                     | 0.11 (-0.0001, 0.087)  | 0.051 |
| ECW/ICW ratio                  | 0.171 (3.033, 17.058) | 0.005 |
| HbA1c, mmol/mol (%)            | 0.151 (0.007, 0.036)  | 0.004 |
| Creatinine, μmol/L             | 0.030 (-0.007, 0.012)  | 0.640 |
| log-logistic of urinary ACR    | -0.200 (-0.347, -0.097) | <0.001 |
| Duration of diabetes           | 0.092 (-0.003, 0.031)  | 0.101 |
| Current smoker                 | -0.046 (-0.339, 0.129) | 0.378 |
| Exercise habit                  | -0.008 (-0.174, 0.148)  | 0.874 |
| Usage of RAS inhibitor         | 0.023 (-0.272, 0.420)  | 0.674 |
| Usage of SGLT-2 inhibitor      | -0.056 (-0.670, 0.205) | 0.297 |
| Usage of diuretics             | 0.086 (-0.106, 1.075)  | 0.107 |

ICW: intracellular water, ECW: extracellular water, ACR: albumin to creatinine ratio, RAS: renin-angiotensin-system, SGLT-2: sodium glucose cotransporter-2.

Current smoker was defined as never- and ex-smoker (0), or current smoker (1); exercise habit was defined as non-regular exerciser (0), regular exerciser (1).

Discussion
We investigated the association of fluid volume imbalance and albuminuria in patients with type 2 diabetes without ESRD based on our hypothesis that fluid imbalance occurred in diabetic patients without ESRD, and was associated with changes in albuminuria. Our study’s findings support the hypothesis.

Previous studies have demonstrated the association of fluid overload and increased risk of eGFR decline and all-cause or cardiovascular mortality in patients with ESRD and patients on dialysis.
Our study's mean ECW/TBW ratio of 0.390 ± 0.01 was lower than that reported in recent studies of patients with CKD stage 4 or 5 (0.39783–0.512) (26,29,30). This finding suggests that fluid imbalance is less likely to occur in patients with early nephropathy than in patients with ESRD and hemodialysis.

Water shift from ICW to ECW led to change the ECW/ICW ratio (9). Cell volume is regulated by apoptosis, which is a morphological hallmark of programmed cell death (31). The loss in cell volume during apoptosis may play a role in change in balance between ICW and ECW content. In addition, uremic status may also cause cell shrinkage. Previous studies have reported that erythrocytes may undergo suicidal death or eryptosis associated with cell shrinkage, which can be stimulated by uremic toxins (32).

Albuminuria is known to reflect endothelial dysfunction and subclinical inflammation caused by oxidative stress and inflammatory cytokines (8,12,33). Kidney endothelial dysfunction plays an important role in the development of albuminuria by reducing vascular relaxation and inflammatory cell infiltration (34). Under physiological conditions, tubule-glomerular feedback (TGF) signaling maintains stable glomerular filtration rate (GFR) by modulating pre-glomerular arteriole tone. Early in nephropathy, chronic hyperglycemic conditions impair SGLT-2 mediated reabsorption of sodium and glucose in the proximal tubule. Thus, despite increased GFR, the macula densa is exposed to low sodium concentrations. This impairment of TGF signaling likely leads to inadequate arteriole tone and increased renal perfusion. As a result, impairment of TGF cause increased body fluid and fluid imbalance (35,36,37). Previous study show that both human and animals with volume overload have significantly higher pro-inflammatory cytokines such as IL-6 or TNF-α (38), which can be the result of kidney endothelial dysfunction and impairment of TGF. Further, inflammation-induced hypoalbuminemia and increased vascular permeability enhance extravascular fluid shift, thereby resulting in ECF volume overload (13). In fact, the ECW/TBW ratio, which is substantially the same as the ECW/ICW ratio, has been used as a marker of ECW excess (8,39,40).

Increase in ECW/ICW ratio caused by fluid overload effects vascular and endothelial level by oxidative stress, chronic activation of the renin-angiotensin system, sympathetic activation and an increase of
inflammation, which leads to atherosclerosis (41). The excess volume status would increase renal efferent pressure and cause glomerular hypertension and eventual decline in eGFR (25). Even in subclinical state, early changes in volume and cardiac stretch, venous congestion or subclinical atherosclerosis may contribute to reduced kidney function. Taking these findings together, increase of ECW/ICW is associated with presence of albuminuria and change in albuminuria. In addition, SGLT-2 inhibitor or diuretics causes extracellular fluid loss, however it was almost the same result if we exclude patients with SGLT-2 inhibitor or diuretics.

These results should be interpreted considering the study’s limitations. First, the sample size may not have been adequate to determine if a significant relationship existed between albuminuria and ECW/ICW ratio resulting in selection bias. Second, our assessment of lifestyle (exercise habit, alcohol consumption) and medication intake only at baseline means that changes in these factors during follow-up might have influenced change in urinary ACR. Lastly, the inclusion of only Japanese diabetic patients without kidney failure could limit the generalization of the findings to non-Japanese patients.

Despite the limitations, the study also was a well-designed epidemiological study of patients with type 2 diabetes. Measurement of body fluid composition by BIA has the advantage of being non-invasive, fast, and reproducible, making it high versatile and constructive. Finally, we addressed threats to internal validity by adjusting for confounding variables and laboratory measurements. This reduced risk of biases.

**Conclusion**

In conclusion, to our knowledge, this is the first study to show that imbalance of body composition evaluated by ECW/ICW ratio is independently associated with presence and increment of albuminuria even in diabetic patients without ESRD. Therefore, we recommend that clinicians pay more attention to monitoring of fluid status among patients with type 2 diabetes mellitus, and maintaining fluid balance in early stage of diabetic nephropathy.

