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Relationship between serum total carbon dioxide concentration and bicarbonate concentration in patients undergoing hemodialysis

Keiji Hirai[1], Susumu Ookawara[1], Junki Morino[1], Saori Minato[1], Shohei Kaneko[1], Katsunori Yanai[1], Hiroki Ishii[1], Momoko Matsuyama[1], Taisuke Kitano[1], Mitsutoshi Shindo[1], Haruhisa Miyazawa[1], Kiyonori Ito[1], Yuichirou Ueda[1], Tatsuro Watano[2], Shinji Fujino[2], Kiyoka Omoto[2], Yoshiyuki Morishita[1]

[1]*Division of Nephrology, First Department of Integrated Medicine, Saitama Medical Center, Jichi Medical University, Saitama, Japan*
[2]*Department of Laboratory Medicine, Saitama Medical Center, Jichi Medical University, Saitama, Japan*

Background: Few studies have investigated the relationship between serum total carbon dioxide (CO_2) concentration and bicarbonate ion (HCO_3^-) concentration in patients undergoing hemodialysis. We determined the agreement and discrepancy between serum total CO_2 and HCO_3^- concentrations and the diagnostic accuracy of serum total CO_2 for the prediction of low (HCO_3^- < 24 mEq/L) and high (HCO_3^- ≥ 24 mEq/L) bicarbonate concentrations in hemodialysis patients.

Methods: One hundred forty-nine arteriovenous blood samples from 84 hemodialysis patients were studied. Multiple linear regression analysis was used to determine factors correlated with HCO_3^- concentration. Diagnostic accuracy of serum total CO_2 was evaluated using receiver operating characteristic curve analysis and a 2 × 2 table. Agreement between serum total CO_2 and HCO_3^- concentrations was assessed using Bland–Altman analysis.

Results: Serum total CO_2 concentration was closely correlated with HCO_3^- concentration ($\beta = 0.858$, $P < 0.001$). Area under the curve of serum total CO_2 for the identification of low and high bicarbonate concentrations was 0.989. Use of serum total CO_2 to predict low and high bicarbonate concentrations had a sensitivity of 100%, specificity of 50.0%, positive predictive value of 96.5%, negative predictive value of 100%, and accuracy of 96.6%. Bland–Altman analysis showed moderate agreement between serum total CO_2 and HCO_3^- concentrations. Discrepancies between HCO_3^- and serum total CO_2 concentrations (serum total CO_2 − HCO_3^- ≤ −1) were observed in 89 samples.

Conclusion: Serum total CO_2 concentration is closely correlated with HCO_3^- concentration in hemodialysis patients. However, there is a non-negligible discrepancy between serum total CO_2 and HCO_3^- concentrations.

Keywords: Acid base balance, Bicarbonate, Hemodialysis, Serum total carbon dioxide

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Correspondence: Keiji Hirai
Division of Nephrology, First Department of Integrated Medicine, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma-cho, Omiya-ku, Saitama-shi, Saitama-ken 330-8503, Japan. E-mail: keijihirai@kfy.biglobe.ne.jp

Introduction

Metabolic acidosis is a commonly identified complication in patients undergoing hemodialysis, and it can contribute to bone mineral loss, protein energy wasting, cardiovascular disease, and higher mortality risk [1–4]. Therefore, early and accurate diagnosis of metabolic acidosis is important to prevent cardiovascular events and increases in the risk of mortality.

In Japan, blood-gas analyzers are available in most hospitals. Therefore, bicarbonate ion (HCO_3^-) concentration, measured in arteriovenous blood samples, has been widely used to assess metabolic acidosis in hemodialysis patients [5]. Lower HCO_3^- concentration has been reported to be associated with higher risk of cardiac dysfunction, peripheral vascular disease, and death in patients undergoing hemodialysis [6,7]. Therefore, HCO_3^- is a significant predictor of cardiovascular disease and mortality in hemodialysis patients. However, these blood-gas analyses require a specific measuring device and syringe, in addition to blood samples used for these analyses [8].

