Subclinical fluid overload is significantly associated with coronary artery calcification in patients with chronic kidney disease

Seohyun Park

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Tae-Hyun Yoo

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YONSEI UNIVERSITY

This certifies that the Master's Thesis of
Seohyun Park is approved.

Thesis Supervisor: Tae-Hyun Yoo

Thesis Committee Member: Shin-Wook Kang

Thesis Committee Member: Sungha Park

The Graduate School
Yonsei University

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ABSTRACT

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Seohyun Park

Department of Medicine
The Graduate School, Yonsei University

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Background:

Cardiovascular (CV) disease is prevalent and most common cause of mortality in dialysis patients. Extracellular fluid (ECF) excess is frequently observed and an independent predictor of CV morbidity in patients with advanced chronic kidney disease (CKD). However, there are few studies about the relationship between fluid excess and CV risks even in patients with early stage CKD. The aim of present study is to investigate the association between extracellular fluid (ECF) status measured using bioelectrical impedance analysis and coronary artery calcification score (CACS) as a surrogate for CV disease in CKD patients with relatively preserved renal function.

Method:

Data were retrieved from the prospective observational cohort of

Cardiovascular and Metabolic Disease Etiology Research Center-High Risk (NCT02003781). Extracellular water (ECW) and total body water (TBW) were assessed by bioelectrical impedance analysis and CACS was measured by multidetector computed tomography. After exclusion of patients with significant volume overload (ECW/TBW >0.4) and substantially impaired renal function (eGFR <45.0 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$), patients were divided into four groups according to the quartiles of their ECW-to-TBW ratio (ECW/TBW). Coronary artery calcification (CAC) was defined as CACS more than 400 agatston units.

Result:

A total of 1481 patients was analyzed and the mean was 59.8 ± 11.3 years; 759 (51.2%) were men; and the mean eGFR was 85.0 ± 16.9 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$. The patients in the increasing quartiles of the ECW/TBW showed older, higher blood pressure, and much prevalent co-morbid conditions such as diabetes compared to those in lower quartiles. CACS [1^{st} to 4^{th} quartile; 0.0 (0.0 – 73.9), 9.95 (0.0 – 133.3), 12.3 (0.0 – 144.6), vs. 59.6 (0.0 – 307.3), P for trend <0.001] and CAC [1^{st} to 4^{th} quartile; 23 (6.2%), 36 (9.7%), 44 (12.0%), vs. 76 (20.5%), P for trend <0.001] significantly increased in accordance with increasing ECW/TBW quartiles. ECW/TBW showed an independent association with CAC after adjustment for multiple confounders (per 0.01 increase in ECW/TBW; odds ratio 2.890, 95%

confidence interval 1.787 – 4.674, P <0.001). In receiver operating characteristic (ROC) analyses, the area under the ROC curve (AUC) for CAC risk prediction was significantly increased by adding ECW/TBW to a model consisting of traditional (AUC; 0.784 vs. 0.736, P = 0.013) or non-traditional factors (AUC; 0.784 vs. 0.749, P = 0.038).

Conclusion:

In conclusion, subclinical fluid excess in CKD patients with preserved renal function was associated with the increasing risk of CAC. This result suggests that the assessment of ECF can help to determine CV risk in patients with early stage CKD.

Key words: extracellular fluid excess, coronary artery calcification, chronic kidney disease

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I. INTRODUCTION

Cardiovascular (CV) disease is prevalent and the leading cause of death in chronic kidney disease (CKD) patients.[1] In addition, decreased renal function was found to be associated with the pathogenesis of CV disease and a risk of CV events was particularly accelerated with eGFR levels less than 30 mL/min/1.73 m^2.[2] In dialyzed patients with end-stage renal disease (ESRD) compared with the general population, traditional risk factors as well as non-traditional risk factors might attributable to high CV morbidity, which was known to increase up to 20 times after adjusting for age, sex, race and diabetes.[1,3] However, recent studies conducted by McCullough PA et al. have shown that the prevalence of CV disease and risk of CV event also increased even in early stage CKD patients with mild renal insufficiency.[4]

Several non-traditional risk factors have been associated with greater incidence of CV disease in dialyzed or non-dialyzed CKD patients. Among

them, extracellular fluid (ECF) excess is frequently accompanied and is known to be associated with adverse clinical outcomes in patients with advanced CKD and dialysis population.[5,6] Several possible explanations have been proposed for increasing incidence of CV disease in CKD including chronic inflammation[7] and the activation of renin-angiotensin system[8]. However, to date, the majority of clinical studies have focused on the cardio-metabolic risks of fluid overload in advanced CKD patients. There is lack of evidences about the association between fluid status and CV risks in patients with early stage CKD.

Vascular calcification is common in CKD patients and is combined in up to about 80% of ESRD patients. Accumulating evidences suggested that the disturbances in mineral metabolism, such as elevated serum calcium and phosphorus levels play an important role in the development of vascular calcification.[9] In addition, some studies also suggested that fluid overload can induce the vascular calcification via endothelial dysfunction in CKD patients. Both studies by Thambyrajah J et al.[10] and Bolton CH et al.[11] demonstrated that endothelial function measured by flow-mediated dilatation of brachial artery was reduced in non-dialyzed CKD patients. Patients with endothelial dysfunction had enhanced coronary artery calcium score (CACS)[12] and adverse CV outcomes[13].

Coronary artery calcification (CAC) is expressed as a CACS measured by multi-detector computed tomography. CAC is known as an index

of atherosclerosis and useful predictor of CV risk in asymptomatic patients.[14] In patients with CKD, CAC is extremely occurred than general population[15] and it is independently associated with all-cause and CV mortality, including coronary heart disease.[16] Unlike general population, medial calcification occurs mainly in patients with CKD, and abnormal mineral metabolisms, anemia, inflammation and uremic toxins are known to be risk factors.[17] However, CAC is also observed in early stage CKD[18,19] in which these risk factors relatively insignificant and the mechanisms have not yet been sufficiently investigated.

Therefore, this study was aimed to evaluate the association between ECF status measured using bioelectrical impedance analysis (BIA) and CACS as a surrogate for CV disease in early stage CKD patients with relatively preserved renal function.

II. MATERIALS AND METHODS

1. Ethics statement

This study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the institutional review board (IRB) at Yonsei University Health System (YUHS) Clinical Trial Center. All patients provided written informed consent before entering the study (IRB No. 4-2013-0581).

2. Study population

The study population was selected from the CV and Metabolic Diseases Etiology Research Center-High Risk Cohort (CMERC-HI) at YUHS between November 2013 and December 2016. The CMERC-HI is an ongoing, nationwide, and prospective cohort study aiming at developing more specific preventive strategies for patients with a high risk of CV disease (clinicaltrials.gov NCT02003781). Patients who fitted at least one of the following descriptions were enrolled in the study: high-risk patients with hypertension, namely hypertensive patients who had an estimated GFR (eGFR) of ≥60 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ and target organ damage, or hypertensive patients with eGFR <60 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$; diabetic patients with random urine albumin to creatinine ratio (uACR) ≥30 mg/g; patients with end-stage renal disease (ESRD) undergoing dialysis; first-degree relatives of patients

with early-onset acute myocardial infarction at the age of <55 years in men and <65 years in women; patients with asymptomatic atherosclerotic CV disease (abdominal aorta diameter ≥3 cm or ankle-brachial index <0.9, or carotid plaque or carotid intima-media thickness ≥0.9 mm, or asymptomatic old cerebrovascular accident, or >30% stenosis in at least one major coronary artery); patients with rheumatoid arthritis aged >40 years who are taking methotrexate or steroid; patients with atrial fibrillation and CHA2DS2-VASc score ≥1; or kidney transplant recipients at >3 months after transplantation. The exclusion criteria were as follows: age <20 years, a history of acute coronary syndrome, symptomatic coronary or peripheral artery disease, heart failure, life expectancy of <6 months, pregnant state in women, and a history of contrast allergy and related adverse effects. Since this study is focused on the association between subclinical volume overload and CAC in early stage CKD patients, patients with advanced CKD (stage 3b to 5), ESRD undergoing dialysis, kidney transplantation, or overt ECF excess by BIA were excluded. In addition, patients who did not measure CACS, ECF status, or serum creatinine was excluded. Finally, a total of 1481 patients were analyzed in the present analysis **(Figure 1)**.

