

ORIGINAL ARTICLE

Time-updated anion gap and cardiovascular events in advanced chronic kidney disease: a cohort study

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ABSTRACT

Background. Studies examining associations between metabolic acidosis and cardiovascular events in chronic kidney disease (CKD) have shown conflicting results and have not differentiated between normal anion gap (hyperchloremic) acidosis and high anion gap acidosis. We aimed to examine the impact of normal and high anion gap acidosis, separately, on the risk of cardiovascular events among patients with CKD.

Methods. This retrospective cohort study included 1168 patients with an estimated glomerular filtration rate (eGFR) of 10–60 mL/min/1.73 m² and available data on anion gap. We analyzed the association of time-updated high anion gap (anion gap ≥ 9.2) with the rate of cardiovascular events using marginal structural models (MSMs) to account for time-dependent confounding. We also analyzed the association between time-updated normal anion gap acidosis (anion-gap-adjusted bicarbonate level ≤ 22.8 mEq/L) and cardiovascular events.

Results. The mean baseline eGFR of the cohort was 28 mL/min/1.73 m². The prevalence rates of high anion gap in CKD stages G3a, G3b, G4 and G5 were 20%, 16%, 27% and 46%, respectively. During a median follow-up period of 2.9 years, 132 patients developed cardiovascular events (3.3/100 patient-years). In MSMs, high anion gap was associated with a higher rate of cardiovascular events [hazard ratio (HR) 1.87; 95% confidence interval (95% CI) 1.13–3.09; $P = 0.02$] and the composite of cardiovascular events or all-cause death (HR 3.28; 95% CI 2.19–4.91; $P < 0.001$). Normal anion gap acidosis was not associated with cardiovascular events (HR 0.74; 95% CI, 0.47–1.17; $P = 0.2$).

Conclusions. Among patients with advanced CKD, high anion gap was associated with an increased risk of cardiovascular events.

Keywords: anion gap, cardiovascular events, chronic kidney disease, marginal structural model, metabolic acidosis

INTRODUCTION

The kidney has a predominant role in the maintenance of acid-base homeostasis. As kidney function deteriorates, metabolic acidosis occurs primarily because of diminished ammoniagenesis in the proximal tubules [1]. Metabolic acidosis leads to various pathological conditions in chronic kidney disease (CKD), such as skeletal muscle catabolism [2], decreased bone mass [3], functional impairment [4] and progression of kidney diseases [5–9]. Additionally, metabolic acidosis may contribute to an

ness in the proximal tubules [1]. Metabolic acidosis leads to various pathological conditions in chronic kidney disease (CKD), such as skeletal muscle catabolism [2], decreased bone mass [3], functional impairment [4] and progression of kidney diseases [5–9]. Additionally, metabolic acidosis may contribute to an

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excess cardiovascular risk among patients with CKD. Acidemia may promote atherosclerosis through the inflammation of endothelial cells [10], oxidation of low-density lipoprotein [11] and upregulation of angiotensin II [12]. In a cross-sectional study of patients with CKD, an inverse correlation between serum bicarbonate levels and brachial-to-ankle pulse wave velocity was reported [13]. Furthermore, a randomized trial of patients with CKD stages G3b and G4 showed that oral sodium bicarbonate improved vascular endothelial function [14].

Nevertheless, previous cohort studies examining the association between serum bicarbonate levels and the risk of cardiovascular events have shown inconsistent results [7, 15–18] and suffered from two important limitations. First, bicarbonate levels were measured only at baseline, thus their time-series alterations typically seen during CKD progression were not considered. Second, the subtypes of metabolic acidosis, that is, normal anion gap (hyperchloremic) acidosis and high anion gap acidosis, were not distinguished. These two types of acidosis may lead to different clinical consequences because the accumulated acids are completely disparate. In particular, anion gap consists of various uremic acids that can induce peculiar cardiovascular toxicities [19]. Importantly, the treatment approach is also different for these two types of acidosis as oral sodium bicarbonate is expected to be ineffective for reducing anion gap.

