Comparison of Hypertonic and Isotonic Reference Electrode Junctions for Measuring Ionized Calcium in Whole Blood: A Clinical Study

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We measured ionized calcium concentrations in whole blood from 91 patients who had no clinical or biochemical evidence of disturbed calcium homeostasis and who had a wide range of serum albumin concentrations. We used both a standard Ciba-Corning 634 analyzer, which has a membrane-restricted saturated KCl reference electrode bridge, and a modified instrument with a 150 mmol/L NaCl bridge. After adjusting the externally standardized values from each instrument for their least-squares regressions on pH, there was a significant correlation between ionized calcium and albumin only with the standard analyzer. In contrast, only values from the modified instrument correlated with serum chloride; this was not explained by ionic strength or organic anion interferences. We conclude that there is unlikely to be any major advantage in using a membrane-restricted isotonic NaCl reference electrode for in vitro clinical measurements, although it may be of value for in vivo monitoring. The importance of measuring serum albumin when using most commercial ionized calcium analyzers is emphasized.

Indexing Terms: monitoring therapy in vivo · variation, source of · albumin · chloride · electrolytes

There is now considerable evidence that measurement of ionized calcium by most commercial instruments is complicated by the positive interference of protein (1-4). This effect has been demonstrated both in simple solutions and in samples from hospital patients, and is such that spurious hypocalcemia may be diagnosed in hypoalbuminemic patients while true hypercalcemia may be masked. The clinical implications become more important as the number of analyzers for ionized calcium in extralaboratory locations increases. The protein interference appears to be a consequence of the composition of the reference electrode (5-7), which invariably contains an electrolyte solution that is hypertonic with respect to plasma. In vitro experiments have demonstrated that substituting isotonic NaCl for the standard reference electrode solution avoids this effect (5-8). Although this modification itself has the disadvantage of making certain designs of analyzer susceptible to interference by the ionic strength of the sample, this was not true for the membrane-restricted reference electrode of the Ciba-Corning 634 analyzer (Ciba-Corning Diagnostics Ltd., Halstead, UK) (8).

By removing the effect of protein, we expected that the reference limits for ionized calcium in samples of whole blood from a population with normal calcium homeostasis but with a wide range of serum albumin values would be significantly narrower when measured by an analyzer with an isotonic reference electrode than by a conventional instrument. Thus true disturbances in ionized calcium would be more readily detected in patients whose serum albumin was low or unknown. We report here the first study of ionized calcium in such a population with measurements made on both types of instrument.

Patients and Methods

The subjects for this study were 91 adult patients at St. James's University Hospital. Of these, 44 were inpatients on general medical wards and 47 were outpatients. None had any clinically evident calcium or acid-base disturbance. All patients included in the study had normal serum concentrations of creatinine, and intact parathyroid hormone was within the reference range of 10–65 ng/L (Allegro Intact PTH; Nichols Institute Diagnostics, San Juan Capistrano, CA). The procedures followed were in accordance with the standards of the Ethical Committee of St. James's University Hospital.

Venous blood was collected into 2-mL syringes containing 50 μL of calcium-titrated heparin (Radiometer Ltd., Crawley, UK), giving a final concentration of 24 IU of heparin per milliliter of whole blood (9). Samples were stored anaerobically on ice prior to analysis.

Both ionized calcium and pH were measured in duplicate on a Ciba-Corning 634 analyzer within 2 h of collection, and ionized calcium only was measured on a similar machine fitted with a reference electrode filled with 150 mmol/L NaCl instead of the usual saturated KCl solution. This instrument also had a minor electronic modification to allow the voltage to be backed off to within the range required by the software for calibration (8). Once the voltage had been so adjusted, it was not altered during the period of the study. The instruments were calibrated according to the manufacturer's instructions, but readings were made against external calibrators consisting of aqueous solutions of 0.5 and 1.5 mmol/L calcium chloride in 150 mmol/L sodium chloride (Nova Biomedical Corp., Waltham, MA).