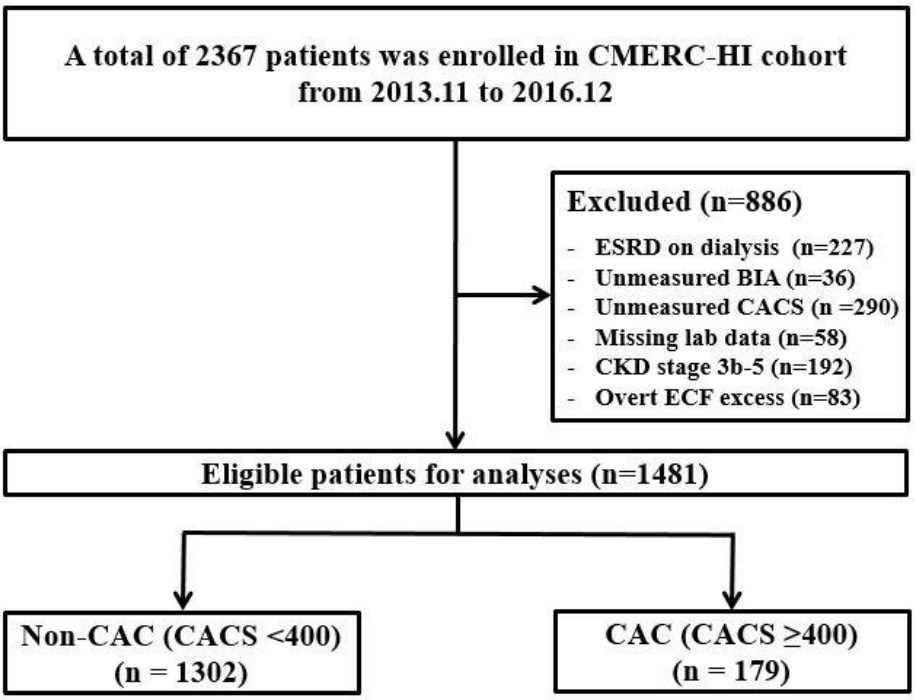

Figure 1. Flow diagram of the study population

Of 2367 high risk cardiovascular disease patients, 886 patients were not met the inclusion criteria in present study. A total of 1481 patients with CKD stage 1 to 3a without overt ECF excess were analyzed. Abbreviations: CMERC-Hi, Cardiovascular and Metabolic Diseases Etiology Research Center-High Risk Cohort; ESRD, end stage renal disease; BIA, bioelectrical impedance analysis; CAC, coronary artery calcification; CACS, coronary artery calcium score; CKD, chronic kidney disease; ECF, extracellular fluid

3. Clinical and biochemical data collection

All participants underwent baseline evaluations, including an initial standardized questionnaire. Demographic and clinical data including age, sex, height, weight, and comorbidities such as diabetes, hypertension, or CV disease including coronary artery occlusive disease, cerebrovascular accident, ischemic heart disease, and peripheral arterial occlusive disease were collected at the time of cohort enrollment. Data such as smoking history, and medication history were also collected. Participants were considered to have diabetes mellitus that defined as a history of diabetes, were receiving anti-diabetic treatment, or had fasting plasma glucose levels of ≥126 mg/dL. Participants were considered to have hypertension if they had a self-reported history of hypertension, antihypertensive medication use, or a blood pressure (BP) of ≥140/90 mm Hg. Laboratory parameters included complete blood cell count, blood urea nitrogen, creatinine, albumin, calcium, phosphate, high-sensitivity C-reactive protein levels, lipid profiles, and uACR. The eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation based on serum creatinine.[20] CKD stages were categorized according to eGFR as proposed by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative.[21]

4. Measurement of extracellular fluid status

Fluid status was assessed by means of BIA (InBody 720 Body

Composition Analysis; BioSpace, Seoul, Korea). A direct segmental multi-frequency BIA method was used with the tetra-polar eight-point tactile electrode system, with 30 impedance measurements obtained by using six frequencies (1, 5, 50, 250, 500, and 1000 kHz) at each of five segments (right arm, left arm, trunk, right leg, and left leg), and reactance was measured with 15 impedance measurements by using three frequencies (5, 50, and 250 kHz) at each of the five segments. Briefly, BIA measures the total body water (TBW) and extracellular water (ECW) using the mechanism that high frequency passes through cell membrane and low frequency does not pass, and intracellular water (ICW) is calculated. Because excess ECW results in edema, ECF status was defined as the ECW-to-TBW ratio (ECW/TBW), and the ECW/TBW ≥0.400 was defined as an overt ECF excess state (Biospace Co. Ltd., Seoul, Korea).[22] All measurements were performed by trained staff in accordance with the manufacturer's recommendations.

5. Assessment of coronary artery calcification

CAC was defined using the CACS as measured by a 320-row computed tomography system (Aquilion ONE; Toshiba Medical Systems, Tokyo, Japan). This system allows images to be reconstructed from a single cardiac phase by collecting images during a single breath in subjects in the supine position on the table. Dual scanograms were used for planning the examination and determining the anatomical range. A non-enhanced

prospective electrocardiogram-gated scan was performed to measure the CACS with the following parameters: rotation time, 275 ms; slice collimation, 0.5 mm; slice width, 3.0 mm; tube voltage, 100 kV; and automatic tube current modulation (SURE Exposure 3D standard; Toshiba Medical Systems Corporation, Otawara, Japan). Images were analyzed in a core workstation with dedicated software (V.4.4.11.82.3430. Beta;TeraRecon, Foster City, CA, USA). Agatston calcium scores were calculated to quantify the extent of CAC. A total CACS of 0 was defined as no CAC, 1–399 as subclinical CAC, and ≥400 as clinically evident CAC.[23,24]

6. Evaluation of arterial stiffness

The arterial stiffness was assessed by carotid to femoral pulse wave velocity (PWV) and PWV was measured by using the SphygmoCor system (AtCor Medical, Sydney, Australia) in this study. SphygmoCor simultaneously records carotid pulse wave and electrocardiogram and then records femoral pulse wave and electrocardiogram. The transit time between carotid and femoral pressure waves was calculated using the foot-to-foot method. PWV is calculated from measurements of pulse transit time and distance traveled by the pulse wave. The distance traveled by the pulse wave was calculated automatically as the difference between the two distances, that is subtracting the sternal notch-right carotid site from right femoral site-sternal notch distances.[25] The PWV of more than 10 m/sec was defined as having

arterial stiffness.[26]