Although we recently reported that time-updated high anion gap was significantly associated with an increased risk of CKD progression [20], it is currently unknown whether this is also true for the risk of cardiovascular events. In this study, we analyzed the association of the two types of metabolic acidosis with cardiovascular outcomes among patients with advanced CKD. Marginal structural models (MSMs) were used to estimate the impact of time-updated exposures on the outcomes to account for time-dependent confounders [21, 22].

MATERIALS AND METHODS

Study population

The details of the study design have been described elsewhere [20, 23]. This was a retrospective cohort study of individuals who visited the Department of Nephrology at Osaka University Hospital as outpatients between January 2009 and December 2018. We enrolled patients (i) aged 20 years or older; (ii) whose estimated glomerular filtration rate (eGFR) was 10–60 mL/min/1.73 m²; and (iii) who had no history of renal replacement therapy (RRT). In addition, patients were required to have simultaneously measured data on venous bicarbonate, serum sodium and serum chloride, which are necessary to calculate anion gap. Patients were followed up from the date of the first measurement of anion gap until the date of RRT initiation, death, the last visit to our hospital or the end of the study period (31 May 2019).

The study protocol was approved by the ethics committee of Osaka University Hospital (approval number 20274). The informed consent was waived because of the retrospective nature of the study design.

Study outcomes

The primary outcome was the first occurrence of one of the following cardiovascular events: coronary artery disease (angina pectoris or myocardial infarction) requiring percutaneous coronary intervention and/or coronary artery bypass graft; stroke (cerebral infarction and intracranial hemorrhage); hospitalization for decompensated heart failure; or cardiovascular death. Additionally, we analyzed the composite of cardiovascular

events and all-cause death. Detailed information about the outcome data were obtained from a chart review of the hospital records and carefully ascertained by nephrologists.

Exposure

The main exposure was the time-updated high anion gap, which was obtained every 3 months throughout the study period. The anion gap was calculated using the following formula: anion gap = sodium (mEq/L) – chloride (mEq/L) – bicarbonate (mEq/L).

A high anion gap was defined as an anion gap ≥ 9.2 . This cut-off point was determined based on the top 25th percentile of our cohort because the cut-off point of the anion gap is not formally determined but is laboratory specific.

Another exposure was time-updated normal anion gap (hyperchloremic) acidosis, which was also obtained every 3 months. To define normal anion gap acidosis, we first calculated the anion-gap-adjusted bicarbonate level: anion-gap-adjusted bicarbonate = bicarbonate (mEq/L) + anion gap – 9.2 (if the anion gap was ≥ 9.2).

Normal anion gap acidosis was defined as an anion-gap-adjusted bicarbonate level ≤ 22.8 mEq/L. This cut-off point was based on the 25th percentile of our cohort as well.

Covariates

The following baseline data were extracted from the medical records: age, sex, body mass index, smoking history, blood pressure, cardiothoracic ratio (CTR), diabetes mellitus and cardiovascular comorbidities (coronary artery disease, congestive heart failure, stroke, aortic disease and valvular heart disease). CTR was measured using chest radiographs.

Laboratory and prescription data were collected every 3 months during the study period using an automated data extraction system. If there were multiple data within a 3-month interval, then a datum measured on the day nearest to each of the 3-month time points was adopted. Laboratory data included serum albumin, creatinine, sodium, potassium, chloride, calcium, phosphate, uric acid, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein (CRP), hemoglobin, urinary protein-to-creatinine ratio (UPCR) and venous blood gas (pH, bicarbonate and PCO₂). Prescription data included loop diuretics, thiazide diuretics, spironolactone, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta-blockers, statins and sodium bicarbonate.

The eGFR was calculated using the following formula for the Japanese population [24]: $eGFR \text{ (mL/min/1.73 m}^2\text{)} = 194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} (\times 0.739 \text{ if female})$.

Serum calcium levels were corrected as follows using serum albumin levels if the serum albumin level was < 4.0 g/dL [25]: adjusted calcium (mg/dL) = serum calcium (mg/dL) + $0.8 \times 4 - \text{serum albumin (g/dL)}$.

Venous bicarbonate levels and pH were measured using blood gas analyzers. Blood gas measurement was performed by trained medical technicians in the central laboratory of our hospital under rigorous quality control standards [22].