Serum total carbon dioxide (CO_2) concentration can be readily measured, along with serum creatinine, urea, and electrolytes, using a biochemical analyzer in a clinical setting [9]. Furthermore, serum total CO_2 has been shown to closely correlate with HCO_3^- concentration in patients with chronic kidney disease (CKD) who are not undergoing renal replacement therapy [10]. However, few studies have investigated the relationship between serum total CO_2 and HCO_3^- concentrations in patients undergoing hemodialysis. Therefore, in the present study, we aimed to analyze the agreement between these two parameters in patients undergoing hemodialysis.

Methods

Ethical approval of the study protocol

This study was carried out in accordance with the ethical principles contained within the Declaration of Helsinki and its subsequent amendments. The study protocol was approved by the Ethics Committee of Saitama Medical Center, Jichi Medical University (S17-052; Saitama, Japan). The requirement for informed consent was waived and an opt-out method was used due to the retrospective design of the study.

Inclusion and exclusion criteria

Inclusion criteria were: 1) age > 20 years; 2) CKD stage G5D; and 3) simultaneous measurement of serum total CO_2 and HCO_3^- concentrations. Exclusion criteria were 1) peritoneal dialysis and 2) renal transplantation.

Study design

This was a single-center, retrospective, cross-sectional study. We analyzed patient data obtained from medical records at the Division of Nephrology, Saitama Medical Center, between April 2016 and March 2018. Laboratory data in the form of blood tests and blood-gas analyses that had been obtained simultaneously were analyzed.

Relationship between serum total CO_2 and HCO_3^- concentrations was analyzed using Pearson's correlation coefficient. Independent factors correlated with HCO_3^- concentration were identified using multiple linear regression analysis. Diagnostic accuracy of serum total CO_2 for the identification of low and high bicarbonate concentrations was analyzed using receiver operating characteristic (ROC) curve analysis and a 2 × 2 table. Agreement between serum total CO_2 and HCO_3^- concentrations was analyzed using Bland–Altman analysis. Relationship between serum total CO_2 concentrations measured using blood-gas analyses and an enzymatic method was analyzed using Pearson's correlation coefficient. Agreement of serum total CO_2 concentrations between the blood-gas and enzymatic methods was evaluated using Bland–Altman analysis.

Laboratory methods

Blood parameters were measured at the Department of Clinical Laboratory, Saitama Medical Center. Blood samples were obtained from an arteriovenous fistula just before the commencement of the first hemodialysis session in a week. Samples of arteriovenous blood were collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes from the arteriovenous fistula and centrifuged within 15 minutes to obtain serum. Serum total CO_2 was measured within 15 minutes after centrifugation in an automated biochemical analyzer (JCA-BM6070; JEOL, Tokyo, Japan), as were biochemical parameters (hemoglobin, total protein, serum albumin, blood urea

nitrogen, serum creatinine, sodium, potassium, chloride, calcium, phosphate, magnesium, and glucose). Serum total CO_2 was determined by an enzymatic method using a commercial kit (Toyobo, Osaka, Japan) in an automated biochemical analyzer. Serum total CO_2 was measured by monitoring the oxidation of nicotinamide adenine dinucleotide (NADH) at 405 nm using the coupled assay of phosphoenolpyruvate carboxylase and malate dehydrogenase. The decrease in NADH concentration is proportional to the concentration of serum total CO_2 in the sample, allowing measurement of serum total CO_2 concentration [9]. Single-pool Kt/V was calculated using the formula of Daugirdas [11].

Samples of arteriovenous blood for gas analyses were collected in a heparinized blood-gas syringe from the arteriovenous fistula at the same time samples were collected for other blood tests, and analyzed within 10 minutes to obtain the pH value and partial pressure of carbon dioxide (pCO_2). The pH and pCO_2 of blood were measured using a blood-gas analyzer (Rapidlab-1265; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). HCO_3^- concentration was calculated from the measured pH and pCO_2 using the Henderson–Hasselbalch equation [12]:

$$pH = 6.1 + \log([HCO_3^-]/pCO_2 \times 0.03).$$

Table 1. Patient characteristics and medications

Characteristic	Value
Number of patients	84
Number of samples	149
Age (yr)	67.1 ± 11.5
Sex (male)	61 (72.6)
Body mass index (kg/m^2)	22.1 ± 3.9
Hemodialysis duration (mo)	15.5 (3.7–38.8)
Diabetes mellitus	53 (63.1)
Corticosteroid	11 (13.1)
β-blocker	38 (45.2)
Renin–angiotensin system inhibitor	44 (52.4)
Aldosterone receptor antagonist	1 (1.2)
Loop diuretic	45 (53.6)
Thiazide diuretic	18 (21.4)
Potassium binder	7 (8.3)
Phosphate binder	49 (58.3)
Calcium-containing phosphate binder	31 (36.9)
Calcium-free phosphate binder	34 (40.5)
Vitamin D analog	43 (51.2)
Cinacalcet	11 (13.1)
Single pool Kt/V	1.3 (1.0–1.5)

Data are presented as number only, mean ± standard deviation, number (%), or median (interquartile range).

Statistical analyses

Statistical analyses were performed using JMP ver. 11 (SAS Institute, Cary, NC, USA). Continuous variables with a normal distribution were expressed as means ± standard deviations while those with a non-normal distribution were expressed as medians and interquartile ranges. Categorical variables were expressed as numbers and percentages. Hemodialysis duration and single pool Kt/V were not normally distributed; therefore, these valuables were transformed using the natural logarithm. Relationships between two variables were evaluated using Pearson's correlation coefficient. Linear regression analysis was used to identify parameters that were independently correlated with HCO_3^- concentration. Linearities between dependent and independent variables were examined using spline analysis, and linear relationships were found between HCO_3^- concentration and the other variables. Parameters that were significantly correlated with HCO_3^- concentration in simple linear regression analyses were included in a subsequent multiple linear regression analysis. Multi-collinearity was examined by calculating variance inflation factors for all the independent variables; no multi-collinearity was detected for any of these variables. Diagnostic accuracy of serum total CO_2 was determined using ROC curve analysis and a 2 × 2 table. Area

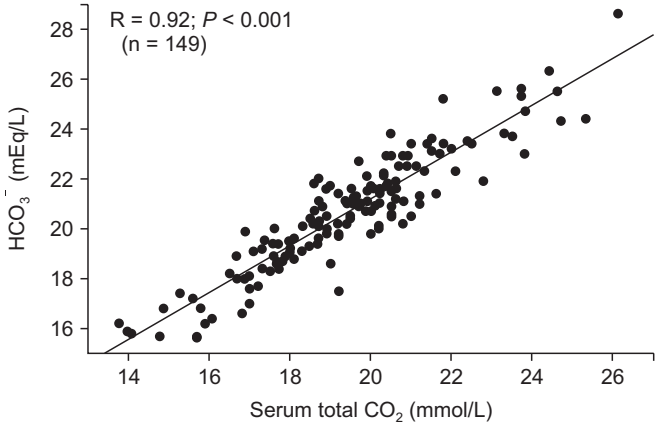

Figure 1. Relationship between serum total CO_2 and HCO_3^- concentration.
CO_2, carbon dioxide; HCO_3^-, bicarbonate ion.

under the curve (AUC), sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated for the identification of low (HCO_3^- < 24 mEq/L) and high (HCO_3^- ≥ 24 mEq/L) bicarbonate concentrations based on the cut-off value for HCO_3^- of 24 mEq/L established in a previous study [3]. Agreement between serum total CO_2 and HCO_3^- concentrations was assessed using the Bland–Altman method. $P < 0.05$ was considered to indicate statistical significance.

Results

Patient characteristics

Characteristics of the patients and their medications are shown in Table 1. A total of 149 blood samples from 84 patients (61 males and 23 females; mean age: 67.1 ± 11.5 years; body mass index: 22.1 ± 3.9 kg/m^2) were obtained. Mean single pool Kt/V was 1.3 (1.0–1.5) and 63.1% of the participants had diabetes mellitus. Proportions of par-

Table 2. Simple and multiple linear regression analyses of parameters correlated with HCO_3^- concentration

