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

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Background: Few studies have investigated the relationship between serum total carbon dioxide (CO₂) concentration and bicarbonate ion (HCO₃⁻) concentration in patients undergoing hemodialysis. We determined the agreement and discrepancy between serum total CO₂ and HCO₃⁻ concentrations and the diagnostic accuracy of serum total CO₂ for the prediction of low (HCO₃⁻ < 24 mEq/L) and high (HCO₃⁻ ≥ 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 HCO₃⁻ concentration. Diagnostic accuracy of serum total CO₂ was evaluated using receiver operating characteristic curve analysis and a 2 × 2 table. Agreement between serum total CO₂ and HCO₃⁻ concentrations was assessed using Bland–Altman analysis.

Results: Serum total CO₂ concentration was closely correlated with HCO₃⁻ concentration (β = 0.858, P < 0.001). Area under the curve of serum total CO₂ for the identification of low and high bicarbonate concentrations was 0.989. Use of serum total CO₂ 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 CO₂ and HCO₃⁻ concentrations. Discrepancies between HCO₃⁻ and serum total CO₂ concentrations (serum total CO₂ - HCO₃⁻ ≤ -1) were observed in 89 samples.

Conclusion: Serum total CO₂ concentration is closely correlated with HCO₃⁻ concentration in hemodialysis patients. However, there is a non-negligible discrepancy between serum total CO₂ and HCO₃⁻ concentrations.

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

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]:

$$\text{pH} = 6.1 + \log([\text{HCO}_3^-]/\text{pCO}_2 \times 0.03).$$

### 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

### 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).

### 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 ($\text{HCO}_3^- < 24 \text{ mEq/L}$) and high ($\text{HCO}_3^- \geq 24 \text{ mEq/L}$) bicarbonate concentrations based on the cut-off value for $\text{HCO}_3^-$ of 24 mEq/L established in a previous study [3]. Agreement between serum total CO$_2$ and $\text{HCO}_3^-$ 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-

| Parameter                                      | Simple linear regression analysis | Multivariate linear regression analysis using variables with $P < 0.05$ in univariate analyses |
|------------------------------------------------|-----------------------------------|-----------------------------------------------------------------------------------------|
| 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                                                                                   |
| 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                                                                                   |
| Blood urea nitrogen (mg/dL)                   | −0.148                            | 0.07                                                                                   |
| Creatinine (mg/dL)                            | −0.273                            | $< 0.001$                                                                               |
| 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                                                                                   |
| Total calcium (mg/dL)                         | 0.289                             | $< 0.001$                                                                               |
| Phosphate (mg/dL)                             | −0.285                            | $< 0.001$                                                                               |
| Magnesium (mg/dL)                             | 0.084                             | 0.31                                                                                   |
| Blood glucose (mg/dL)                         | −0.035                            | 0.67                                                                                   |
| Serum total CO$_2$ (mmol/L)                   | 0.922                             | $< 0.001$                                                                               |

$\text{HCO}_3^-$, bicarbonate ion; Ln, logarithm.
Participants 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.

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 $-1.24 \pm 0.92$, 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 |
|---------------------------------------------------------------|
| Serum total CO$_2$                                           |
| Low serum total CO$_2$ (HCO$_3^-$ < 24 mEq/L)                |
| High serum total CO$_2$ (HCO$_3^-$ ≥ 24 mEq/L)               |
| Total                                 |
| Low bicarbonate                                              | 138 | 5 | 143 |
| High bicarbonate                                             | 1   | 5 | 6   |
| Total                                                      | 139 | 10 | 149 |

CO$_2$, carbon dioxide; HCO$_3^-$, bicarbonate ion.
The mean difference was $-2.38 \pm 0.95$ (n = 149), 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). The mean difference was $-1.24 \pm 0.92$ (n = 149) (Fig. 3A).

**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$_3$: \[ \text{H}^+ + \text{Cl}^- + \text{Na}^+ + \text{HCO}_3^- = \text{Na}^+ + \text{Cl}^- + \text{H}_2\text{CO}_3 \] [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 blood 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$_2 < \text{HCO}_3^-$ 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.

Acknowledgments

We thank all the staff of the Department of Clinical Laboratory (Saitama Medical Center, Jichi Medical University, Saitama, Japan) for their excellent work. We also thank Mark Cleasby, PhD, from the Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

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 Kyoka 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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