Statistical analysis

Marginal structural models. The MSMs were used to analyze the association between time-updated exposures and outcomes. An MSM is a statistical method of estimating the effects of time-varying exposures on outcomes in the presence of time-varying confounders [26, 27]. In this study, eGFR was considered the main time-dependent confounder because

eGFR affects both the exposures (anion gap and bicarbonate level) and outcomes (cardiovascular events), whereas eGFR might be influenced by previous exposures (anion gap and bicarbonate level). The detailed method of MSM is described in Supplementary data, Statistical analyses.

Imputation for missing data. All missing data at baseline were imputed by multiple imputation by chained equation (MICE) using all baseline covariates. Linear regression imputation was used for continuous variables with missing data (body mass index, systolic blood pressure, hemoglobin, potassium, calcium, phosphate, albumin, CRP, UPCR and CTR). Logistic regression imputation was used as a dichotomous variable with missing data (smoking). We created five imputed datasets that were analyzed separately and combined using Rubin's rules. Missing data during follow-up were imputed by the last observation carried forward method.

Sensitivity analyses. We conducted the following two additional analyses.

First, we repeated the same analyses with different cut-off values for the high anion gap (≥ 10) [27].

Second, anion gap was adjusted for albumin [Equation (1)] and for potassium, albumin, phosphate and pH [Equation (2)] [28]. The cut-off points of these adjusted anion gap were determined based on the top 25th percentile of our cohort.

Equation (1): Albumin-adjusted anion gap = sodium (mEq/L) – chloride (mEq/L) – bicarbonate (mEq/L) + 2.5×4 – albumin (g/dL) if albumin < 4.0 g/dL [28]. A high albumin-adjusted anion gap was defined as an albumin-adjusted anion gap ≥ 10.4 .

Equation (2): Fully adjusted anion gap = sodium (mEq/L) + potassium (mEq/L) – chloride (mEq/L) – bicarbonate (mEq/L) – $10 \times$ albumin (g/dL) \times (0.123 \times pH 0.631) – phosphate (mEq/L) \times (0.309 \times pH 0.469) [29]. A high fully adjusted anion gap was defined as a fully adjusted anion gap ≥ 1.9 .

Statistical analyses were performed using STATA version 16.1 (STATA Corporation, College Station, TX, USA).

RESULTS

Study patients

Among 3161 patients whose baseline eGFR was 10–59.9 mL/min/1.73 m², 1168 (37%) with available anion gap data were included in the subsequent analysis. Baseline data except for eGFR were similar among those with and without anion gap data; eGFR was slightly lower for those with anion gap data (Supplementary data, Table S1).

Baseline characteristics

The mean baseline eGFR of the 1168 study patients was 28 mL/min/1.73 m², standard deviation (SD) 13 mL/min/1.73 m². The prevalence rates of CKD stages G3a, G3b, G4 and G5 were 14%, 27%, 42% and 17%, respectively.

The baseline characteristics according to anion gap at baseline are shown in Table 1. Patients with high anion gap had lower bicarbonate levels and eGFR and higher phosphate and uric acid levels compared with those without high anion gap. The pH levels were similar between the groups.

Compared with patients without normal anion gap (hyperchloremic) acidosis, patients with normal anion gap

acidosis had lower bicarbonate, pH and eGFR and higher chloride, phosphate, uric acid and UPCR (Table 1).

Anion gap

The mean anion gap at baseline was 7.9 (SD 2.4). High anion gap (anion gap ≥ 9.2) was observed in 307 patients (26%). The prevalence rates of high anion gap were 20%, 16%, 27% and 46% in CKD stages G3a, G3b, G4 and G5, respectively.

During the median follow-up of 2.9 years (quartile 1–quartile 3, 1.1–5.2 years), anion gap was measured at an average of 5.3 times (SD 7.1 times). Among 861 patients without high anion gap at baseline, 545 (63%) developed high anion gap during follow-up.