7. Statistical analyses

Baseline characteristics were analyzed according to the quartiles of ECW/TBW. Depending on their distribution, continuous parameters are either presented as means ± standard deviation for normally distributed variables or medians with interquartile range for skewed variables. Categorical data are expressed as numbers of participants and percentages. The normality of distribution was ascertained by using the Kolmogorov-Smirnov test, and skewed continuous parameters were logarithmically transformed before use in parametric procedures. To compare the differences among the quartiles of ECW/TBW, analysis of variance (ANOVA) test, or Kruskal-Wallis test were used for continuous variables, and the chi-square test for categorical variables. Multiple linear regression analysis was performed to identify factors independent correlates of ECW/TBW. Multiple logistic regression analysis was conducted to evaluation the independent association of ECW/TBW and CAC. Incremental adjustments were performed. Model 1 included demographic characteristics such as age, sex, waist to hip ratio, hypertension, diabetes, history of CV disease and smoking status, and pulse pressure, model 2 added eGFR to model 1, model 3 inserted biochemical variables including hemoglobin, albumin, lipid profiles, calcium, phosphate, and uACR to model 2, and model 4 put medications including aspirin, antiplatelet agent, statins,

and number of antihypertensive drugs. Moreover, the independent association of ECW/TBW with CAC was also evaluated by cubic splines. The predictive power of ECF status for CAC was determined by using adjusted receiver operating characteristic (ROC) curves. In addition, to estimate the effect of arterial stiffness on the association between ECF excess and CAC in patients with early stage CKD, the subjects were divided by presence of arterial stiffness (PWV $\geq$9.7 and PWV <9.7 m/sec)[27]. All analyses were performed by using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) and R package, version 3.3.2[28]. The level of statistical significance was defined as $P < 0.05$.

III. RESULTS

1. Baseline characteristics

The baseline characteristics according to the ECW/TBW are presented in **Table 1**. The mean patient age was 59.8 ± 11.3 years, 759 (51.2%) were men, and the mean eGFR was 85.0 ± 16.9 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$. Of the total subject, 1250 (84.8%), 585 (39.7%), and 130 (9.6%) had hypertension, diabetes, and the history of CV disease, respectively. And among 1481 patients, 626 (42.3%), 702 (47.4%), and 153 (10.3) patients were CKD stage 1, 2, and 3a, respectively. The mean value of ECW/TBW in total subjects was 0.384 ± 0.009, and the mean value of ECW/TBW was 0.373 ± 0.010 in the first quartile, 0.381 ± 0.002 in the second quartile, 0.387 ± 0.002 in the third quartile, and 0.394 ± 0.003 in the fourth quartile, respectively. Patients in the higher quartiles were significantly older, had higher proportion of women, a higher prevalence of diabetes and more smoking history than patients in the lower quartiles of ECW/TBW. In addition, systolic BP and pulse pressure were significantly increased according to increasing quartiles of ECW/TBW. In addition, patients group in higher quartiles of ECW/TBW had higher CACS [1^{st} to 4^{th} quartile; 0.0 (0.0 – 73.9), 9.95 (0.0 – 133.3), 12.3 (0.0 – 144.6), vs. 59.6 (0.0 – 307.3), P for trend <0.001] and higher prevalence of CAC [1^{st} to 4^{th} quartile; 23 (6.2%), 36 (9.7%), 44 (12.0%), vs. 76 (20.5%), P for trend <0.001] compared to those of a lowest quartile group. In addition,

patients with higher ECW/TBW showed significantly lower in diastolic BP and body mass index (BMI). Also, hemoglobin, total cholesterol, low-density lipoprotein (LDL), albumin, and calcium were decreased according to quartiles of ECW/TBW **(Table 1)**.

Table 1. Baseline characteristics of the subjects according to the quartiles of ECW-to-TBW ratio

	Total (n=1481)	Q1 (n=371)	Q2 (n=372)	Q3 (n=368)	Q4 (n=370)	P
Age (years)	59.8 ± 11.3	52.9 ± 11.0	58.7 ± 10.0	62.3 ± 10.2	65.5 ± 9.9	<0.001
Men (%)	759 (51.2)	296 (79.8)	214 (57.5)	146 (39.7)	103 (27.8)	<0.001
BMI (kg/m^2)	25.4 ± 3.8	26.7 ± 4.2	25.3 ± 3.4	24.6 ± 3.5	24.9 ± 3.6	<0.001
WHR	0.92 ± 0.07	0.93 ± 05	0.92 ± 0.07	0.92 ± 0.07	0.93 ± 0.07	0.005
Hypertension (%)	1250 (84.8)	318 (86.2)	311 (83.8)	306 (83.4)	315 (85.8)	0.638
Diabetes (%)	585 (39.7)	103 (28.0)	117 (31.6)	157 (42.8)	208 (56.7)	<0.001
CVD (%)	130 (9.6)	33 (9.5)	34 (10.1)	34 (10.2)	29 (8.6)	0.877
Smoking (%)	633 (42.7)	243 (65.5)	176 (47.3)	131 (35.6)	83 (22.4)	<0.001
SBP (mmHg)	128.1 ± 13.1	126.4 ± 11.1	127.2 ± 13.2	128.3 ± 14.0	130.0 ± 14.0	0.002
DBP (mmHg)	77.3 ± 8.1	78.7 ± 7.6	77.8 ± 7.4	76.7 ± 7.7	76.1 ± 9.5	<0.001
PP (mmHg)	50.9 ± 9.8	47.7 ± 7.3	49.5 ± 9.8	52.0 ± 9.9	54.4 ± 10.6	<0.001
CACS [a]	16 (0 – 163)	0 (0 – 74)	10 (0 – 133)	12 (0 – 145)	60 (0 – 307)	<0.001
CAC (%)	179 (12.1)	23 (6.2)	36 (9.7)	44 (12.0)	76 (20.5)	<0.001
Laboratory findings						
WBC (1000 cells/μL)	6.78 ± 1.91	7.13 ± 1.82	6.60 ± 1.92	6.65 ± 1.83	6.75 ± 2.02	0.001
Hemoglobin (g/dL)	13.9 ± 1.8	15.0 ± 1.4	14.2 ± 1.6	13.5 ± 1.6	12.8 ± 1.7	<0.001

