



The Association for Clinical Biochemistry & Laboratory Medicine

Better Science, Better Testing, Better Care

Albumin-adjusted calcium: a position paper

Foreward

This position paper provides interim guidance on albumin-adjusted calcium and was prepared by a working group established by the Clinical Practice Section of the Association for Clinical Biochemistry and Laboratory Medicine. The guidance is 'interim' in the sense that the working group recognised the need for a systematic review on this topic to support evidence based practice recommendations. A review has now been commissioned by the Clinical Sciences Reviews Committee of the ACB which will incorporate practice based recommendations and will be available in due course. Guidance based on a systematic literature review inevitably will have a more rigorous foundation than the collective wisdom of a working group. Furthermore the review may identify additional points not covered in the present document

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Albumin-adjusted calcium: a position paper

Introduction

There is evidence of variability in the reporting of albumin-adjusted calcium by UK laboratories. The variability encompasses:

- i) whether it is reported [*i.e.* not all laboratories report adjusted calcium]
- ii) what equation is used
- iii) if total and adjusted calcium are both/singly reported
- iv) whether the test is called 'adjusted' or 'corrected' calcium

The purpose of the present paper is to make recommendations on the calculation of reported and adjusted calcium and to identify areas of further research. There has been no recent systematic review of the biomedical literature base on this topic; such a review would be of value in supporting practice guideline development.

Recommendation 1: a systematic review of the biomedical literature base relating to the adjustment of total serum calcium should be undertaken.

Background

The average human contains around 25moles of calcium, the majority of which [99%] is in the skeleton. Only around 1% is found in soft tissues and the extracellular fluid compartment. In addition to its important mechanical role in the skeleton, calcium in soft tissues and the extracellular compartment plays many important roles in muscle function, hormone secretion and action, coagulation, cell membrane potential and cell permeability.

Disturbances in calcium metabolism are common and the measurement of serum or plasma calcium is an important and commonly requested test.

Calcium is present in serum in three different physiological states:

- protein bound calcium [principally albumin but also globulins] - 40%
- calcium complexed with small anions
[including bicarbonate, lactate, citrate, phosphate] - 10%
- free ionised calcium – 50%

It is generally considered that free ionised calcium [accounting for around 50% of the total] is the biologically active species and the concentration of which is tightly regulated by hormones including PTH and Vitamin D.

The majority of protein bound calcium is albumin associated. This binding is charge dependent and is therefore affected by the ambient p H with acidosis reducing and alkalosis increasing binding resulting in increased and decreased ionised calcium concentrations respectively . Altered concentrations of other proteins such as globulins may also affect total calcium *e.g.* paraproteinaemia may be associated with increased calcium binding and increased total serum calcium.

Although free ionised calcium represents the biologically active species, free ionised calcium measurement [generally referred to as ‘ionised calcium’] by ion selective electrode is not universally available and the measurement of total serum calcium remains by far the most commonly requested test to assess blood calcium status. However it has been recognised for over 50 years that variation in albumin concentration will give rise to variation in total calcium concentration in the absence of any abnormality in the free ionised calcium concentration.^{1,2}

This has led to the concept of ‘adjusting’ or ‘correcting’ the measured total calcium concentration to take into account the albumin concentration. The terms ‘adjusted’ and ‘corrected’ calcium have been used interchangeably; ‘adjusted’ calcium is the preferred term as ‘corrected’ implies an error in the initial result which has now been eliminated.

Recommendation 2: The preferred term is adjusted calcium.

Over the years many equations for adjusting calcium have been published.³⁻⁵ These have generally been based on the liner regression of serum calcium on albumin and derived in single laboratory using a specific calcium and albumin methods. There are significant differences between the reported adjustment equations which most probably relate to to case-mix as well as differences in the analytical techniques employed, in particular for the measurement of albumin *e.g.* the use of bromocresol green v. bromocresol purple, the particular formulation of the binding dye and the analytical platform on which the assay is performed. To what extent differences in calcium and albumin assay methods contributes to differences in reported equations is less clear.

One commonly used equation which continues to feature widely in popular medical textbooks (and therefore familiar to clinical staff) states:⁶

Adjusted calcium = total calcium + 0.02 [40- albumin]

(Where calcium units are mmol/L and albumin units g/L)

This equation was derived for a calcium O-cresophthalein complexone methods and a bromocresol green albumin method). However this equation maybe invalid when applied to calcium and albumin results generated by alternative assays.