Parameter	Simple linear regression analysis		Multivariate linear regression analysis using variables with $P < 0.05$ in univariate analyses	
	Standard coefficient	*P* value	Standard coefficient	*P* value
Age (yr)	0.053	0.52		
Sex (male: yes or no)	–0.119	0.15		
Body mass index (kg/m^2)	–0.138	0.09		
Ln-hemodialysis duration (mo)	–0.054	0.52		
Diabetes mellitus (yes or no)	–0.156	0.06		
Corticosteroid (yes or no)	0.068	0.41		
β-blocker (yes or no)	0.018	0.83		
Renin–angiotensin system inhibitor (yes or no)	–0.171	0.04	0.038	0.26
Aldosterone receptor antagonist (yes or no)	–0.143	0.08		
Loop diuretic (yes or no)	0.007	0.93		
Thiazide diuretic (yes or no)	–0.045	0.59		
Potassium binder (yes or no)	0.020	0.81		
Phosphate binder (yes or no)	–0.083	0.32		
Calcium-containing phosphate binder (yes or no)	–0.006	0.95		
Calcium-free phosphate binder (yes or no)	–0.010	0.90		
Vitamin D analogue (yes or no)	–0.030	0.72		
Cinacalcet (yes or no)	–0.000	1.00		
Ln-single pool Kt/V	0.059	0.48		
Total protein (g/dL)	0.052	0.53		
Serum albumin (g/dL)	–0.061	0.46		
Hemoglobin (g/dL)	–0.192	0.02	–0.037	0.33
Blood urea nitrogen (mg/dL)	–0.148	0.07		
Creatinine (mg/dL)	–0.273	< 0.001	–0.060	0.11
Uric acid (mg/dL)	–0.048	0.56		
Sodium (mEq/L)	0.131	0.11		
Potassium (mEq/L)	–0.033	0.69		
Chloride (mEq/L)	–0.191	0.02	–0.087	0.009
Total calcium (mg/dL)	0.289	< 0.001	0.133	< 0.001
Phosphate (mg/dL)	–0.285	< 0.001	–0.024	0.49
Magnesium (mg/dL)	0.084	0.31		
Blood glucose (mg/dL)	–0.035	0.67		
Serum total CO_2 (mmol/L)	0.922	< 0.001	0.858	< 0.001

HCO_3^-, bicarbonate ion; Ln, logarithm.

ticipants taking various medications were as follows: corticosteroid, 13.1%; β-blocker, 45.2%; renin–angiotensin system inhibitor, 52.4%; aldosterone receptor antagonist, 1.2%; loop diuretic, 53.6%; thiazide diuretic, 21.4%; potassium binder, 8.3%; phosphate binder, 58.3%; calcium-containing phosphate binder, 36.9%; calcium-free phosphate binder, 40.5%; vitamin D analogue, 51.2%; and cinacalcet, 13.1%.

Relationship between serum total CO_2 and HCO_3^- concentrations

Fig. 1 shows the correlation between serum total CO_2 and HCO_3^- concentrations. Serum total CO_2 concentration was correlated closely with HCO_3^- concentration (r = 0.92; $P < 0.001$). Serum total CO_2 level was also correlated closely with blood-gas total CO_2 concentration (r = 0.92; $P < 0.001$).

Factors associated with HCO_3^- concentration

Simple linear regression analyses showed that HCO_3^- concentration was significantly negatively correlated with hemoglobin, creatinine, chloride, and phosphate concentrations, and with the use of a renin–angiotensin system inhibitor. HCO_3^- concentration was significantly positively correlated with serum total calcium and total CO_2. A multiple linear regression analysis was performed using variables that showed significant correlations with HCO_3^- concentration in simple linear regression analyses (Table 2). This analysis revealed that chloride (standard coefficient [β] = −0.087, $P = 0.009$), total calcium (β = 0.133, $P < 0.001$), and serum total CO_2 (β = 0.858, $P < 0.001$) were independently correlated with HCO_3^- concentration.