eGFR (mL/min/ 1.73 m^2)	85.0 ± 16.9	87.6 ± 18.1	86.1 ± 17.0	85.8 ± 15.7	80.4 ± 15.8	<0.001
Creatinine (mg/dL) [a]	0.9 (0.7 – 1.1)	1.0 (0.8 – 1.1)	0.9 (0.7 – 1.2)	0.8 (0.7 – 1.1)	0.9 (0.7 – 1.3)	<0.001
Total cholesterol (mg/dL)	174.9 ± 37.6	182.6 ± 42.6	174.4 ± 36.6	172.6 ± 33.7	169.7 ± 35.5	<0.001
LDL (mg/dL)	96.3 ± 30.4	101.9 ± 32.3	96.1 ± 30.6	95.5 ± 29.2	91.8 ± 28.8	<0.001
HDL (mg/dL)	50.3 ± 13.2	48.5 ± 12.2	50.7 ± 13.6	51.3 ± 13.3	50.5 ± 13.3	0.030
Triglyceride (mg/dL)	139.7 ± 81.7	161.5 ± 97.0	141.1 ± 83.1	127.8 ± 69.3	128.6 ± 70.2	<0.001
Albumin (g/dL)	4.28 ± 0.29	4.37 ± 0.26	4.30 ± 0.26	4.26 ± 0.27	4.18 ± 0.32	<0.001
Calcium (mg/dL)	9.19 ± 0.41	9.25 ± 0.35	9.22 ± 0.45	9.16 ± 0.40	9.13 ± 0.45	<0.001
Phosphate (mg/dL)	3.60 ± 0.51	3.50 ± 0.47	3.58 ± 0.52	3.63 ± 0.50	3.70 ± 0.54	<0.001
uACR [a]	3.0 (0.9 – 23.8)	2.7 (0.8 – 24.2)	2.9 (0.9 – 25.7)	2.6 (0.8 – 18.4)	4.1 (1.1 – 27.0)	0.217
Medications						
Aspirin (%)	381 (25.7)	104 (28.0)	94 (25.3)	86 (23.4)	97 (26.2)	0.534
Antiplatelet (%)	266 (18.0)	49 (13.2)	59 (15.9)	78 (21.2)	80 (21.6)	0.005
Statins (%)	790 (53.3)	185 (49.9)	188 (50.5)	199 (54.1)	218 (58.9)	0.053
Anti-HTN drug (%) [b]	1140 (77.0)	287 (77.4)	288 (77.4)	276 (75.0)	289 (78.1)	0.765
Number of anti-HTN drugs [a]	1 (1 – 2)	1 (1 – 2)	1 (1 – 2)	1 (1 – 2)	1 (1 – 2)	0.597
Diuretics (%)	340 (23.0)	84 (22.6)	91 (24.5)	75 (20.4)	90 (24.3)	0.518
ECW/TBW	0.384 ± 0.009	0.373 ± 0.010	0.381 ± 0.002	0.387 ± 0.002	0.394 ± 0.003	<0.001

***Note*:** [a] Mann-Whitney U test, [b] Antihypertensive drugs included angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, renin inhibitor, calcium channel blocker, β-blocker, vasodilator, and α-blocker, [c] RAAS blockade included angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, and renin inhibitor.

***Abbreviation*:** ECW, extracellular water; TBW, total body water; WHR, waist to hip ratio; PP, pulse pressure; CKD, chronic kidney disease; CACS, coronary artery calcium score; CAC, coronary artery calcification; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein; HDL, high density lipoprotein; uACR, urine albumin to creatinine ratio; HTN, hypertension

2. Factors associated with ECW-to-TBW ratio

The relationships between ECW/TBW and clinical and biochemical variables are explored using univariate and multivariable linear regression analyses, which are presented in **Table 2**. In multivariate linear regression after adjustment for confounders, age, women, and prevalence of diabetes and use of antiplatelet agent increased with increasing quartiles of ECW/TBW. On the other hand, BMI and serum hemoglobin and albumin levels decreased with increasing quartiles of ECW/TBW.

Table 2. Univariate and multivariate linear regression analyses of factors related to the ECW-to-TBW ratio

	Univariate		Multivariate 1		Multivariate 2		Multivariate 3	
	β	**P**	**β**	**P**	**β**	**P**	**β**	**P**
Age (per 1 year)	0.028	<0.001	0.023	<0.001	0.024	<0.001	0.024	<0.001
Men (vs. Women)	-0.586	<0.001	-0.463	<0.001	-0.275	0.003	-0.262	0.005
BMI (per 1 kg/m^2)	-0.039	<0.001	-0.042	<0.001	-0.028	0.001	-0.028	0.001
WHR (per 0.01)	0.005	0.182						
Hypertension	0.025	0.711	-0.063	0.318	-0.095	0.190	-0.090	0.231
Diabetes	0.356	<0.001	0.231	<0.001	0.204	<0.001	0.184	0.002
CVD	0.016	0.834	-0.134	0.082	-0.129	0.159	-0.140	0.132
Smoking history	0.806	<0.001	-0.051	0.456	-0.006	0.935	-0.013	0.869
SBP (per 1 mmHg)	0.005	0.025						
DBP (per 1 mmHg)	-0.014	<0.001						
PP (per 1 mmHg)	0.019	<0.001	0.007	0.003	0.006	0.053	0.006	0.059
Laboratory findings								
eGFR (per 1 mL/min/1.73 m^2)	-0.007	<0.001			0.001	0.482	0.001	0.470
Creatinine (per 1 log) [a]	0.916	0.045						
WBC (per 1000 cells/μL)	-0.035	0.008			0.018	0.203	0.019	0.193
Hb (per 1 g/dL)	-0.202	<0.001			-0.097	<0.001	-0.097	<0.001

T. cholesterol (per 1 mg/dL)	-0.003	<0.001	-0.002	0.396	-0.002	0.388
HDL (per 1 mg/dL)	0.003	0.095	0.004	0.769	0.004	0.184
LDL (per 1 mg/dL)	-0.003	<0.001	0.001	0.175	0.001	0.706
Triglyceride (per 1 mg/dL)	-0.001	<0.001	0.000	0.937	0.000	0.898
Albumin (per 1 g/dL)	-0.659	<0.001	-0.284	0.010	-0.288	0.009
Calcium (per 1 mg/dL)	-0.188	0.001	-0.056	0.401	-0.049	0.463
Phosphate (per 1 mg/dL)	0.221	<0.001	-0.104	0.060	-0.093	0.094
uACR (per 1og) [a]	0.024	0.378	0.024	0.498	0.022	0.538
Medications						
Aspirin	-0.056	0.312			-0.057	0.393
Antiplatelet	0.207	0.001			0.168	0.025
Statins	0.081	0.094			0.040	0.463
Anti-HTN drug	0.025	0.659				
Number of anti-HTN drugs [a]	0.107	0.664			-0.159	0.667
Diuretics	0.033	0.563			0.026	0.748

***Note*:** [a] log transformed

Abbreviations: ECW, extracellular water; TBW, total body water ratio; BMI, body mass index; WHR, waist to hip ratio; CVD, history of cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; WBC, white blood cell; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein; HDL, high density lipoprotein; uACR, urine albumin to creatinine ratio; HTN, hypertension

3. Independent association between ECW-to-TBW ratio and CAC

ECW/TBW was significantly associated with the risk of CAC in the univariate logistic regression analysis [Odds ratio (OR) = 1.931, 95% confidential interval (95% CI) = 1.545 – 2.412, P <0.001]. In addition, age, sex, waist to hip ratio, diabetes, history of CV disease and smoking, pulse pressure, eGFR, lipid profiles, serum calcium levels, the use of aspirin, antiplatelet agents, and statins, and total number of antihypertensive drug were also associated with the presence of CAC **(Table 3)**. Even after incrementally adjusting the demographic characteristics and laboratory variables from model 1 to model 4, ECF status had an independent association with CAC (model 1: OR = 2.045, 95% CI = 1.476 – 2.833, P <0.001; model 2: OR = 1.997, 95% CI = 1.441 – 2.767, P <0.001; model 3: OR = 2.872, 95% CI = 1.781 – 4.632, P <0.001; model 4: OR = 2.890, 95% CI = 1.787 – 4.674, P <0.001).

Cubic spline curve showed that the risk of CAC increased steadily with higher ECW/TBW. **Figure 2-A** showed that the crude ORs for the risk of CAC was increased from approximately 0.385 of ECW/TBW. After adjustment for confounding factors such as age, sex, waist to hip ratio, hypertension, diabetes, history of CV disease and smoking, pulse pressure, eGFR, hemoglobin, albumin, lipid profiles, proteinuria, medication history of aspirin, antiplatelet agent, and statins, and total number of antihypertensive drugs, the cubic spline curve

demonstrated a similar trend that the risk of CAC increased along with the ratio of ECW/TBW increase above 0.385 **(Figure 2-B)**.

