Original Article

Albumin-corrected or ionized calcium in renal failure?
What to measure?

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Abstract
Background. Secondary hyperparathyroidism is frequently observed in patients with chronic renal failure, and clinical treatment guidelines have been published. Despite this, a large proportion of patients do not reach the target levels for calcium, phosphorus, calcium × phosphorus product, or intact parathyroid hormone. The use of albumin-corrected calcium is recommended as calcium measurement, but it is the concentration of ionized calcium that is biologically active. We hypothesized that in clinical practice, the use of ionized calcium rather than albumin-corrected calcium would influence the calcium classification of the individual patient.

Methods. Blood samples from 34 patients in chronic haemodialysis were analysed for evaluation of mineral metabolism according to K/DOQI guidelines. Blood for analysis of total and ionized calcium was drawn simultaneously. As ionized calcium is pH dependent, samples were analysed at the actual pH of the individual patient.

Results. For both methods, a similar number of patients were characterized as normocalcaemic. The use of albumin-corrected calcium caused one patient (3%) to be classified as hypocalcaemic, and 10 patients (26%) as hypercalcaemic whereas with ionized calcium, five (15%) and three patients (9%) were classified as hypo- and hypercalcaemic, respectively.

Conclusions. According to present guidelines, the difference in calcium classification of patients might have clinical implications for the prescription of vitamin D, and on the choice of phosphate binders.

Keywords: calcium; chronic renal failure; ionized calcium; secondary hyperparathyroidism

Introduction
Secondary hyperparathyroidism (SHPT) is frequently observed in patients with chronic renal failure [1,2]. SHPT is a result of hypocalcaemia, hyperphosphataemia, and reduced levels of 1,25 dihydroxvitamin D3 [2,3], and is not only associated with renal bone disease but also with excess cardiovascular morbidity and mortality in these patients [1,4,5].

Clinical guidelines for the treatment of disturbances in mineral and bone metabolism in patients with chronic kidney disease have recently been published [6]. The target concentrations for calcium, phosphorus and calcium × phosphorus product are close to the normal range even in patients with chronic kidney disease stage 5.

The main factor for regulation of parathyroid hormone (PTH) secretion is the extra cellular ionized calcium concentration [2,3]. In clinical guidelines, the target level for serum calcium is given as albumin-corrected total calcium concentration and no target level is given for the concentration of ionized calcium [6].

Measured total calcium in serum consists of ~15% bound to organic and inorganic anions, about 40% bound to albumin, and the remaining as biologically active ionized calcium. A variety of formulae have been proposed to permit calculation of the albumin-corrected total calcium or ionized calcium from the total calcium and protein concentration, but no data support the use of such algorithms [7,8]. However the K/DOQI guidelines recommend the use of albumin-corrected total calcium for routine clinical interpretation of calcium [6], and the use of ionized calcium is recommended only when more exact values are required. The sample collection and handling are crucial for an accurate measurement of ionized calcium [7,9].

We have evaluated the use of ionized calcium as opposed to albumin-corrected total calcium in an unselected group of patients in chronic haemodialysis. We hypothesized that the calcium classification of
patients would be different in individual patients depending on the use of ionized- or albumin-corrected calcium levels.

**Subjects and methods**

We investigated all patients undergoing chronic haemodialysis in our department, 23 men and 11 women, median age (range) 59.1 years (18.0–85.4). The median duration (range) in haemodialysis was 21.3 months (2.5–79.7). All patients were dialyzed three times a week, 4–5 h per treatment, using a dialysate calcium concentration of 1.50 mmol/l in all patients except for one patient with a dialysate concentration of 1.25 mmol/l. All patients were treated according to K/DOQI guidelines, 30 patients receiving sevelamer, two patients calcium carbonate and seven patients oral alfacalcidol.

All blood samples were collected midweek prior to next dialysis, and after the patient had been seated for at least 10 min. The patients were not in a fasting state. Arterialized venous blood was collected from the patient’s fistula (n = 29) or venous blood from the permanent dialysis catheter (n = 5). If a tourniquet was required, it was released for more than a minute prior to sampling. All samples were analysed in the hospital laboratory except for intact parathyroid hormone (iPTH), which was analysed in The Hormone Laboratory, University Hospital of Bergen, using a two-site enzyme-labelled immunometric assay (Immulite® 2000 intact PTH). Samples for the measurement of ionized calcium were collected in heparin-free SST gel tubes, blood for pH determination in 80 IU electrolyte-balanced heparin tubes, and the other samples in heparinized tubes. Samples for pH determination were stored and transported on ice. Samples were transported to the hospital laboratory within 30 min. The SST gel tubes were centrifuged within 2 h. Total calcium and albumin were analysed using a photometric technique (Roche Modular automated clinical chemistry analyzer). The calcium assay was calibrated once a day. The albumin-corrected method was estimated with the following equation: albumin-corrected calcium = total calcium + [0.02 × (41.3-albumin g/l)] [10]. Ionized calcium and pH were analysed using an ion-selective electrode (Radiometer Copenhagen ABL 625), and the pH in the sample at the time of analysis was compared to the actual blood pH of the patient at the time of blood collection. The hospital laboratory is a participant in the Danish Institute for External Quality Assurance for laboratories in Health Care (DEKS) and of the Finnish external quality assurance (Labquality). The reference ranges for the laboratory values are given in Table 1.