Hematocrit was measured on an Ames Microspin microcentrifuge (Bayer Diagnostics, Slough, UK) by using an aliquot of blood remaining from the ionized calcium analysis. A separate sample of venous serum collected at the same time was analyzed on a Parallel analyzer (American Monitor Ltd., Burgess Hill, UK) for sodium and potassium (both by an indirect ion-selective electrode, with between-batch analytical CVs of 0.7% at
139 mmol/L and 1.5% at 4.1 mmol/L, respectively), chloride (mercuric 2,4,6-tripyridyl-s-triazine, CV 1.7% at 100 mmol/L), bicarbonate (phosphoenolpyruvate carboxylase/malate dehydrogenase, CV 2.8% at 30 mmol/L), total calcium (alkaline o-cresolphthalein complexone, CV 1.9% at 2.48 mmol/L), phosphate (polyvinylpyridilone-catalyzed molybdate, CV 1.4% at 1.02 mmol/L), magnesium (alkaline calmagite, CV 1.4% at 0.92 mmol/L), albumin (bromcresol green, CV 1.4% at 40 g/L) and total protein (biuret, CV 1.1% at 68 g/L).

The data were analyzed by the method of least squares.

Results

Ionized calcium measurements on the modified instrument were significantly more precise than with the standard analyzer (CVs of patients' duplicates were 0.42% and 0.85%, respectively, P < 0.001). The between-batch analytical CV for pH was 0.21% at 7.35. The mean (±SD) of the reported ionized calcium values was significantly lower (P < 0.001) when determined by the analyzer with the isotonic NaCl junction: 1.03 (0.051) vs 1.26 (0.068) mmol/L.

The observed ranges and 95% limits (mean ± 2 SD) for the measured values of sodium, potassium, chloride, bicarbonate, total calcium, phosphate, magnesium, albumin, total protein, hematocrit, and pH are given in Table 1, together with those for the calculated anion gap [(Na + K) − (Cl + HCO_3)] , globulin (total protein − albumin), and albumin-adjusted total calcium (total calcium + 0.018 [47 − albumin]).

As expected, ionized calcium correlated significantly with pH when measured by both instruments (hypertonic: r = 0.51, P < 0.001; isotonic: r = 0.41, P < 0.001). The software of the Ciba-Corning 634 contains an algorithm to calculate ionized calcium at pH 7.40. However, because this equation was derived from a population with normal protein concentrations by using the standard instrument, it is not valid for the isotonic version, nor for samples with abnormal concentrations of proteins (10). We therefore derived our own equations to adjust ionized calcium for pH, using the observed least-squares regressions of ionized calcium on pH for the patients we studied (who had a wide range of protein concentrations), as follows:

\[
\text{pH-adjusted } Ca^{2+} = \text{measured } Ca^{2+} + \text{slope } pH - \text{intercept} + \text{mean } Ca^{2+}
\]

The equations used for the two instruments were:

\[
\text{pH-adjusted } Ca^{2+} = \text{measured } Ca^{2+} + 0.83 pH - 6.14 \text{ (hypertonic)}
\]

\[
\text{pH-adjusted } Ca^{2+} = \text{measured } Ca^{2+} + 0.55 pH - 4.04 \text{ (isotonic)}
\]

After adjustment for pH, there was no residual correlation between ionized calcium and hydrogen ion activity. The pH-adjusted ionized calcium correlated weakly but significantly with both hematocrit (positively) and phosphate (negatively); the correlations did not differ between the instruments (Table 2).

Only measurements made on the standard analyzer correlated significantly (positively) with albumin (Figure 1). Although pH-adjusted ionized calcium values from the modified instrument were not affected by albumin, they did correlate significantly with both serum chloride (positively, Figure 2) and bicarbonate (negatively). Because these two anions were themselves correlated (r = −0.34, P = 0.001), chloride could be adjusted for bicarbonate concentration in the same way that ionized calcium was adjusted for pH. When this was done, the correlation between pH-adjusted ionized calcium and chloride remained significant (r = 0.48, P < 0.001). However, the converse was not true; adjustment of bicarbonate concentration for serum chloride weakened its correlation with pH-adjusted ionized calcium such that it was no longer significant.

No significant correlations were found between pH-adjusted ionized calcium and sodium, potassium, magnesium, globulin, or anion gap, when measured on either instrument.