Normal anion gap (hyperchloremic) acidosis

The mean anion-gap-adjusted bicarbonate level was 24.9 mEq/L (SD 3.8 mEq/L). Normal anion gap acidosis (anion-gap-adjusted bicarbonate level ≤ 22.8 mEq/L) was found in 315 (27%) patients at baseline; the prevalence rates were 5%, 14%, 32% and 53% in CKD stages G3a, G3b, G4 and G5, respectively. Among 853 patients without normal anion gap acidosis at baseline, 192 (23%) developed normal anion gap acidosis during follow-up.

Marginal structural model

Overall, 132 patients (11%) developed cardiovascular events (3.3/100 patient-years) (19 coronary artery events, 35 strokes, 57 hospitalization for decompensated heart failure and 21 cardiovascular causes). There were 127 all-cause deaths (11%) (3.2/100 patient-years). Additionally, 293 (25%) patients started renal replacement therapy and 239 (20%) were lost to follow-up.

In the MSMs, high anion gap (anion gap ≥ 9.2) was associated with a 1.87-fold higher rate of cardiovascular events compared with normal anion gap (anion gap < 9.2) [95% confidence interval (95% CI) 1.13–3.09; $P = 0.02$] (Figure 1, Table 2). High anion gap was also associated with a higher rate of composite of cardiovascular events and all-cause death [hazard ratio (HR) 3.28; 95% CI 2.19–4.91; $P < 0.001$]. There were no significant interactions between anion gap and pre-specified baseline covariates (age, sex, diabetes mellitus, cardiovascular comorbidities, UPCR and eGFR).

In contrast, normal anion gap acidosis (anion-gap-adjusted bicarbonate level ≤ 22.8 mEq/L) was not associated with cardiovascular events (HR 0.74; 95% CI 0.47–1.17; $P = 0.2$) or the composite of cardiovascular events and all-cause death (HR 1.11; 95% CI 0.80–1.54; $P = 0.5$) (Table 3).

Sensitivity analyses

When a different cut-off value (≥ 10) was used for high anion gap, high anion gap was still significantly associated with higher rates of cardiovascular events and the composite of cardiovascular events and all-cause death (Table 2).

Both albumin-adjusted and fully adjusted anion gap were significantly associated with higher rates of cardiovascular events and the composite of cardiovascular events and all-cause death (Table 2).

DISCUSSION

In this retrospective cohort study, we found for the first time that high anion gap was significantly associated with an