There is good evidence that hypoalbuminaemic patients may be classified differently i.e as normocalcaemic, hypocalcemic, or hypercalcaemic by different adjustment equations.^{4,5} Although this has the potential to result in misdiagnosis it is unclear to what extent patient outcomes have been adversely affected by the use of inappropriate adjustment equations.

Nevertheless given the differences in published adjustment equations, it would appear desirable that individual laboratories derive local equations based on the regression of calcium on albumin concentration in patients specific for their calcium and albumin methods. Few robust studies have been undertaken which have compared the accuracy of adjustment equations against ionised calcium measurement in correctly classifying patients as hypo - , hyper- or normo-calcaemic.⁵

Current UK Practice

There is evidence from the UK and elsewhere of wide variability in practice.⁷ Firstly not all laboratories report adjusted calcium and secondly, there is variability in what adjustment equation is used and how this is derived. A WEQAS survey in 2012 found that 42.6% of laboratories surveyed and which reported adjusted calcium used the traditional adjustment equation of:⁷

Adjusted calcium = total calcium + 0.02 [40- albumin]

As indicated above, this equation may not be applicable to the calcium and albumin methods used by individual laboratories. For laboratories using different equations, it is unclear whether these were locally generated or literature derived [with or without local validation]. It is unclear whether there has been in change in UK practice since this survey was undertaken

Recommendation 3:

A further survey should be undertaken to assess current UK practice in reporting adjusted calcium.

There is no scientific basis to support this variability in practice [i.e. whether adjusted calcium is reported and what adjustment equation should be used] which has the potential to result in patients being managed differently depending in which laboratory the blood sample is analysed

Recommendation 4:

There should be a harmonised approach to the adjustment of calcium for albumin concentration in the UK.

There is general agreement that adjusted calcium provides better diagnostic accuracy in classifying patients as hypo - , hyper- or normo-calcaemic.⁵ Failure of laboratories to report adjusted calcium may result in clinical staff calculating adjusted calcium using inappropriate adjustment equations.

Recommendation 5:

All laboratories providing total calcium measurements should report adjusted calcium.

Given the variability of adjustment equations between different albumin and calcium analytical methods it is proposed that laboratories should use equations based on the regression of calcium on albumin concentration specific for their calcium and albumin methods and analytical platform.^{5,8} This may either be using a locally derived equation or by validation of an existing equation.

Recommendation 6:

Laboratories should use locally derived equations specific to their calcium/albumin methods and analytical platforms rather than unvalidated literature derived equations.

How to generate a locally derived equation

Descriptions of how to derive local adjustment equations are given in the biomedical literature.^{5,8,9}

Data for regression analysis should be extracted from the laboratory information system for calcium and albumin measurements on individual patients. The aim is to include data on patients with a broad range of albumin concentrations and in whom there are no other conditions that might affect calcium homeostasis, such as renal disease, endocrine disease, malignancy, or vitamin D deficiency.

For patients with multiple calcium/ albumin measurements, only the first result (single result per patient) should be collected. Albumin concentrations have generally been restricted to the range 20-50g/L. Although there is evidence that the adult derived adjustment equations may be valid in children down to the age of one year, only data from adult patients *i.e* > 18 years should be analysed.

It is necessary to exclude patients in whom there are other conditions that might affect calcium homeostasis.^{5,8} The following patient groups with conditions that might influence calcium metabolism should be excluded:

- Patients with renal impairment [creatinine > 200umol/L or urea >15mmol/L]
- Hypomagnesaemia [hypokalaemia as a surrogate marker *i.e.* K >3.5 and < 5.5mmol/L]
- Liver disease [ALT/ALP > upper reference limit]
- Total calcium concentration <2.0 and >2.7 mmol/L
- Hypo/hyperparathyroidism *i.e.* PTH outside the healthy population reference range
- Vitamin D deficiency
- Vitamin D toxicity
- Hypoadrenalism
- Patients on parenteral nutrition
- Patients with malignancy

The identification of patients fulfilling these exclusion categories may be logistically challenging. Only a small fraction of patients for example will have had other relevant blood tests taken, in particular PTH and vitamin D measurement. It may be difficult from the laboratory information system to identify reliably certain categories of patients *e.g.* those on parenteral nutrition or with malignancy.

Therefore a pragmatic approach appropriate to local circumstances might be used based on the source of the test request e.g. excluding patients from nephrology, endocrinology, oncology, and haematology.