Table 3. Logistic regression analyses of ECW-to-TBW ratio for the presence of CAC

	Univariate		Model 1		Model 2		Model 3		Model 4	
	OR	**P**	**OR**	**P**	**OR**	**P**	**OR**	**P**	**OR**	**P**
Age (per 1 year)	1.051	<0.001	1.031	0.007	1.028	0.020	1.029	0.067	1.027	0.100
Men (vs. Women)	1.995	<0.001	2.917	<0.001	2.822	0.001	3.956	0.002	3.820	0.003
BMI (per 1 kg/m^2)	0.983	0.428								
WHR (per 0.01)	1.041	0.001	0.996	0.788	0.994	0.717	0.991	0.662	0.989	0.613
Hypertension	1.0561	0.080	1.283	0.398	1.231	0.484	1.315	0.461	1.185	0.656
Diabetes	2.143	<0.001	1.872	0.004	1.916	0.003	1.874	0.028	1.816	0.039
CVD	1.801	0.016	1.575	0.120	1.633	0.095	1.841	0.100	1.640	0.192
Smoking history	1.691	0.001	0.940	0.831	0.908	0.737	0.770	0.472	0.767	0.469
ECW/TBW (per 0.01)	1.931	<0.001	2.045	<0.001	1.997	<0.001	2.872	<0.001	2.890	<0.001
SBP (per 1 mmHg)	1.013	0.048								
DBP (per 1 mmHg)	1.002	0.870								
PP (per 1 mmHg)	1.021	0.016	0.998	0.832	0.977	0.779	1.002	0.901	1.001	0.944
Laboratory findings										
WBC (1000 cells/μL)	1.029	0.502								
Hemoglobin (per 1 g/dL)	0.926	0.091					1.009	0.926	1.011	0.911
eGFR (per 1 mL/min/1.73 m^2)	0.975	<0.001			0.992	0.224	0.996	0.659	0.996	0.684
Creatinine (per 1 log) [a]	7.286	0.137								
Total cholesterol (per 1 mg/dL)	0.990	<0.001					1.009	0.252	1.009	0.249

LDL (per 1 mg/dL)	0.988	<0.001	0.981	0.031	0.981	0.034
HDL (per 1 mg/dL)	0.990	0.139	1.001	0.928	1.003	0.811
Triglyceride (per 1 mg/dL)	0.999	0.486	0.999	0.926	1.000	0.831
Albumin (per 1 g/dL)	0.668	0.138	0.984	0.975	0.940	0.904
Calcium (per 1 mg/dL)	1.472	0.039	1.754	0.074	1.831	0.058
Phosphate (per 1 mg/dL)	0.802	0.162	1.014	0.957	0.993	0.980
uACR (per 1og) [a]	0.973	0.786	1.020	0.909	1.010	0.953
Medications						
Aspirin	1.830	<0.001			1.272	0.397
Antiplatelet	1.701	0.004			1.493	0.171
Statins	1.383	0.046			1.065	0.814
Anti-HTN drug	1.264	0.240				
Number of anti-HTN drugs (per 1 log) [a]	5.827	0.028			2.372	0.507
Diuretics	1.146	0.459				

Note: [a] Log transformed
Model 1: adjusted for age, sex, waist to hip ratio, hypertension, diabetes, history of cardiovascular disease and smoking and pulse pressure
Model 2: Model 1 + eGFR
Model 3: Model 2 + hemoglobin, albumin, lipid profiles, calcium, phosphate, and urine albumin to creatinine ratio[a]
Model 4: Model 3 + medication of aspirin, antiplatelet agent, and statins, and number of anti-HTN drugs[a]
Abbreviations: ECW, extracellular water; TBW, total body water ratio; CAC, coronary artery calcification; OR, odds ratio; BMI, body mass index; WHR, waist to hip ratio; CVD, history of cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; WBC, white blood cell; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein; HDL, high density lipoprotein; uACR, urine albumin to creatinine ratio; HTN, hypertension

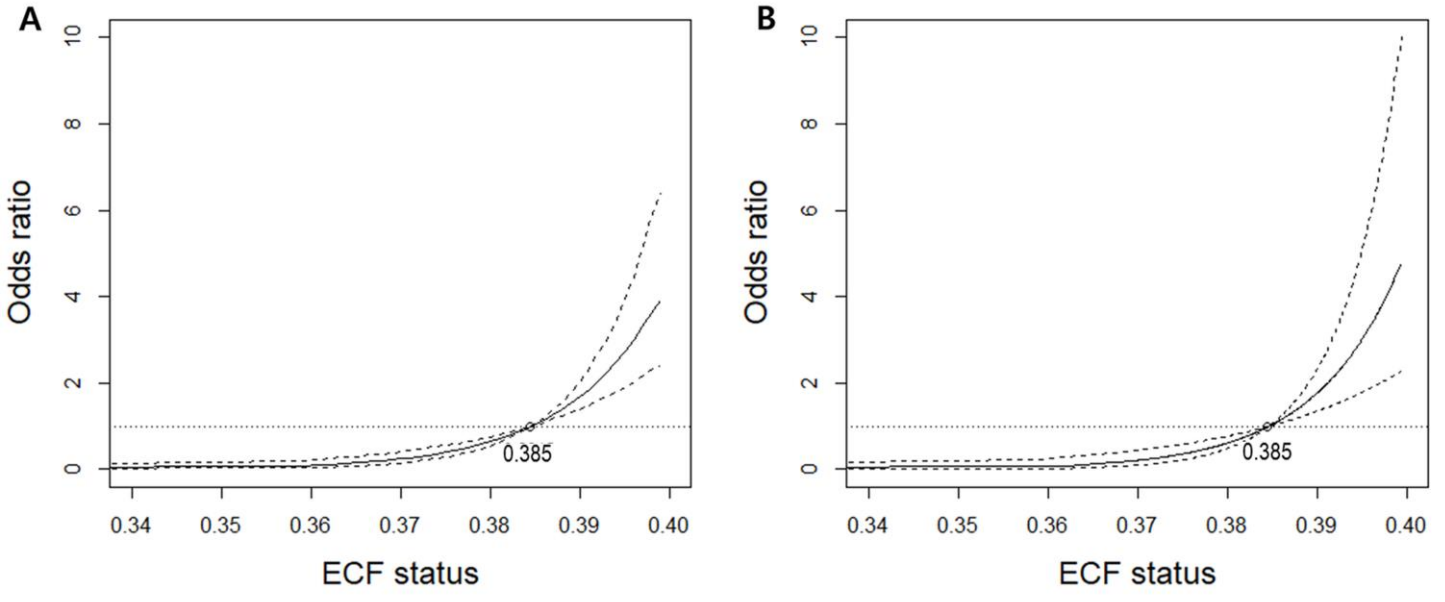

Figure 2. Cubic spline plot of ECW-to-TBW ratio on the risk of CAC

The graph shows the odds ratio of CAC with ECW/TBW as a spline curve in the unadjusted (A) and fully adjusted model (B). In the fully adjusted model, the odds ratio was derived after adjustment for age, sex, waist to hip ratio, hypertension, diabetes, history of CVD and smoking, pulse pressure, estimated glomerular filtration rate, hemoglobin, albumin, lipid profiles, calcium, phosphate, log transformed urine albumin to creatinine ratio, medication history of aspirin, antiplatelet agent, and

statins, and log transformed total number of antihypertensive drugs. The odds ratio of CAC was greater than 1 when the ECW/TBW was greater than about 0.385 in both the unadjusted and adjusted model. The dotted line represents the 95% confidence interval. ***Abbreviations:*** ECW, extracellular water, TBW, total body water; CAC, coronary artery calcification; CVD, cardiovascular disease