Table 1. Baseline characteristics of the study participants

	n (%) of missing values	Total n = 1168	High anion gap		Normal anion gap acidosis	
			No n = 861	Yes n = 307	No n = 853	Yes n = 315
Demographics						
Age, years	0 (0)	65 (15)	66 (14)	63 (15)	65 (14)	64 (15)
Male, n (%)	0 (0)	780 (67)	598 (70)	182 (59)	580 (68)	200 (63)
Body mass index, kg/m ²	75 (6)	23.1 (4.4)	23.0(4.2)	23.4 (4.9)	23.1 (4.2)	23.0 (4.9)
Systolic blood pressure, mmHg	66 (6)	131 (21)	132 (20)	130 (21)	131 (21)	132 (20)
Diastolic blood pressure, mmHg	66 (6)	74 (14)	74 (14)	75 (15)	74 (14)	75 (13)
Diabetes mellitus, n (%)	0 (0)	573 (49)	412 (48)	161 (52)	425 (50)	148 (47)
Smoking history, n (%)	151 (13)	451 (44)	336 (46)	115 (41)	321 (44)	130 (46)
Cardiovascular comorbidities, n (%)	0 (0)	276 (24)	209 (24)	67 (22)	214 (25)	62 (20)
Cardiothoracic ratio, %	99 (8)	49 (7)	48 (6)	49 (7)	49 (7)	49 (7)
Laboratory data						
pH	0 (0)	7.34 (0.05)	7.34 (0.05)	7.35 (0.07)	7.35 (0.05)	7.32 (0.06)
PCO ₂ , mmHg	0 (0)	46.4 (7.7)	48.0 (7.1)	41.8 (7.2)	48.8 (6.6)	39.7 (6.3)
HCO ₃ , mEq/L	0 (0)	24.5 (4.0)	25.3 (3.7)	22.4 (4.3)	26.3 (2.8)	19.7 (2.5)
Albumin, g/DL	83 (7)	3.6 (0.7)	3.6 (0.7)	3.7 (0.7)	3.7 (0.7)	3.5 (0.7)
Creatinine, mg/DL	0 (0)	2.2 (1.0)	2.0 (0.9)	2.5 (1.1)	2.0 (0.9)	2.7 (1.0)
eGFR, mL/min/1.73 m ²	0 (0)	28 (13)	30 (13)	24 (13)	31 (13)	21 (9)
Hemoglobin, g/DL	45 (4)	11.5 (2.1)	11.6 (2.1)	11.3 (2.1)	11.8 (2.1)	10.7 (1.7)
Sodium, mEq/L	0 (0)	139 (4)	139 (3)	139 (5)	139 (3)	138 (4)
Chloride, mEq/L	0 (0)	106 (5)	107 (4)	105 (6)	105 (5)	110 (5)
Potassium, mEq/L	1 (<1)	4.6 (0.7)	4.6 (0.6)	4.4 (0.8)	4.5 (0.6)	4.8 (0.7)
Adjusted calcium, mg/dL	109 (9)	9.1 (0.6)	9.2 (0.5)	9.1 (0.7)	9.2 (0.6)	9.0 (0.6)
Phosphate, mg/dL	155 (13)	3.7 (0.7)	3.6 (0.7)	3.9 (0.9)	3.6 (0.7)	3.9 (0.9)
Uric acid, mg/dL	91 (8)	7.0 (1.8)	6.9 (1.7)	7.4 (2.2)	7.0 (1.7)	7.4 (2.0)
LDL-C, mg/dL	241 (21)	107 (37)	107 (37)	107 (37)	109 (37)	99 (36)
HDL-C, mg/dL	352 (30)	51 (18)	51 (18)	50 (20)	52 (18)	47 (18)
C-reactive protein, mg/dL	204 (17)	0.1 (0, 0.4)	0.1 (0, 0.3)	0.2 (0, 0.8)	0.1 (0, 0.4)	0.1 (0, 0.6)
UPCR, g/gCre	441 (38)	0.7 (0.2, 2.4)	0.7 (0.2, 2.3)	0.7 (0.2, 2.8)	0.6 (0.1, 2.1)	0.9 (0.3, 3.2)
Medications						
Sodium bicarbonate, n (%)	0 (0)	162 (14)	126 (15)	36 (12)	102 (12)	60 (19)
ACEIs/ARBs, n (%)	0 (0)	583 (50)	446 (52)	137 (45)	418 (49)	165 (52)
Beta-blockers, n (%)	0 (0)	282 (24)	209 (24)	73 (24)	215 (25)	67 (21)
Loop diuretics, n (%)	0 (0)	385 (33)	286 (33)	99 (32)	286 (34)	99 (31)
Thiazide diuretics, n (%)	0 (0)	199 (17)	128 (15)	71 (23)	148 (17)	51 (16)
Spirolactone, n (%)	0 (0)	169 (14)	138 (16)	31 (10)	130 (15)	39 (12)
Statins, n (%)	0 (0)	353 (30)	260 (30)	93 (30)	269 (32)	84 (27)

A high anion gap is defined as an anion gap ≥ 9.2 .

Normal anion gap acidosis is defined as an adjusted bicarbonate level ≤ 22.8 mEq/L.

Data are expressed as mean (standard deviation) or median (interquartile range) for continuous variables and as number (%) for categorical variables.

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

increased risk of cardiovascular events among patients with CKD stages G3–G5. This association was independent of various relevant clinical factors including time-updated eGFR and consistent when anion gap was corrected for albumin, potassium, phosphate and pH. High anion gap was also associated with an increased risk of the composite of cardiovascular events and all-cause death, although the association between anion gap and mortality has been reported previously [30]. On the other hand, normal anion gap (hyperchloremic) acidosis was not associated with cardiovascular outcomes. Our findings suggest the clinical importance of high anion gap in terms of the excess cardiovascular burden in patients with advanced CKD.