Ideally 1000 values should be collected with at least 30 data points for each whole integer albumin concentration.

Perform linear regression analysis of calcium on albumin using appropriate statistical software. Least squares or Deming regression may be used. Calculate the slope of the best fit line, the 'Y' axis intercept (which represents the projected calcium concentration at zero albumin concentration) and the correlation coefficient (r).

The adjustment equation is given as follows:

$$\text{Adjusted [Ca]} = \text{Total [Ca]} - (\text{slope} \cdot [\text{albumin}]) + (\text{mean total [Ca]} - \text{intercept [Ca]})$$

Where

- the slope is the slope of the best fit regression line
- the mean total calcium is the mid-point of the healthy population calcium reference range. For the Pathology Harmony proposed reference range of 2.2 to 2.6mmol/L, this will be 2.4 mmol/L.

This adjustment equation should remain valid in the absence of any change in the albumin or calcium assay, change in formulation of assay reagents by the manufacturer, change in reagent source or analytical platform, or any evidence pointing to a change in analytical performance of the albumin or calcium assays. Nevertheless it would seem prudent that the validity of the equation over time is monitored.⁸ The required frequency will depend on the stability of the calcium and albumin assays. However in the event of any change in assay or assay performance it will be necessary to derive a new adjustment equation. Laboratories should participate in an accredited adjusted calcium external quality assurance scheme.

Recommendation 7:

The validity of the adjustment equation should be monitored with time [the required frequency will depend on the stability of calcium and albumin assays].

Recommendation 8:

The adjustment equation should be recalculated in the event of any change in the albumin or calcium assay, change in the formulation of assay reagents by the manufacturer, change in reagent source or analytical platform or any evidence pointing to a change in the analytical performance of the albumin or calcium assays.

Recommendation 9:

Laboratories reporting adjusted calcium should participate in an accredited External Quality Assurance scheme for adjusted calcium.

Given that there is uncertainty in the range of clinical states over which adjusted calcium concentration may be valid *i.e.* evidence that it may not be valid in critically patients, dialysis patients, neonates, presence of jaundice,¹⁰⁻¹² it appears appropriate that the measured total calcium is reported alongside the adjusted calcium concentration.

Recommendation 10:

The measured total calcium concentration should be reported alongside the adjusted calcium concentration.

Further Research

Although widely adopted, there remain a number of uncertainties regarding the use of adjusted calcium. There have been few robust studies which have compared the diagnostic value of adjusted calcium in correctly defining calcium status against the gold standard of ionised calcium measurements. There is uncertainty about the situations in which adjusted calcium should not be reported. For example there is evidence suggesting that it may be unreliable in critically ill patients, in dialysis patients, neonates and in the presence of jaundice.⁹⁻¹¹ Finally there is an absence of evidence on the extent to which the reporting of adjusted calcium using locally derived equations [rather than literature derived equations] results in improved patient outcomes; it is recognised that such evidence will be difficult to obtain.

This document was prepared by a working group established by the Clinical Practice Section of the Association for Clinical Biochemistry and Laboratory Medicine. See Appendix for membership of the working group.

Summary of recommendations

Recommendation 1: a systematic review of the biomedical literature base relating to the adjustment of total serum calcium should be undertaken.

Recommendation 2: The preferred term is adjusted calcium.

Recommendation 3: A further survey should be undertaken to assess current UK practice in reporting adjusted calcium.

Recommendation 4: There should be a harmonised approach to the adjustment of calcium for albumin concentration in the UK.

Recommendation 5: All laboratories providing total calcium measurements should report adjusted calcium.

Recommendation 6: Laboratories should use locally derived equations specific to their calcium/albumin methods and analytical platforms rather than unvalidated literature derived equations.

Recommendation 7: The validity of the adjustment equation should be monitored with time [the required frequency will depend on the stability of calcium and albumin assays].

Recommendation 8: The adjustment equation should be recalculated In the event of any change in the albumin or calcium assay, change in the formulation of assay reagents by the manufacturer, change in reagent source or analytical platform or any evidence pointing to a change in the analytical performance of the albumin or calcium assays.

Recommendation 9: Laboratories reporting adjusted calcium should participate in an accredited External Quality Assurance scheme for adjusted calcium.

Recommendation 10: The measured total calcium concentration should be reported alongside the adjusted calcium concentration.

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Appendix

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