**Abbreviations**

ACR, albumin to creatinine ratio; ANOVA, Analysis of Variance; BIA, bioelectrical impedance analysis; BMI, Body mass index; CVD, cardiovascular disease; DKD, diabetic kidney disease; ECW, extracellular
water; ECW/ICW, extracellular water to intracellular water ratio; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HSD, honestly significant difference; ICW, intracellular water; RAS, renin angiotensin aldosterone; SGLT-2, sodium-glucose cotransporter 2; SMI, skeletal muscle index; TBW, total body water; TGF, tubule-glomerular feedback.

Declarations

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. And informed consent was obtained from all individual participants included in the study. This study was approved by the Medical Ethics Committee of the Kyoto Prefectural University of Medicine and Kameoka Municipal Hospital.

Consent for publication: Not applicable.

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

Competing interests: Y.H. has received grant support from Asahi Kasei Pharma and honoraria from Mitsubishi Tanabe Pharma Corporation and Novo Nordisk Pharma Ltd. S.M. has received honoraria from Novo Nordisk Pharma Ltd., Abbott Japan Co., Ltd., AstraZeneca plc, Kowa Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd. T.S. has received honoraria from Ono Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Co, Astellas Pharma Inc., Kyowa Hakko Kirin Co., Ltd., Sanofi K.K., MSD K.K., Kowa Pharmaceutical Co., Ltd., Taisho Toyama Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Kissei Pharmaceutical Co., Ltd., Novo Nordisk Pharma Ltd., Eli Lilly Japan K.K. N.N. has received honoraria from Novo Nordisk Pharma Ltd., Abbott Japan Co., Ltd., AstraZeneca plc, Kowa Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd. U.E. has received grant support from the Japanese Study Group for Physiology and Management of Blood Pressure, the Astellas Foundation for Research on Metabolic Disorders (Grant number: 4024). Donated Fund Laboratory of Diabetes therapeutics is an endowment department,
supported with an unrestricted grant from Ono Pharmaceutical Co., Ltd., and received personal fees from AstraZeneca plc, Astellas Pharma Inc., Daiichi Sankyo Co., Ltd., Kyowa Hakko Kirin Company Ltd., Kowa Pharmaceutical Co., Ltd., MSD K.K., Mitsubishi Tanabe Pharma Corp., Novo Nordisk Pharma Ltd., Taisho Toyama Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd. and Johnson & Johnson K.K. M.A. has received honoraria from Novo Nordisk Pharma Ltd., Abbott Japan Co., Ltd., AstraZeneca plc, Kowa Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd. M.H, has received grant support from Asahi Kasei Pharma, Nippon Boehringer Ingelheim Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Daiichi Sankyo Company, Limited, Sanofi K.K., Takeda Pharmaceutical Company Limited, Astellas Pharma Inc., Kyowa Kirin Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Novo Nordisk Pharma Ltd., and Eli Lilly Japan K.K. M.Y. has received personal fees from MSD K.K., Sumitomo Dainippon Pharma Co., Ltd., Kowa Company, Limited, AstraZeneca PLC, Takeda Pharmaceutical Co., Ltd, Kyowa Hakko Kirin Co., Ltd., from Daiichi Sankyo Co., Ltd., Kowa Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd. M.F. has received grants from Nippon Boehringer Ingelheim Co., Ltd., Kissei Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Co, Daiichi Sankyo Co., Ltd., Sanofi K.K., Takeda Pharmaceutical Co., Ltd., Astellas Pharma Inc., MSD K.K., Kyowa Hakko Kirin Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Kowa Pharmaceutical Co., Ltd., Novo Nordisk Pharma Ltd., Ono Pharmaceutical Co., Ltd., Sanwa Kagaku Kenkyusho Co., Ltd. Eli Lilly Japan K.K., Taisho Pharmaceutical Co., Ltd., Terumo Co., Teijin Pharma Ltd., Nippon Chemiphar Co., Ltd., and Johnson & Johnson K.K. Medical Company, and received honoraria from Nippon Boehringer Ingelheim Co., Ltd., Kissei Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Corp., Daiiichi Sankyo Co., Ltd., Sanofi K.K., Takeda Pharmaceutical Co., Ltd., Astellas Pharma Inc., MSD K.K., Kyowa Kirin Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Kowa Pharmaceutical Co., Ltd., Novo Nordisk Pharma Ltd., Ono Pharmaceutical Co., Ltd., Sanwa Kagaku Kenkyusho Co., Ltd., Eli Lilly Japan K.K., Taisho Pharmaceutical Co., Ltd., Bayer Yakuhin, Ltd., AstraZeneca K.K., Mochida Pharmaceutical Co., Ltd., and Combi Corp. The other authors have nothing to disclose.

**Funding:** none.
Author’s contributions: H.N. contributed to the design of this work, analysis of the data and wrote the manuscripts. Y.H. contributed to the conception and design of the work, analysis and interpretation of the data and draft the discussion. A.K., R.S., and F.T. contributed to the design of the work, acquisition the data and revise the work critically for important intellectual content. Y.Y., R.B., T.O., N.K., S.M., T.S., H.O., N.N., E.U., and M.A. contributed to the acquisition the data and draft the discussion. M.H. contributed to the design of the work and interpretation of the data and revise the work critically for important intellectual content. M.Y. contributed to the acquisition the data and draft the discussion. M.F. contributed to the conception and design of the work, and interpretation of the data and revise the work critically for important intellectual content. All the authors permitted final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgments: We thank all of the staff members of Kyoto Prefectural University of Medicine and Kameoka Municipal Hospital. We would like to thank Editage (www.editage.com) for English language editing.

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Figures
Figure 1

Inclusion and exclusion flow chart of participants.