In an earlier cohort of haemodialysis patients we evaluated the method for sampling and analysing the ionized calcium by comparing the concentrations of ionized calcium and pH in the heparin free tubes and in electrolyte-balanced heparin tubes, which were transported to the laboratory on ice and analysed immediately after arrival. The mean difference in ionized calcium by the two methods was –0.001 ± 0.017 mmol/l. We expected 95% of the difference between the two methods to lie within ±2 SD of the mean, which is defined as the limit of agreement [11]. For ionized calcium concentration variability this limit of agreement was –0.035 to 0.033 mmol/l. The pH variability was –0.023 ± 0.030 pH units, and the limit of agreement was –0.083 to 0.037 pH units. According to the plots, both the ionized calcium concentration and pH variability are thus acceptable, and confirm the reliability of the method in clinical practice.

**Statistical analysis**

Age and time in haemodialysis were not normally distributed and the results are reported as median and range. Descriptive statistics are used for laboratory values. Non-parametric testing, Mann–Whitney, was used to compare continuous observations between two groups of patients. The agreement between categorical assessments was done with kappa statistics, and the degree of agreement between continuous variables was estimated according to Bland and Altman [11]. The arithmetic mean and the absolute differences between the measurements in the same patient were calculated, and an agreement plot was constructed. Any systematic differences between the measurements would result in the mean of the differences being significantly different from zero. The wider the scatter between the points in the direction of the y-axis, the worse will be the agreement. We would expect 95% of the differences to lie within ±2SD of the mean, which is defined as the limit of agreement [11]. The statistical analysis was performed using the StatView packages.

**Results**

Twenty-three patients (68%) had albumin-corrected total calcium within the target level of K/DOQI guidelines defined as the normal range for the laboratory, one patient (3%) had hypocalcaemia, and 10 patients (29%) had hypercalcaemia. Further, if the lower end of the normal range for albumin-corrected calcium, 2.10–2.37 mmol/l, had been used as recommended by the K/DOQI guidelines, then only seven patients (21%) would have been classified as normocalcaemic. Twenty-seven patients (79%) would have been classified as hypercalcaemic if non-corrected total calcium was used, mean

### Table 1. Reference ranges for laboratory values

<table>
<thead>
<tr>
<th>Reference range K/DOQI guidelines</th>
<th>Albumin-corrected total calcium Age 18–49; 2.17–2.47mmol/l</th>
<th>Normal range local laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;50; 2.17–2.53 mmol/l</td>
<td>Non-corrected total calcium 2.15–2.55 mmol/l</td>
<td>ND</td>
</tr>
<tr>
<td>Ionized calcium 1.14–1.32 mmol/l</td>
<td>Phosphorus 0.90–1.40 mmol/l</td>
<td>ND</td>
</tr>
<tr>
<td>0.13–1.78</td>
<td>Target range CKD stage 5</td>
<td>16.5–33.0 pmol/l</td>
</tr>
<tr>
<td>Albumin 35–50 g/l</td>
<td>iPTH 0.7–5.6 pmol/l</td>
<td>ND</td>
</tr>
</tbody>
</table>
| 10 min. The patients were not in a fasting state. Arterialized venous blood was collected from the patient’s fistula (n = 29) or venous blood from the permanent dialysis catheter (n = 5). If a tourniquet was required, it was released for more than a minute prior to sampling. All samples were analysed in the hospital laboratory except for intact parathyroid hormone (iPTH), which was analysed in The Hormone Laboratory, University Hospital of Bergen, using a two-site enzyme-labelled immunometric assay (Immulite® 2000 intact PTH). Samples for the measurement of ionized calcium were collected in heparin-free SST gel tubes, blood for pH determination in 80 IU electrolyte-balanced heparin tubes, and the other samples in heparinized tubes. Samples for pH determination were stored and transported on ice. Samples were transported to the hospital laboratory within 30 min. The SST gel tubes were centrifuged within 2 h. Total calcium and albumin were analysed using a photometric technique (Roche Modular automated clinical chemistry analyzer). The calcium assay was calibrated once a day. The albumin-corrected method was estimated with the following equation: albumin-corrected calcium = total calcium + [0.02 × (41.3-albumin g/l)] [10]. Ionized calcium and pH were analysed using an ion-selective electrode (Radiometer Copenhagen ABL 625), and the pH in the sample at the time of analysis was compared to the actual blood pH of the patient at the time of blood collection. The hospital laboratory is a participant in the Danish Institute for External Quality Assurance for laboratories in Health Care (DEKS) and of the Finnish external quality assurance (Labquality). The reference ranges for the laboratory values are given in Table 1.

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Calcium concentrations (SD) were 2.39 ± 0.15 mmol/l. The mean albumin concentration (SD) was 37.2 ± 4.5 g/l (range 25.0–46.0).

