Adjustment of serum total calcium for albumin concentration: effects of non-linearity and of regression differences between laboratories

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SUMMARY. It has been reported that the relationship between serum total calcium and albumin concentrations in hospital patients deviates from linearity at low albumin concentrations. We searched a large laboratory computer data base for adult patients with discretionary requests for serum calcium analysis but with no other data suggestive of disturbances of calcium homeostasis, and collected a minimum of 100 calcium values at each of a wide range of albumin concentrations. We confirmed the earlier observation, but found that the use of a single regression to derive an adjustment of total calcium for albumin gave only small differences of no clinical significance.

To investigate whether equations to adjust total calcium for albumin can be transferred between laboratories, three laboratory computers were searched for calcium requests from patients likely to have a low prevalence of calcium disturbances. The regressions of total calcium on albumin differed significantly between laboratories, but within each laboratory gave adjusted calcium values identical with those in health. Although the errors resulting from applying an equation from one laboratory to another were small and unlikely to be of major clinical significance, we recommend that where possible laboratories should derive adjustment equations from their own data.

Additional key phrases: reference ranges; probit analysis

Ideally plasma calcium homeostasis should be assessed by measuring the physiologically active ionized fraction. Total calcium concentration is potentially unreliable because normally approximately 42% is protein-bound, largely to albumin, and this fraction varies with abnormalities in protein concentration.[1,2] In the absence of acid base disturbances, a more practical method is to adjust the calcium value for the albumin concentration using an equation based on their regression. The use of this technique with prospective analyses over a wide range of albumin concentrations has been shown to give adjusted calcium values with confidence limits identical with the total calcium reference range.[3]

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In 1986, Ashby *et al.*[4] published an investigation of the relationship between calcium and albumin in a large data base derived from two non-discretionary analysers after excluding data from patients with abnormal results suggestive of bone, liver or kidney disease. They made two main observations. First, the relationship between calcium and albumin appeared to be linear only over the albumin concentration range 32–50 g/L. Secondly, the two analysers gave data that fitted different equations, even though the analytical principles were the same.

We have made an extensive search of one laboratory data base to re-examine linearity and have also examined the relationship between total calcium and albumin in data from three different hospitals to determine whether equations for calcium adjustment can be transferred between laboratories.

PATIENTS AND METHODS

Criteria for the selection of patients from computer records

Three laboratory computers (TelePath Systems Ltd, Birmingham, UK) were searched for patients aged over 18 years with calcium and albumin measurements. Patients were excluded if their serum urea concentration exceeded 15 mmol/L, their serum creatinine concentration exceeded 200 μmol/L or their serum potassium concentration was less than 3·5 mmol/L. Aspartate transaminase or alkaline phosphate activity greater than the laboratory upper reference limit were also grounds for exclusion. All patients attending departments of endocrinology, haematology, oncology or nephrology were excluded, as were patients under the care of the general surgeons who used total parenteral nutrition. Only one result per individual patient was collected and the data for each hospital were analysed separately. The search of the Leeds General Infirmary database was extended so that at least 100 total calcium values were collected from eligible patients at each albumin concentration (rounded to integers) over the range 20–55 g/L.

Analytical methods

Measurements were made at Leeds General Infirmary on Hitachi 747 and 717 analysers (Boehringer–Mannheim plc, Lewes, UK) using the manufacturer's *o*-cresolphthalein complexone and bromocresol green (BCG) reagents; at St James's University Hospital both on a Dax analyser (Bayer plc, Basingstoke, UK) using the manufacturer's *o*-cresolphthalein complexone and BCG reagents and on a CX7 analyser (Beckman International (UK) Ltd, High Wycombe, UK) using arsenazo III dye for calcium and Bayer's BCG reagent for albumin, and at York District Hospital on a Hitachi 717 analyser using *o*-cresolphthalein complexone and BCG reagents from Randox (Crumlin, UK). The analytical imprecision (coefficients of variation) at normal concentrations over 30 days was 0·71–1·43% for calcium and 1·09–1·70% for albumin for the three laboratories. All methods were linear for albumin and calcium over a wide range. None of the laboratories showed significant or consistent bias for either calcium or albumin on external quality assurance schemes.