4. Predicting ability of ECW-to-TBW ratio for CAC

I further explored that ECF status improved the risk predictability of CAC compared to traditional and non-traditional factors related to prevalence of CAC. To confirm the additional predictability for the risk of CAC, three multivariate logistic regression models were conducted; Model A was composed of traditional risk factors for CAC, model B is organized by adding the non-traditional risk factors to the model A, and model C included in ECW/TBW to model B. Traditional factors were age, sex, waist to hip ratio, history of hypertension, diabetes and CV disease, smoking history and lipid profiles. And non-traditional factors consisted of factors such as eGFR, high-sensitivity C-reactive protein (hs-CRP), serum calcium and phosphate levels, urine albumin to creatinine ratio, and medication histories of aspirin, antiplatelet agent or statins. In the c-statistical analysis, adding ECW/TBW to traditional risk factors significantly increased the area under the ROC curve (AUC) for CAC as compared with model A (0.784 vs. 0.736, P = 0.013). The AUC was also significantly increased when ECW/TBW was added to model B (0.784 vs. 0.749, P = 0.034). However, in the post-hoc analysis using the Bonferroni correction method, the significance remained only in comparison model C and model A. The addition of non-traditional factors to traditional factors and traditional factors only did not show any difference in predictive power (0.749 vs. 0.736, P = 0.247). **(Figure 3, Table S4)**

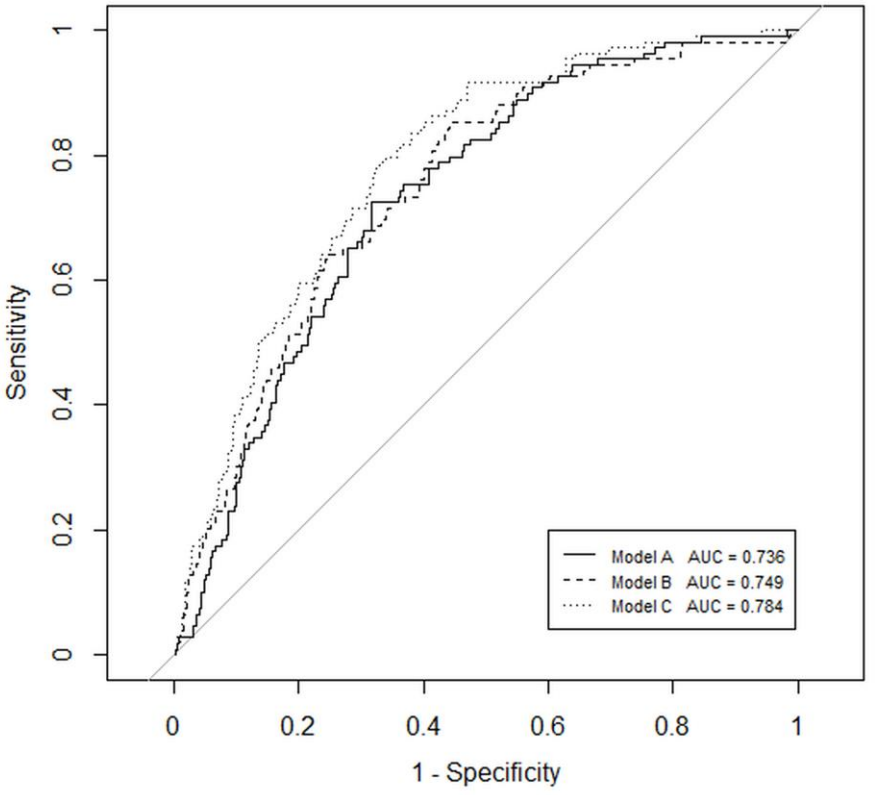

Figure 3. ROC analysis showing improvement of CAC risk prediction by ECW-to-TBW ratio

Model A included conventional factors such as age, sex, waist to hip ratio, history of hypertension, diabetes and cardiovascular disease, smoking history and lipid profiles. Model B included Model A and non-traditional factors such as estimated glomerular filtration rate, hs-CRP, serum calcium and phosphate levels, urine albumin to creatinine ratio, and medication histories of aspirin, antiplatelet agent or statins. Model C included Model B and ECW/TBW. Model C showed a statistically significant higher the area under the ROC curve (AUC) than Model A and B, and post-hoc adjusted p-value obtained using the Bonferroni correction method was statistically significant only in Model C and Model A. ***Abbreviations:*** ROC, receiver operating characteristics; CAC, coronary artery calcification; ECW, extracellular water, TBW, total body water; hs-CRP, high-sensitivity C-reactive protein

Table 4. ROC analysis showing improvement of CAC risk prediction by ECW-to-TBW ratio

	AUC (95% CI)	raw p-value for differences	
		Model A	Model B
Model A	0.736 (0.690-0.780)	-	
Model B	0.749 (0.703-0.795)	0.247	-
Model C	0.784 (0.743-0.826)	0.013*	0.038

Note: * Post-hoc adjusted P-value is 0.039 that is obtained by the Bonferroni correction method

Model A included conventional factors such as age, sex, waist to hip ratio, history of hypertension, diabetes and cardiovascular disease, smoking history and lipid profiles. Model B included Model A and non-traditional factors such as estimated glomerular filtration rate, hs-CRP, serum calcium and phosphate levels, urine albumin to creatinine ratio, and medication histories of aspirin, antiplatelet agent or statins. Model C included Model B and ECW/TBW.

Abbreviations: ROC, receiver operating characteristics; CAC, coronary artery calcification; ECW, extracellular water, TBW, total body water; hs-CRP, high-sensitivity C-reactive protein; AUC, the area under the ROC curve

5. Relationship arterial stiffness and ECW-to-TBW ratio with CAC

To investigate the effect of arterial stiffness on the risk of CAC according to the ECF status, analyses was conducted by dividing PWV into two categories; normal PWV (PWV ≤10 m/sec) and high PWV (PWV >10 m/sec). In all patients, the risk of CAC increased significantly in the higher quartiles compared to the first quartile of ECW/TBW, and the odds ratio also tended to increase as the quartiles ***(Figure 4-A)***. As shown in ***Figure 4-B***, in the normal PWV group, fully adjusted odds ratios indicating the risk of CAC rose upward rapidly with increasing quartiles of ECW/TBW compared to 1st quartile (compared to the 1st quartile, 2nd quartile: OR = 3.598, 95% CI = 0.885 – 18.590, P = 0.090; 3rd quartile: OR = 5.787, 95% CI = 1.325 – 32.468, P = 0.028; and 4th quartile, OR = 27.223, 95% CI = 6.116 – 161.583, P <0.001). In high PWV group, however, there was no significant relationship between ECF status and CAC in any quartiles. In other words, the relationship between CAC and ECW/TBW was disappeared in the presence of arterial stiffness. ***(Figure 4, Table 5)***