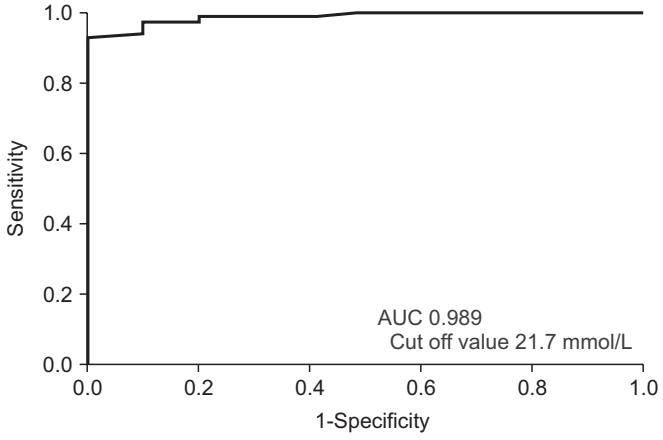

Figure 2. Receiver operating characteristic curve of serum total CO_2 for the identification of low (HCO_3^- < 24 mEq/L) and high (HCO_3^- ≥ 24 mEq/L) bicarbonate concentrations.
AUC, area under the curve; CO_2, carbon dioxide; HCO_3^-, bicarbonate ion.

Diagnostic accuracy of serum total CO_2 for the prediction of low and high bicarbonate concentrations

The serum total CO_2 ROC curve for the identification of low (HCO_3^- < 24 mEq/L) and high (HCO_3^- ≥ 24 mEq/L) bicarbonate concentrations is shown in Fig. 2. The AUC was 0.989, and the optimal cut-off value was 21.7 mmol/L. The 2 × 2 tables, stratified according to serum total CO_2 and HCO_3^- concentrations for low and high bicarbonate groups, are shown in Table 3. The diagnostic accuracy measures of serum total CO_2 for the prediction of low and high bicarbonate concentrations were as follows: sensitivity (99.3%), specificity (50.0%), positive predictive value (96.5%), negative predictive value (83.3%), accuracy (96.0%), pre-test probability (93.3%), positive post-test probability (96.5%), and negative post-test probability (16.7%).

Agreement between serum total CO_2 and HCO_3^- concentrations

Bland–Altman analysis showed a moderate agreement between serum total CO_2 and HCO_3^- concentrations. Mean difference was −1.24 ± 0.92, and more than 95% of the points were included within the limits of agreement

Table 3. 2 × 2 tables stratified according to serum total CO_2 and HCO_3^- concentration for low and high bicarbonate samples

	HCO_3^-		Total
	Low bicarbonate (HCO_3^- < 24 mEq/L)	High bicarbonate (HCO_3^- ≥ 24 mEq/L)	
Serum total CO_2			
Low serum total CO_2 (Serum total CO_2 < 24 mmol/L)	138	5	143
High serum total CO_2 (Serum total CO_2 ≥ 24 mmol/L)	1	5	6
Total	139	10	149

CO_2, carbon dioxide; HCO_3^-, bicarbonate ion.