Derivation of equations to adjust calcium for albumin

The adjustment equation for each population was derived as follows. The least squares regression coefficient of calcium on albumin and the calcium intercept at zero albumin concentration were calculated. The intercept value was taken to be the mean non-protein-bound calcium concentration of the population. This value was subtracted from the mean of the reference range to obtain the average normal protein-bound calcium. Individual calcium values were then adjusted for albumin by subtracting the product of the slope of the regression and the albumin concentration from the measured total calcium and adding a constant, the average normal protein-bound concentration, using the equation:

$$\text{Adjusted calcium} = \text{total calcium} - (\text{slope} * \text{albumin}) + (\text{mean normal total calcium} - \text{intercept calcium})$$

For example, if the regression equation were

$$\text{Calcium (mmol/L)} = [0{\cdot}025 * \text{albumin(g/L)}] + 1{\cdot}40\text{(mmol/L)}$$

and the reference range for total calcium were 2·20–2·60 mmol/l (mean 2·40), then

$$\text{Adjusted calcium} = \text{total calcium} - [0{\cdot}025 * \text{albumin}] + [2{\cdot}40\text{–}1{\cdot}40]\ \text{mmol/L}$$

Reference ranges

Reference ranges for total calcium concentration were derived from the analysis of samples from 200 healthy blood donors at Leeds General Infirmary and from 117 blood donors and 290 general practitioner haematology out-patients at St James's. The 95% confidence limits were 2·25–2·60 mmol/L and 2·20–2·60 mmol/L, respectively. The York range of 2·10–2·60 mmol/L was derived from hospital data.

Statistics

Least squares regression and probit analysis were performed using the Astute® statistics add-in package for Microsoft Excel (DDU Software, University of Leeds, Leeds, UK). Probit (normal probability) plots of the adjusted calcium values were constructed to assess the distribution and best straight lines were drawn to exclude outliers and determine the 2·5 and 97·5 percentile values.

RESULTS

Relationship of calcium with albumin across a wide range of albumin values

The relationship between mean total serum calcium and albumin concentrations derived from the Leeds General Infirmary database, with results from a minimum of 100 patients at each integral albumin concentration over the range 20–51 g/L, is shown in Fig. 1. The regression of

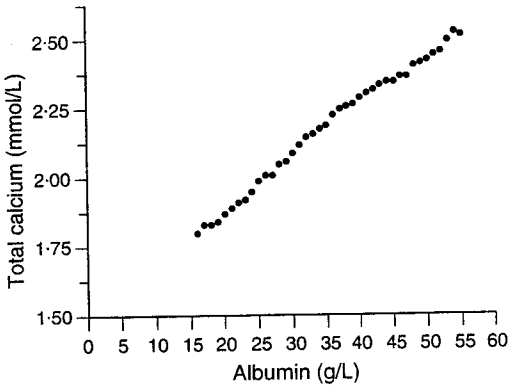

FIGURE 1. *Mean total serum calcium concentration against albumin concentration in selected patients from one laboratory: at least 100 data points were collected at each integral albumin concentration from 20 to 51 g/L.*

calcium on albumin over the albumin range 16–35 g/L inclusive had a slope of 0·0219 (standard error, SE, 0·0004) mmol/g and over the range 37–55 g/L inclusive had a slope of 0·0155 (SE 0·0005) mmol/g ($P<0·001$). The regression lines met at an albumin concentration of 36 g/L and a calcium concentration of 2·22 mmol/L. The overall slope was 0·0188 (SE 0·0003) mmol/g and the intercept 1·5162 (SE 0·0119) mmol/L. The predicted calcium concentrations using the overall slope were higher than those using the slope derived from the lower albumin concentration by 0·005 mmol/L at an albumin concentration of 25 g/L and lower at 0·011 mmol/L at an albumin of 30 g/L.

Comparison of derived adjustment equations between hospitals

The regression equations of serum total calcium on albumin concentration from consecutive adult patients at each hospital who did not fail the exclusion criteria showed significant differences between hospitals (Table 1). The derived adjustment equations were:

Leeds General Infirmary:

$$\text{Adjusted calcium} = \text{total calcium} - (0·0194 * \text{albumin}) + 0·857$$

or

$$\text{Adjusted calcium} = \text{total calcium} + 0·0194\ (44·2 - \text{albumin}) \quad (1)$$

St James's University Hospital:

$$\text{Adjusted calcium} = \text{total calcium} - (0·0160 * \text{albumin}) + 0·744$$

or

$$\text{Adjusted calcium} = \text{total calcium} + 0·0160\ (46·5 - \text{albumin}) \quad (2)$$

York District Hospital:

$$\text{Adjusted calcium} = \text{total calcium} - (0·0232 * \text{albumin}) + 0·981$$

or

$$\text{Adjusted calcium} = \text{total calcium} + 0·0232\ (42·3 - \text{albumin}) \quad (3)$$

The York regression equation had the steepest slope and the predicted calcium values were lower than those from the equation with the shallowest slope (St James's) by 0·057 mmol/L at an albumin concentration of 25 g/L and by 0·021 mmol/L at an albumin of 30 g/L.