Anion gap in patients with CKD consists of a wide array of anionic substances. Among them, phosphate is likely to be involved in our findings because the accumulation of phosphate may promote atherogenesis as well as vascular calcification,

thus contributing to high cardiovascular risks [31–33]. However, we showed that the association between anion gap and cardiovascular risk was independent of time-updated serum phosphate levels and was maintained when anion gap was corrected for serum phosphate levels. This indicates that anions other than phosphate may also have been involved in our findings. Gut-derived uremic anions such as indoxyl sulfate, indole-3 acetic acid, *p*-cresyl sulfate and trimethylamine N-oxide are known to induce endothelial inflammation and oxidative stress [34], arteriosclerosis [35, 36], vascular calcification [37, 38], cardiac hypertrophy and fibrosis [39, 40]. Cohort studies have reported a significant association between these uremic anions and cardiovascular events in patients with CKD [41–43]. It is important to note, however, that these substances represent only a fraction of the entire uremic solute [44, 45]. Therefore, many other accumulated anions in CKD patients may also contribute to cardiovascular risks. More detailed and

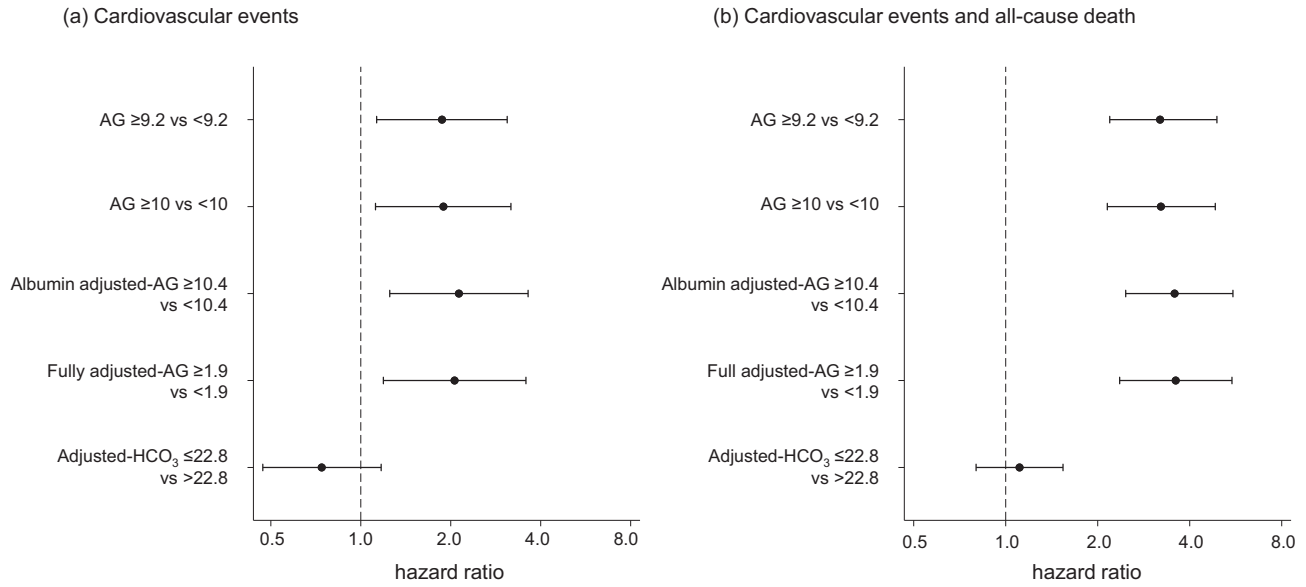


FIGURE 1: Hazard ratios for (a) cardiovascular events and (b) composite of cardiovascular events or all-cause death in marginal structural models. The dots represent hazard ratios and the solid lines represent 95% confidence intervals. Albumin-adjusted anion gap = sodium (mEq/L) – chloride (mEq/L) – bicarbonate (mEq/L) + 2.5 × 4 – albumin (g/dL) (if albumin <4 g/dL). Fully adjusted anion gap = sodium (mEq/L) + potassium (mEq/L) – chloride (mEq/L) – bicarbonate (mEq/L) – 10 × albumin (g/dL) × (0.123 × pH 0.631) – phosphate (mEq/L) × (0.309 × pH 0.469). Adjusted bicarbonate = bicarbonate (mEq/L) (+ anion gap – 9.2, if the anion gap was ≥9.2). Normal anion gap acidosis is defined as an adjusted bicarbonate level ≤22.8 mEq/L. AG, anion gap.