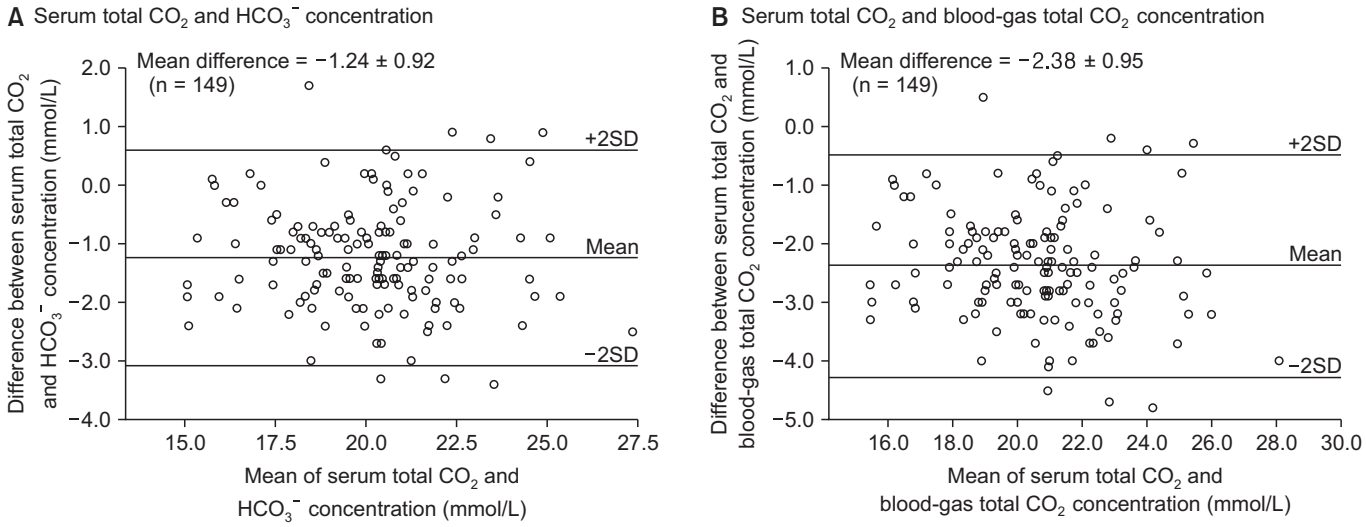

Figure 3. Bland–Altman analyses of the agreement between serum total CO_2 and HCO_3^- concentration, and between serum total CO_2 and blood-gas total CO_2 concentration. (A) Bland–Altman plot comparing serum total CO_2 and HCO_3^- concentration. (B) Bland–Altman plot comparing serum total CO_2 and blood-gas total CO_2 concentration.
CO_2, carbon dioxide; HCO_3^-, bicarbonate ion; SD, standard deviation.

Table 4. Comparison of acid-base balance parameters among groups divided according to the difference between serum total CO_2 and HCO_3^- concentration

	Serum total CO_2 < HCO_3^- (serum total CO_2 − HCO_3^- ≤ −1)	Serum total CO_2 = HCO_3^- (−1 <serum total CO2 − HCO_3^- < 1)	Serum total CO2 > HCO_3^- (serum total CO2 − HCO_3^- ≥ 1)
Number of samples	89	59	1
Serum total CO_2 (mmol/L)	19.2	19.7	19.2
Blood-gas total CO_2 (mmol/L)	22.2*	21.3	18.7
HCO_3^- (mEq/L)	21.0*	20.1	17.5
pCO_2 (mmHg)	37.6*	36.4	39.0
pH	7.37	7.36	7.27

CO_2, carbon dioxide; HCO_3^-, bicarbonate ion; pCO_2, partial pressure of carbon dioxide.
*P < 0.05 vs. the serum total CO_2 = HCO_3^- group.

(mean difference between the two methods ± 2 standard deviations) (Fig. 3A). This analysis also showed a moderate agreement between serum total CO_2 and blood-gas total CO_2 concentrations. The mean difference was −2.38 ± 0.95, and more than 95% of the points were included within the limits of agreement (mean difference between the two methods ± 2 standard deviations) (Fig. 3B). Additionally, we divided the samples into three groups according to the difference between serum total CO_2 and HCO_3^- concentrations: serum total CO_2 < HCO_3^- (serum total CO_2 − HCO_3^- ≤ −1), serum total CO_2 = HCO_3^- (−1 < serum total CO_2 − HCO_3^- < 1), and serum total CO_2 > HCO_3^- (serum total CO_2 − HCO_3^- ≥ 1). The number of samples in each group were 89, 59, and 1, respectively (Table 4). HCO_3^-, blood-gas total CO_2, and pCO_2 were significantly higher in the serum total CO_2 < HCO_3^- group than in the serum total CO_2 = HCO_3^- group (each P < 0.05).

Discussion

In the present study, we assessed the relationship between serum total CO_2 and HCO_3^- concentrations in hemodialysis patients, and found that serum total CO_2 concentration was closely correlated with HCO_3^- concentration. We also found that serum total CO_2 concentration had high diagnostic accuracy for the prediction of low and high bicarbonate concentrations in hemodialysis patients.