Validation of regression equations

At Leeds General Infirmary and St James's the adjusted calcium equations were validated by applying them prospectively to subsequent data sets collected using the same exclusion criteria. Submission of the second sets of data to the previously derived equations gave 95% confidence limits for the adjusted calcium values which were identical to the original sets of data from each hospital.

DISCUSSION

We have confirmed the non-linearity of the relationship between serum total calcium and albumin concentration reported by Ashby *et al.*,[4] finding instead two straight lines. The values at which they intersected, 2·22 mmol/L for calcium and 37 g/L for albumin (Fig. 1), were each at the lower limit of their respective reference range. Inspection of the data of Ashby *et al.*[4] (see their Fig. 3) also suggests the presence of two lines intersecting at similar values. This raises the possibility that the increase in globulins that commonly accompanies a decrease in albumin may play a role in the steeper slope at lower albumin concentrations. BCG binds to globulins to a small extent and gives values higher than true albumin.[5] However, when a method with a short reaction time was used, as it was in our laboratories, BCG was found to measure a constant globulin component over the whole range of albumin concentrations encountered in patients.[6] Thus, this explanation seems unlikely unless interaction with globulins varies with the formulation of BCG reagent.

TABLE 1. *Regression data of total serum calcium on albumin from three hospital laboratory (lab) databases. A = Leeds General infirmary, B = St James's University Hospital, C = York District Hospital*

	Lab A	Lab B	Lab C	A versus B	B versus C	A versus C
Sample size	700	760	193			
r	0·714	0·643	0·790	$P>0·03$	$P>0·03$	$P>0·03$
Slope (mmol/g)	0·01944	0·0160	0·0232	$P<0·001$	$P<0·02$	$P<0·001$
SEM	0·000722	0·000693	0·00130			
Intercept (mmol/L)	1·568	1·656	1·419	$P<0·05$	$P<0·01$	$P<0·001$
SEM	0·0285	0·0278	0·0498			

SEM = standard error of the mean

An alternative explanation is that the change in slope is a consequence of the recruitment of calcium-binding sites of lower affinity as the relative concentration of albumin increases. This also seems unlikely as experimental evidence indicates that human albumin has 12 identical and negligibly interacting calcium-binding sites.[7]

Whatever the explanation, there would appear to be no benefit to be gained from the use of different regression equations above and below the intersection of the lines, since the differences in adjusted calcium values obtained by using separate equations are of little or no clinical significance.

At St James's Hospital the slope of total calcium on albumin concentration has been re-evaluated over a number of years.[3,6,8] It had not changed significantly from 0·025 mmol/g of albumin until 3 years ago. At that time an astute physician noted that he had started to see rather more marginally low values than formerly. It was found that the slope had become 0·016 mmol/g (and has remained so since[1]) in spite of there having been no change in instrumentation or source of reagents, and no quality control problems. A similar change was reported by another user of the same instrument (Swaminathan R, personal communication). We suspect that a change in the manufacturer's reagent formulation had resulted in reduced reaction of the BCG reagent with globulins. This was not detected on external quality assessment schemes because low albumin specimens invariably have low globulins, presumably because they are manufactured by dilution of whole serum. Differences in BCG formulation between manufacturers might also have accounted for the change in regression noted by Ashby *et al.*[4] when they changed instruments.

We have found significant differences between the regressions of total calcium on albumin in data selected using the same criteria from three laboratory data bases, with slopes ranging from 0·0160 to 0·0232 mmol/g. It is of particular significance that two of the laboratories used the same instruments but obtained reagents from different sources, adding weight to the suggestion that it may be the formulation of BCG reagent that accounts for the differences.

Within each laboratory adjustment of total calcium values for albumin concentration using algorithms derived from their own regressions gave values which, after small numbers of outliers were excluded by probit analysis, were identical with those in health. Although the errors resulting from applying an equation from one laboratory to another are small and unlikely to be of major clinical significance, we recommend that where possible laboratories should derive adjustment equations from their own data and should monitor their validity over time.

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