Using ionized calcium, 26 patients (76%) fell within the reference range for the laboratory. Five patients (15%) had hypocalcaemia, and only three patients (9%) had hypercalcaemia.

Twelve patients (35%) had phosphorus within the target range, four patients (12%) had hypophosphataemia, and 18 patients (53%) had hyperphosphataemia. Nineteen patients (56%) had a calcium × phosphorus product of <4.4 mmol²/l².

Fourteen patients (41%) had iPTH within the target range of 16.5–33.0 pmol/l, 10 patients (29%) had a lower value, and 10 patients (29%) a higher value than the target range for iPTH.

Seven patients (21%) were misclassified as hypercalcaemic by the albumin-corrected calcium compared to ionized calcium concentrations, and 19 patients (56%) were classified as normocalcaemic with both measurements. The use of albumin-corrected total calcium seems to underestimate the prevalence of hypocalcaemia and overestimate the prevalence of hypercalcaemia as compared to use of ionized calcium. The characteristics of these patients are given in Table 2, and there were no significant differences between the groups of patients with respect to the concentrations of albumin, phosphorus or iPTH.

### Discussion

Our results indicate that in our laboratory, the calcium classification of patients, and subsequently the clinician’s therapeutic recommendations, will differ depending on use of ionized calcium or albumin-corrected calcium as reference values.

In our laboratory serum calcium is analysed using a photometric method, and the analysis is compared to a Nordic reference population [10]. The total error of the analysis should be <2.4% [12]. The analytical imprecision in our laboratory for the analysis of calcium is 2.3%. The formula used in our laboratory for albumin correction differs slightly from the K/DOQI recommendations. However, use of the formula recommended by the K/DOQI guidelines does not alter the clinical classification.

The concentration of ionized calcium is pH dependent, since hydrogen ions compete with calcium and also magnesium for binding sites on albumin and other proteins [13]. The ionized calcium concentration decreases with 0.36 mmol/l per 1 pH unit increase. In our laboratory the ionized calcium concentrations are analysed at the actual pH level of the patient and is not corrected to pH 7.40. Metabolic acidosis is prevalent in patients with chronic renal failure, and a correction to pH 7.40 would then have underestimated the actual concentrations of ionized calcium. The pH in the sample is well within the limit for the actual pH in the patients at the sample time, and we have also evaluated the method for changes in ionized calcium measured immediately after sampling compared to our standard method. Changes in pH and ionized calcium concentrations from sampling time to time of analysis can therefore not explain the differences in patient’s calcium classification between albumin-corrected calcium and ionized calcium concentrations.

Use of albumin-corrected calcium concentrations may lead to inappropriate clinical decisions with withdrawal of vitamin D, calcium containing phosphate binders and reduction of calcium concentration in the dialysis fluid of a patient classified as hypercalcaemic. In our study, fewer patients would have been classified as hypercalcaemic using ionized calcium than would have been the case if albumin-corrected calcium was used for calcium classification. The number of patients is small with only three patients classified as hypercalcaemic with both measurements, and it is not possible to characterize these patients further.

Although the measurement of ionized calcium maybe more costly, prospective studies comparing albumin-corrected calcium to ionized calcium as biochemical guides for optimizing treatment of SHPT in patients with chronic renal failure, should be considered. In our study albumin-corrected calcium could not substitute for ionized calcium in classifying patients as hypo-, normo- or hypercalcaemic.

**Conflict of interest statement.** None declared.

### Table 2. Number (%) of patients classified as hypo-, normo- or hypercalcaemic using ionized-, albumin-corrected total, and non-corrected total calcium concentrations

<table>
<thead>
<tr>
<th></th>
<th>Ionized calcium</th>
<th>Albumin-corrected total calcium</th>
<th>Non-corrected total calcium</th>
<th>Albumin g/l</th>
<th>Phosphorus mmol/l</th>
<th>iPTH pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypocalcaemia</td>
<td>5 (15%)</td>
<td>1 (3%)</td>
<td>3 (9%)</td>
<td>38.0 (28.0–41.0)</td>
<td>1.9 (1.2–3.2)</td>
<td>29.6 (17.3–58.5)</td>
</tr>
<tr>
<td>Normocalcaemia</td>
<td>26 (76%)</td>
<td>23 (68%)</td>
<td>24 (71%)</td>
<td>38.0 (25.0–46.0)</td>
<td>1.8 (0.8–2.9)</td>
<td>23.5 (3.5–48.4)</td>
</tr>
<tr>
<td>Hypercalcaemia</td>
<td>3 (9%)</td>
<td>10 (29%)</td>
<td>7 (20%)</td>
<td>38.0 (34.0–43.0)</td>
<td>1.3 (1.2–1.9)</td>
<td>44.0 (9.5–45.1)</td>
</tr>
</tbody>
</table>

Concentrations, median (range) of albumin, phosphorus, and iPTH in the different groups of patients in which the patients are classified according to ionized calcium concentrations.
References


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