"Serum total CO_2" is the total concentration of all forms of CO_2 in a serum sample, which includes HCO_3^-, car-

bonate, and dissolved CO_2. In general, serum total CO_2 is approximately equivalent to the HCO_3^- concentration, because most CO_2 exists as HCO_3^- in the blood [9]. Furthermore, serum total CO_2 has been reported to be closely correlated with HCO_3^- concentration in pre-dialysis CKD patients [10]. However, a discrepancy between serum total CO_2 and HCO_3^- concentration, caused by differences in temperature and/or acidity [13], is sometimes identified in patients without renal impairment [14]. In the present study, calcium and chloride concentrations, in addition to serum CO_2, were independently correlated with HCO_3^- concentration in serum. HCO_3^- concentration was reported to be negatively correlated with calcium concentration in CKD stage G5D patients [15]. However, HCO_3^- concentration was positively correlated with calcium concentration in the present study. This discrepancy might be explained by the fact that ~50% of the participants in the present study had been taking a calcium-containing phosphate binder, which has been reported to be positively associated with HCO_3^- concentration [16]. HCO_3^- concentration is known to decrease as chloride concentration increases because of the equilibrium between HCl and $NaHCO_3$: $H^+ + Cl^- + Na^+ + HCO_3^- = Na^+ + Cl^- + H_2CO_3$ [17]. In the present study, chloride concentration was negatively correlated with HCO_3^- concentration, which is compatible with the findings of a previous study [10]. Correlations between calcium or chloride and HCO_3^- concentration were weak, but significant. Serum total CO_2 was closely correlated with HCO_3^- concentration and showed a high level of accuracy for the diagnosis of high or low bicarbonate concentrations. Therefore, serum CO_2 may represent a useful predictor of bicarbonate concentration and whether this is high or low. In our study, arteriovenous blood samples were analyzed. Serum total CO_2 has been reported to correlate strongly with HCO_3^- concentration in both arterial and venous blod samples [10,18]. Arterial pCO_2 and HCO_3^- concentration have been shown to correlate strongly with venous pCO_2 and HCO_3^- concentration, respectively [19]. In the present study, serum total CO_2 showed a close correlation with HCO_3^- concentration in arteriovenous blood samples, which are a mixture of arterial and venous blood. These results suggest that serum total CO_2 is closely correlated with HCO_3^- concentration in arteriovenous blood samples. Further studies are needed to confirm the correlation between serum total CO_2 and HCO_3^- concentrations measured in arteriovenous blood samples in hemodialysis patients.

It has been reported that serum albumin, estimated glomerular filtration rate, and blood glucose are independently correlated with HCO_3^- concentration, in addition to serum total CO_2, in CKD patients not undergoing renal replacement therapy [10]. There are several potential explanations for the differences between our results and those previously published. First, a higher serum albumin concentration has been shown to be associated with metabolic acidosis in pre-dialysis CKD patients [20], and this phenomenon is considered to be at least in part due to the weak acidity of albumin [21]. Loss of albumin into the dialysate and its adsorption onto the dialysis membrane can occur during hemodialysis [22]. Influx of HCO_3^- from the dialysate into the blood occurs during hemodialysis because the HCO_3^- concentration in serum is usually lower than that in the dialysate [4]. Therefore, the reduction in serum albumin due to loss into the dialysate and adsorption onto the dialysis membrane, and the increase in HCO_3^- caused by influx from the dialysate into the blood, might affect the relationship between serum albumin and HCO_3^- in hemodialysis patients. Second, serum HCO_3^- concentration has been reported to decrease as renal function decreases in pre-dialysis CKD patients, and this reduction is considered to be due to the inability of the kidney to synthesize ammonia, regenerate HCO_3^-, and excrete hydrogen ions (H^+) [23]. An increase in urinary glucose as a result of hyperglycemia has been shown to inhibit the excretion of H^+ through the proximal renal tubules *via* the sodium–glucose-coupled transporter, with consequent inhibition of the Na^+–H^+ exchanger because of competition with sodium influx [24,25]. The participants in the present study had been undergoing hemodialysis for a mean of 46.6 months, suggesting that they had little residual renal function [26]. Therefore, loss of residual renal function might explain the lack of correlation between serum creatinine or blood glucose and HCO_3^- concentration in the study.