The 95% limits for pH-adjusted ionized calcium were 0.937–1.113 mmol/L for the modified instrument, significantly narrower than the range of 1.152–1.361 mmol/L for the standard analyzer (P = 1.41, P < 0.05). However,
Discussion

The effect of protein concentration on ionized calcium measurement was first described by Ferreira and Bold (11). They demonstrated that serum had a higher ionized calcium concentration than its ultrafiltrate, but a lower concentration than the retained material. Payne (1) subsequently confirmed a positive effect of albumin by using dialysis. It has been argued by Thode et al. (12, 13) that these findings are not caused by direct interference on the ion-selective electrode but are a predictable consequence of the Donnan equilibrium across a semipermeable membrane, leading to an unequal distribution of calcium ions. However, evidence has accumulated over the past decade that albumin causes a direct interference (3, 4), that the interference is at the reference electrode (5) because of its hypertonic nature, and that it can be eliminated by an isotonic junction (6, 7, 14).

The present study has confirmed the previous finding (3) of a significant interference by albumin on the measurement of pH-adjusted ionized calcium in patients' samples when a hypertonic reference electrode is used. This effect has been demonstrated in different populations with three commercial instruments—the Nova 2, the Radiometer ICA1, and now the Ciba-Corning 634. In patients with no disturbance of calcium homeostasis and with the full range of clinically encountered serum albumin concentrations, the 95% limits of pH-adjusted ionized calcium are wider than those determined for patients or normal subjects with normal albumin concentrations, regardless of which of the three instruments is used.

We have also confirmed in patients' samples earlier observations with simple solutions of NaCl, calcium, and albumin that interference by albumin can be eliminated by the use of an isotonic NaCl reference electrode (6, 8). The albumin effect appears to be caused by the coagulation of sample proteins at the hypertonic reference electrode liquid junction, which alters the residual liquid junction potential (RLJJP) in comparison with that of nonprotein-containing solutions (15). Differences in the RLJJP also account for the lower ionized calcium values when the isotonic reference electrode bridge was used (7). The effect of protein on measured potential has been demonstrated on electrode systems selective for ions other than calcium, including sodium, hydrogen (15), and potassium (7), and is of a similar magnitude for each (~2.2 mV/100 g of protein) (15).

The elimination of albumin interference by using an isotonic NaCl reference electrode significantly narrowed the reference range for pH-adjusted ionized calcium, but because the values were lower, the population CV was unchanged. The lower values may represent the true ionized calcium of the samples (14). However, there was a new positive correlation with serum chloride, and another population of patients with a greater range of serum chloride values may be expected to show a wider reference range for ionized calcium when an isotonic reference electrode is used. Explanation of this refer-
ence electrode-dependent chloride effect on ionized calcium is difficult. An effect of bicarbonate, or of anions that might replace it in metabolic acidosis (6), is not supported by the lack of correlation with either chloride-adjusted bicarbonate or anion gap. Nor is the chloride effect a reflection of ionic strength interference because there was no correlation between pH-adjusted ionized calcium and sodium, the major determinant of ionic strength in plasma. This is consistent with the conclusion from in vitro studies that the Ciba-Corning 634 is no more susceptible to ionic strength interference with an isotonic NaCl reference electrode than with an electrode of hypertonic KCl (8). In that particular study the ionic strength was varied by increasing the concentration of NaCl from 100 to 150 mmol/L. Although this was a wider range of chloride concentrations than encountered in the present study, the standard and modified instruments responded no differently. It would seem prudent, therefore, to be cautious in extrapolating observations made on simple solutions to whole blood.

In terms of the clinical interpretation of results reported by the modified analyzer, a mean difference in serum chloride of 10 mmol/L would give rise to a mean difference in pH-adjusted ionized calcium equivalent to 28% of its reference range. For the unmodified instrument, a mean serum albumin difference of 20 g/L would give a similar ionized calcium difference.

We conclude that a membrane-restricted isotonic NaCl reference electrode appears to offer no major clinical advantage for the measurement of ionized calcium, but we reemphasize the importance of measuring albumin to detect hypoalbuminemia when most standard instruments are used. The effect of an isotonic KCl rather than NaCl reference electrode on samples from patients remains to be explored. Although the use of an isotonic NaCl reference electrode appears to have substituted one type of interference for another, the modified instrument performed no worse than the standard design and was actually more precise. This may be exploited for in vivo monitoring of ionized calcium in patients for whom a reference electrode containing saturated potassium chloride would be potentially dangerous. Possible clinical indications for continuous in vivo monitoring include liver transplantation, exchange transfusion, and hemodialysis—all situations in which ionized calcium concentrations are subject to rapid change.

References