Table 2. Associations between high anion gap and cardiovascular outcomes in marginal structural models

	Outcomes					
	Cardiovascular events			Cardiovascular events and all-cause death		
	HR	95% CI	P-value	HR	95% CI	P-value
Normal anion gap	Ref.	–	–	Ref.	–	–
High anion gap (anion gap ≥9.2)	1.87	1.13–3.09	0.02	3.28	2.19–4.91	<0.001
Normal anion gap	Ref.	–	–	Ref.	–	–
High anion gap (anion gap ≥10)	1.89	1.12–3.18	0.02	3.23	2.15–4.85	<0.001
Normal albumin-adjusted anion gap ^a	Ref.	–	–	Ref.	–	–
High albumin-adjusted anion gap	2.13	1.25–3.63	0.005	3.70	2.47–5.54	<0.001
Normal fully adjusted anion gap ^b	Ref.	–	–	Ref.	–	–
High fully adjusted anion gap	2.06	1.19–3.57	0.01	3.60	2.36–5.50	<0.001

^aAlbumin-adjusted anion gap = sodium (mEq/L) – chloride (mEq/L) – bicarbonate (mEq/L) + 2.5 × 4 – albumin (g/dL) (if albumin <4 g/dL). A high albumin-adjusted anion gap is defined as an albumin-adjusted anion gap ≥10.4.

^bFully adjusted anion gap = sodium (mEq/L) + potassium (mEq/L) – chloride (mEq/L) – bicarbonate (mEq/L) – 10 × albumin (g/dL) × (0.123 × pH 0.631) – phosphate (mEq/L) × (0.309 × pH 0.469). A high fully adjusted anion gap is defined as a fully adjusted anion gap ≥1.9.

Models are adjusted for (i) baseline covariates including age, sex, body mass index, smoking history, systolic blood pressure, diabetes mellitus, cardiovascular comorbidities, cardiothoracic ratio, albumin, eGFR, hemoglobin, sodium, potassium, calcium, phosphate, uric acid, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, UPCR, C-reactive protein, bicarbonate, loop diuretics, thiazide diuretics, spironolactone, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, statins and sodium bicarbonate, and (ii) time-dependent covariates including albumin, eGFR, hemoglobin, sodium, potassium, calcium, phosphate, uric acid, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, UPCR, C-reactive protein, bicarbonate, loop diuretics, thiazide diuretics, spironolactone, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, statins and sodium bicarbonate.

Ref., reference.

comprehensive studies are necessary to identify the exact role of uremic anions as cardiovascular toxins.

In our study, the association between anion gap and cardiovascular events was independent of bicarbonate levels. This suggests that the correction of acidemia using oral alkaline therapy is not sufficient to alleviate the detrimental cardiovascular effects induced by high anion gap. To date, interventions that can decrease anion gap in CKD have not been established. Because gut dysbiosis and intestinal barrier disruption may increase the

production and absorption of gut-derived uremic toxins [46], modification of the microbiome might help decrease anion gap. Animal studies have shown that prebiotics prevent endothelial dysfunction [47] and left ventricular hypertrophy [48]; however, their direct effects on anion gap are currently unknown. The development of anion gap-lowering therapy is desired to test the cardiovascular impact of high anion gap.

We did not observe a significant association between normal anion gap (hyperchloremic) acidosis and cardiovascular events.

Table 3. Associations between normal anion gap (hyperchloremic) acidosis and cardiovascular outcomes in marginal structural models

	Outcome					
	Cardiovascular events			Cardiovascular events and all-cause death		
	HR	95% CI	P-value	HR	95% CI	P-value
Without normal anion gap acidosis	Ref.	–	–	Ref.	–	–
Normal anion gap acidosis	0.74	0.47–1.17	0.2	1.11	0.80–1.54	0.5

Normal anion gap acidosis is defined as an adjusted bicarbonate level ≤ 22.8 mEq/L.