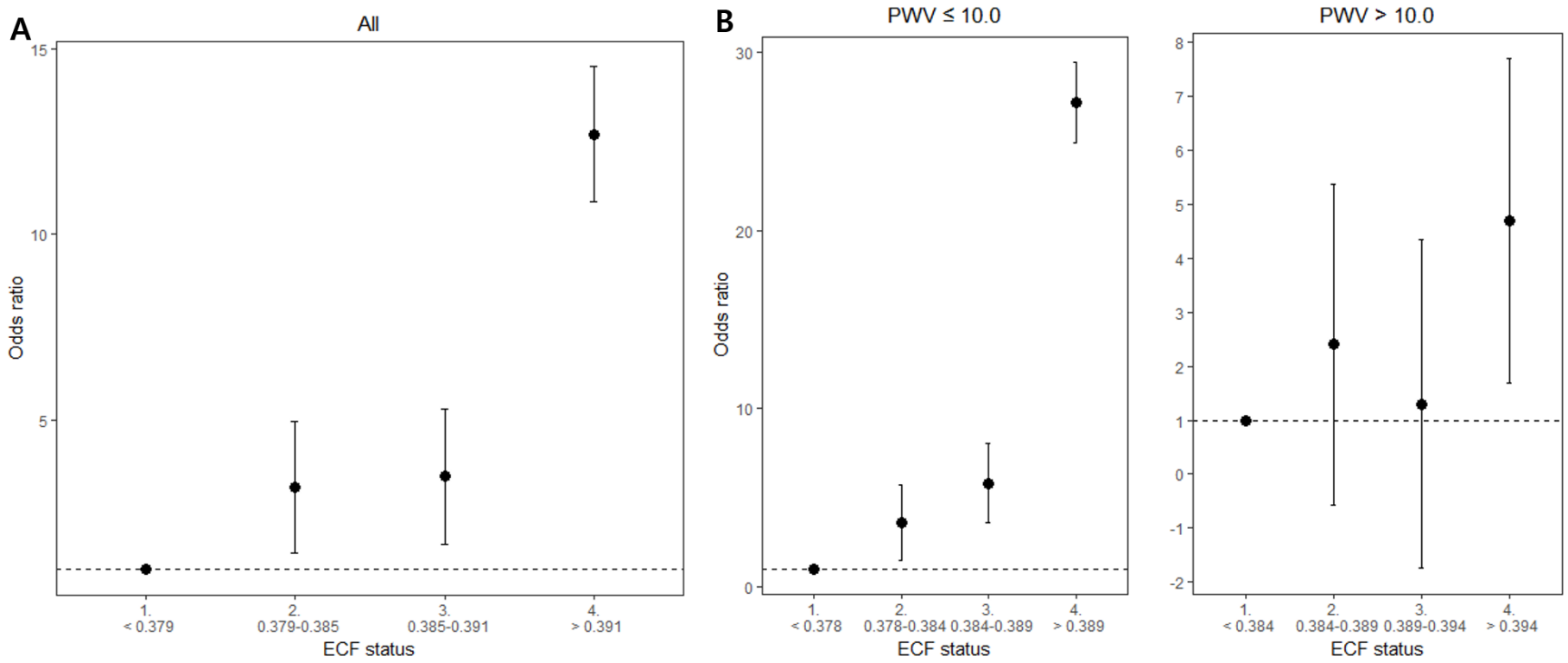

Figure 4. Forest plot showing the effect of arterial stiffness on the relationship between ECW-to-TBW ratio and CAC

It is graphic presentations of fully adjusted ORs and standard errors for the presence of CAC for each quartile of ECW/TBW (A) and the effect of arterial stiffness on the relationship between CAC and ECW/TBW (B). Age, sex, waist to hip ratio, hypertension, diabetes, history of cardiovascular disease and smoking, pulse pressure, estimated glomerular filtration rate, serum hemoglobin, albumin levels, lipid profiles, calcium, phosphate, log transformed urine albumin to creatinine ratio, medication history of aspirin, antiplatelet agent, and statin, and log transformed total number of antihypertensive drugs were adjusted for this analysis and arterial stiffness was defined as PWV >10 m/sec. **Abbreviations:** ECW, extracellular water, TBW, total body water; CAC, coronary artery calcification; PWV, pulse wave velocity; CVD, cardiovascular disease

Table 5. Adjusted association between ECW-to-TBW ratio and CAC in patients with and without arterial stiffness by quartiles of ECW-to-TBW ratio

	All		Central PWV ≤10 m/sec		Central PWV >10 m/sec	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
Q1	reference	-	reference	-	reference	-
Q2	3.214 (1.115 – 10.810)	0.040	3.598 (0.885 – 18.590)	0.090	2.350 (0.313 – 25.754)	0.433
Q3	3.458 (1.138 – 12.206)	0.038	5.787 (1.325 – 32.468)	0.028	1.349 (0.171 – 15.405)	0.788
Q4	12.696 (4.201 – 45.486)	<0.001	27.223 (6.116 – 161.583)	<0.001	4.739 (0.631 – 52.941)	0.157

Note: Adjustment for age, sex, waist to hip ratio, hypertension, diabetes, history of cardiovascular disease and smoking, pulse pressure, estimated glomerular filtration rate, serum hemoglobin, albumin levels, lipid profiles, calcium, phosphate, log transformed urine albumin to creatinine ratio, medication history of aspirin, antiplatelet agent, and statin, and log transformed total number of antihypertensive drugs

Abbreviations: ECW, extracellular water, TBW, total body water; CAC, coronary artery calcification; PWV, pulse wave velocity; OR, odds ratio; CI, confidential interval

IV. DISCUSSION

This study demonstrated that ECF status, even subclinical fluid overload was closely associated with the CAC in patients with early stage CKD. Furthermore, adding subclinical ECF status to traditional and non-traditional risk factors for CV disease increased the predictive power of CAC risk in patients with early stage CKD.

CV disease is a major cause of adverse outcomes in patients with CKD, and reduced renal function itself has been regarded as an important CV risk.[29] In addition, Framingham Heart Study has reported that CV diseases also increased in the early stages of CKD, and a recent report form HOPE study reaffirmed this interactional association.[30,31] The increase in CV disease in patients with early-stage CKD was not fully accounted for by traditional factors alone including age, sex, obesity, hypertension, diabetes, and hyperlipidemia, and was not preventable through the modification of these traditional risk factors.[32,33] Although non-traditional risk factors such as low GFR, inflammation, abnormal metabolism in calcium and phosphate, and albuminuria were also considered as an increasing risk for CV disease, early CKD patients with relatively preserved renal function might be less influenced by these non-traditional risk factors for CV disease.[34-37]. For example, serum calcium and phosphate levels were relatively stable until eGFR was <20 mL/min/1.73 m^2 and intact parathyroid hormone levels began to rise at eGFR levels approximately less than 45

mL/min/1.73 m^2. Unlike other non-traditional factors, this study demonstrated that ECF excess, which was able to accompany with renal impairment, was correlated with CV risk and increased the predictive power of CAC risk in CKD patients. Chronic inflammation mediated by fluid excess was considered preferentially as a potential contributing mechanism for CAC. Recently, Benz K *et al*. reported that calcification of aortic media was prevalent in patients with early stages CKD as well as advanced CKD without elevation of serum calcium-phosphate product. Instead of abnormal mineral metabolism, they observed that proinflammatory molecules such as CD40, CRP, and CD154 were locally up-regulated in calcification site of aortic media.[40] It has been known that ECF excess stimulates systemic immune activation, oxidative stress, and cytokine production through translocation of endotoxin by alteration of gut permeability [41] or through direct damage to vascular endothelial cells via mechanical stretch by increasing hydrostatic pressure. [42] Thus, it assumes that subclinical fluid excess contributes to CAC by provoking inflammatory responses in vascular wall of coronary artery.