Serum total CO_2 is usually higher than HCO_3^- concentration because total CO_2 is equal to the sum of the HCO_3^- concentration and dissolved CO_2, which is calculated from pCO_2 [9]. However, in the present study, HCO_3^- concentration was higher than serum total CO_2 concentration in more than half of the patients. PCO_2 value was higher in the serum total $CO_2 < HCO_3^-$ group than the

serum total CO_2 = HCO_3^- group. It has been reported that elevated pCO_2 could cause a discrepancy between serum total CO_2 and HCO_3^- concentration [14]. Another study reported that HCO_3^- concentration could be overestimated through a change in pK value caused by elevated pCO_2 [8]. These findings might explain the discrepancy between serum total CO_2 and HCO_3^- concentration in our study. We found a discrepancy in serum total CO_2 concentrations between blood-gas analyses and an enzymatic method, despite the fact that they were significantly correlated. Blood-gas analyzers measure pH and pCO_2, and then calculate HCO_3^- concentration using the Henderson-Hasselbalch equation. Subsequently serum total CO_2 is calculated as HCO_3^- + 0.03 × pCO_2 [27]. By contrast, the enzymatic method measures CO_2 released from plasma as a result of the addition of acid. This method measures the CO_2 present as HCO_3^-, dissolved CO_2, and carbamino CO_2 [9]. Differences in measurement principles might explain the discrepancy in serum total CO_2 concentrations between blood gas analyses and the enzymatic method. In the present study, the proportion of samples with a high bicarbonate concentration (HCO_3^- ≥ 24 mEq/L) was substantially lower than that reported in a previous study (6.7% vs. 30%) [3]. There are several possible explanations for this discrepancy. First, mean single pool Kt/V in our study was lower than that recommended by clinical practice guidelines [28]. Second, dialysate HCO_3^- concentrations differ among countries. Dialysate HCO_3^- concentration in hemodialysis is lower in Japan than in other countries [4]. Indeed, a trans nation-wide observational study showed that pre-dialysis HCO_3^- concentration was lowest in Japan among the seven countries that participated in the study [5]. Further studies are required to confirm the correlation between serum total CO_2 and HCO_3^- concentrations in hemodialysis patients treated with increased dialysis efficiency and higher dialysate HCO_3^- concentrations.

Measurement of serum total CO_2 has two main advantages over blood-gas analyses. First, there is no need for a blood gas-syringe, which decreases costs, and the amount of blood that needs to be collected is less for serum total CO_2 measurement than for blood gas measurements. Second, serum total CO_2 can be used to predict low and high bicarbonate concentrations without the need for a blood-gas analyzer. Therefore, measurement of serum total CO_2 can alleviate some of the burden on patients and laboratory staff.

Our study had four main limitations. First, it was a single-center, retrospective, observational study, and may therefore have been subject to patient selection bias. Second, the study cohort was small, especially patients with high HCO_3^- concentrations, which restricts the generalizability of our findings and assessment of the correlation between serum total CO_2 and HCO_3^- concentrations in patients with a high HCO_3^- concentration. Third, we used arteriovenous blood samples for analyses; the results might have been different if arterial blood samples had been used. Fourth, hemodialysis duration varied widely among patients in the present study. Because residual renal function declines in accordance with increasing duration of dialysis, HCO_3^- concentration decreases as dialysis duration increases [29]. Therefore, the large variation in hemodialysis duration might have affected our study results by causing variation in HCO_3^- concentrations. Therefore, further prospective, large-scale, multicenter studies with an adequate number of patients with a high HCO_3^- concentration are required to confirm our findings. In conclusion, serum total CO_2 concentration was closely correlated with HCO_3^- concentration in hemodialysis patients. However, there was a non-negligible discrepancy between serum total CO_2 and HCO_3^- concentrations.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Authors' contributions

Keiji Hirai and Susumu Ookawara conceived and designed the research. Keiji Hirai, Junki Morino, Momoko Matsuyama, Haruhisa Miyazawa, Kiyonori Ito, and Yuichirou Ueda performed research. Saori Minato, Shohei

Kaneko, Katsunori Yanai, Hiroki Ishii, Taisuke Kitano, and Mitsutoshi Shindo collected the data. Keiji Hirai, Tatsuro Watano, Shinji Fujino, and Kiyoka Omoto performed the analysis. Keiji Hirai and Susumu Ookawara wrote the paper. Yoshiyuki Morishita made critical revisions and approved the final version. All authors read and approved the final manuscript.

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