Models were adjusted for (i) baseline covariates including age, sex, body mass index, smoking history, systolic blood pressure, diabetes mellitus, cardiovascular comorbidities, cardiothoracic ratio, albumin, eGFR, hemoglobin, sodium, potassium, calcium, phosphate, uric acid, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, UPCR, C-reactive protein, loop diuretics, thiazide diuretics, spironolactone, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, statins and sodium bicarbonate, and (ii) time-dependent covariates including albumin, eGFR, hemoglobin, sodium, potassium, calcium, phosphate, uric acid, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, UPCR, C-reactive protein, loop diuretics, thiazide diuretics, spironolactone, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, statins and sodium bicarbonate.

Ref., reference.

This might be in contrast to the study by Dobre *et al.*, who have reported a significant association between lower serum bicarbonate levels and increased cardiovascular risk [18]. Although they did not differentiate high and normal anion gap acidosis, their study patients had relatively preserved kidney function (mean eGFR, 72.4 mL/min/1.73 m²), therefore their low bicarbonate levels are expected to be attributable to normal anion gap acidosis. This discrepancy may be explained by the different patient populations comprising the two cohorts, particularly in terms of kidney function. Additionally, the study by Dobre *et al.* involved a highly selected patient population recruited from a randomized controlled trial; they excluded patients with diabetes mellitus, history of stroke or urinary protein ≥ 1 g/day [18]. In contrast, our study was based on real-world data of a population with advanced CKD. Moreover, we analyzed the impact of time-updated exposures with appropriate adjustments for time-updated eGFR using MSMs. This may be critical because there is a strong association between metabolic acidosis and kidney function. On the other hand, some of our patients received sodium bicarbonate for hypobicarbonatemia, which might have weakened the effect of normal anion gap acidosis. Future studies are required to confirm our data regarding the null association between normal anion gap acidosis and the risk of cardiovascular events.

Our study had several strengths. The majority of our patients had advanced CKD, thus more than half of them were exposed to high anion gap during the study period. Our dataset contained time-updated anion gap, which allowed us to capture time-series alterations of anion gap during CKD progression. We assessed clinically meaningful outcomes (cardiovascular events) that were carefully ascertained according to hospital medical records. We used MSMs to account for several time-dependent confounders. Sensitivity analyses using different definition and formulas for anion gap yielded consistent results.

Our study also had some limitations. Because of the observational study design, causality between high anion gap and the incidence of cardiovascular events cannot be proven. Residual confounding was possible despite the extensive adjustment for measured confounders. Incident rates of cardiovascular events are generally much lower among CKD patients in Japan than those in Western countries [49]. Therefore, a null association between normal anion gap acidosis and cardiovascular events may have been only attributable to the limited statistical power. Be-

cause of racial differences, it is unknown whether our findings are applicable to different populations. Furthermore, there was selection bias toward patients with lower eGFR due to the availability of blood gas data. This bias was largely because of the Japanese clinical practice guideline for CKD, which recommends venous blood gas measurement for patients with CKD stages G4 and G5.

CONCLUSION

In conclusion, we found an association between high anion gap and increased risk of cardiovascular events among patients with advanced CKD by using MSMs to account for various time-dependent confounders. Our findings suggest the clinical relevance of high anion gap in terms of the excess cardiovascular burden among patients with CKD. In contrast, normal anion gap acidosis was not associated with cardiovascular events. Because of the selection bias among the study patients, our findings should be verified by future studies involving different patient populations. Differentiation of high and normal anion gap acidosis may advance the clinical management of metabolic acidosis in patients with CKD.

SUPPLEMENTARY DATA

Supplementary data are available at [ckj](#) online.

AUTHORS' CONTRIBUTIONS

Y.A. and Y.S. contributed in conception or design or analysis and interpretation of data or both. Y.A., Y.S., S.K., K.H., Y.D. and T.O. were involved in drafting the article or revising it. J.-Y.K. and Y.I. contributed in providing intellectual content of critical importance to the work described. All authors gave final approval of the version to be published.

DATA AVAILABILITY STATEMENT

The data underlying this article cannot be shared publicly due to the privacy of individuals who participated in the study. The data will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

Nothing to disclose. Results presented in this paper have not been published previously in whole or part, except in abstract format.

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