Another potential mechanism of association between CAC and ECF excess is the phenotypical alteration of contractile vascular smooth muscle cells (VSMCs) to proliferative and osteogenic VSMCs. VSMCs can be converted to osteoblastic cells in response to multiple stimuli such as bone morphogenic proteins, osteopontin, and osteoprogerin, which are induced by chronic pressure overload. [43-45] Thus, we assume that ECF excess induced vascular stretch and

VSMCs activation, eventually leading to vascular calcification in overhydrated patients with CKD. Lastly, activation of RAAS in a vicious cycle with ECF excess can contribute to CAC. In this study, we identify that ECF status and the use of RAAS blockade had negative correlations. According to previous studies, even in an early stage nephropathy, intra-renal RAAS system was activated. [46] Chronic fluid excess triggered by intra-renal RAAS leads to persistent activation of systemic and intra-renal RAAS. Recently, RAAS was known as involving pro-calcified effects, such as promoting osteoinductive signaling, oxidative stress, inflammation, and other multiple signaling pathways.[47] In addition, some studies demonstrated that RAAS blocking agents reduced arterial medial calcification and their mechanisms. [48,49] Therefore, we regarded that activation of RAAS related to fluid excess can contribute to CAC.

In many studies, fluid excess, CAC, and arterial stiffness have been reported as risk factors for CV events, and there has been controversy over causality between these factors. Arterial stiffness, vascular calcification, and fluid excess occur as a consequence of a complex interplay independently or inter-dependently. [50-53] One of the interesting results in this study was that in patients with stiffed arteries, the relationship between fluid overload and vascular calcification was lost. Fluid excess is closely associated with CACS in patients with low PWV, meanwhile, mutual association between CACS and fluid overload is lost in patients with stiff artery (data not shown). In addition, arterial stiffness assessed by PWV is also related with CACS in this population.

These findings suggest that increased arterial stiffness and fluid overload are the risk factors for CAC and both of fluid overload and arterial stiffness are reciprocally associated with increased CACS in CKD patients. However, even in definite association of PWV and fluid overload with CACS, causal-relationship among these factors should be elucidated in future.

This study has distinct strengths. This study is the first study to identify that ECF excess as a non-traditional factor is associated with CAC in patients with early state CKD. In addition, this study was conducted in a prospective and large-scale cohort study that enrolled approximately 1500 patients. All measurements including fluid status, CACS, PWV, and blood pressure were taken in almost all patients by the well-trained measurer with a standardized protocol. In estimating arterial stiffness, carotid to femoral PWV was used to evaluate the aortic stiffness directly.[31] However, this study has several limitations. First, the possibility that existing co-morbid conditions including underlying diseases may affect the relationship between fluid status and vascular calcification cannot be completely ruled out because of the use of cohort data for high-risk patients with CV disease. Second, there is a debate as to whether CAC measured by CACS in patients with CKD could predict CV risk. In severe studies, medial calcification also occurs in patients with impaired renal function in contrast to the general population, thus CACS are less accurate due to the inability of calcium scoring to separate out intimal calcification from medial arteriosclerosis.[54,55] However, recent studies report a significant

association between CACS and CV outcome in patients with renal impairment, and it is clear that both vascular calcification of intima and media are associated with CV risk even though CACS does not differentiate between intima and media calcification. [56-58] Therefore, the high CACS in this study could be considered as s marker of CAC and CV risk. Last, this study was performed as an observational study, therefore any results regarding whether regulation of ECF status can reduce CV risk could not be included. This suggests that interventional studies are needed to confirm that the control of fluid status can be a treatment method to reduce CV risk in patients with early stage CKD.

V. CONCLUSION

In conclusion, subclinical fluid excess even in early CKD patients with preserved renal function was associated with the increasing risk of CAC. This result suggests that in addition to the known traditional and non-traditional factors, the assessment of ECF status can help to determine CV risk and also contribute to develop treatment strategies in patients with early stage CKD.

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ABSTRACT (IN KOREAN)

초기 단계의 만성신부전 환자에게 나타나는 무증상 (subclinical)의 수분 과부하와 관상동맥 석회화 사이의 연관성 고찰

<지도교수 유태현>

연세대학교 대학원 의학과

박서현

배경:

심혈관 질환은 투석 환자의 빈번한 동반 질환이며 주요한 사망원인이다. 진행된 만성신부전 환자에게서 수분 과부하는 자주 발생하는 문제이며, 심혈관 질환을 예측하는 독립 인자로 알려져 있다. 그러나, 초기 단계의 신부전 환자에서 수분 과부하와 심혈관 질환 사이의 연관성에 대한 연구는 한정되어 있다. 따라서 이번 연구에서는 비교적 신기능이 보존된 만성신부전 환자를 대상으로 임피던스로 측정한 세포 외 수분상태와 심혈관 질환의 표지자인 관상동맥 칼슘 점수 (CACS)사이의 연관성을 고찰하고자 하였다.

방법:

이번 연구는 심혈관 질환의 고 위험 군으로 구성된 CMERC-HI (Cardiovascular and Metabolic Disease Etiology Research Center-High Risk, NCT02003781) 코호트에서 명백한 수분 과부하가 없는 만성 신부전 1기에서 3a기에 해당 하는

환자 총 1, 481명을 대상으로 하였다. 수분 상태는 임피던스 분석기로 측정하였고, CACS는 다중절편 방사선 단층촬영 영상을 이용하여 측정하였다. 수분상태는 세포 외 수분양을 총 수분양으로 보정한 값으로 정의하였고, 대상자를 수분상태에 따라 사분위로 나누어 분석했다. 관상동맥 석화화는 관상동맥 칼슘 점수가 400 agatston unit 이상일 때로 정의하였다.

결과:

전체 대상자의 평균 연령은 59.8세 였고 남성이 51.2% 였으며, 평균 사구체여과율은 85.0 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ 였다. 수분상태로 정의한 사분위가 증가 할수록, 연령과 혈압이 증가하였고 당뇨, 고혈압, 및 심혈관 질환의 유병율이 높았다. 또한 사분위에 따라 관상동맥 칼슘 점수가 증가하였고, 관상동맥 석회화 유병율이 증가하였다. 여러 혼란 변수들을 단계 별로 보정하여 로지스틱 회귀분석을 시행한 결과에서, 수분 상태는 관상동맥 석회화와 독립적으로 연관되어 있었다. ROC (receiver operating characteristic) 분석에서, 관상동맥 석회화의 기존 위험인자들에 수분 상태를 추가한 모델에서 ROC 곡선의 면적이 통계적으로 유의하게 증가하였다. 경동맥과 대퇴동맥 사이 맥파 전파 속도가 10 m/sec 이상일 경우로 정의한 동맥 경직이 있을 경우 이러한 수분 상태와 관상동맥 석회화 사이의 연관성은 소실 되었다.

결론:

신기능이 비교적 보존되어 있는 초기 만성 신부전 환자에서도 무증상의 수분 과부하는 유의하게 관상동맥 석회화의 위험성을 높였다. 따라서 초기 단계의 만성 신부전 환자군에서 수분상태를 평가하는 것이 심혈관 질환의 예측에 도움이 될 수 있다.

핵심되는 말: 수분 과부하, 관상동맥 석화화, 만성 신부전