Malays J Pathol 2020; 42(3): 385 – 394

ORIGINAL ARTICLE

Limitations of calculated ionised calcium & adjusted calcium in critically ill patients: Time to consider measured ionised calcium

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Abstract

Introduction: Ionised calcium is a good prognostic and diagnostic tool as opposed to total calcium in critical patients but is not available in most central laboratories and non-intensive care units. To date, four equations to calculate ionised calcium in critical patients have been published. Objectives: (1) Evaluate the four published equations' performance in estimating ionised calcium; (2) Determine the accuracy of calculated ionised and adjusted total calcium in classifying patients according to calcium states; and (3) Identify factors associated with hypocalcaemia in the critically ill population. *Materials and methods:* This is a cross-sectional study involving 281 critically ill patients aged 18-80 years of both genders in a Malaysian tertiary intensive care unit. Performance of the four equations was analysed using Bland-Altman difference plot and Passing Bablok regression analysis. Cross-tabulation was conducted to assess classification accuracy. Mann-Whitney U or Pearson Chi-Square tests were performed to identify variables associated with hypocalcaemia. *Results:* Calculated ionised calcium using all four equations significantly overestimated ionised calcium. Calculated ionised and adjusted total calcium had poor accuracies in classifying hypocalcaemic patients. pH was significantly higher in hypocalcaemics. *Conclusion:* Calculated ionised and adjusted total calcium significantly overestimate ionised calcium in the critically ill. In this specific population, calcium status should only be confirmed with ionised calcium measured by direct ion-selective electrode (ISE).

Keywords: measured ionised calcium; estimated ionised calcium; calculated ionised calcium; ionised calcium formula; adjusted calcium

INTRODUCTION

Calcium plays an important role in structural, neuromuscular, enzymic and signalling processes in the human body. 99% of total body calcium is found in the skeleton with the remaining 0.6% in the interstitium and only 0.4% in plasma.[1] The biologically active form i.e. ionised calcium makes up approximately half of the total circulating calcium while the remaining 40% is bound to albumin and 10% bound to anions such as phosphate, sulphate and citrate.[2,3] Calcium is measured either in its free ionised form by direct ISE method or as total calcium by automated spectrophotometry.

Hypocalcaemia is seen in up to 88% of critically ill patients while hypercalcaemia is less prevalent, affecting largely those with malignancies and hyperparathyroidism.[3,4] Clinical features of hypocalcaemia include stupor, paraesthesia, tetany, convulsions and cardiac arrhythmias.[1] Hypocalcaemia is also a predictor of mortality, prolonged hospital stay and increased APACHE II scores.[3,5-7] While total calcium has lesser pre-analytical issues and is more widely available in medical facilities, it is poorly indicative of actual calcium status especially in critical patients due to disturbances in pH, albumin, phosphate and fatty acid levels.[3,7,8] For this reason, ionised calcium is preferentially measured in critical care.

However, ionised calcium analysis is largely unavailable in most central laboratories due to costing, pre-analytical and technical issues. In most centres, bench-top analysers providing ionised calcium analysis are only available to critical patients in the intensive care unit (ICU). As not all critically ill patients are admitted to

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the ICU due to bed shortages, ward physicians rely on adjusted total calcium values to determine calcium status. However, studies have shown that adjusted calcium poorly agrees with measured ionised calcium in patients with chronic renal disease, renal transplants, parenteral nutrition, polytrauma and other critical illnesses.[9-11] To overcome this, equations to calculate ionised calcium have been formulated although many were derived from healthy subjects and only few tested on critically ill patients. To date, four equations to calculate ionised calcium derived from critical patients have been published - three of which were from the same study by Antonio JM in 2015 (Table 1).[12,13]

To the authors' knowledge, these four equations have not been compared in critically ill patients. Using measured ionised calcium as the reference method, this study aims to:

(i) Evaluate the performance of these four equations in estimating ionised calcium,
(ii) Determine the accuracy of calculated ionised and adjusted total calcium in classifying patients according to calcium states,
(iii) Identify factors associated with hypocalcaemia in the critically ill population,
(iv) Determine the applicability of calculated ionised calcium in critical patients.

MATERIALS & METHOD

Study design & ethics

This research is a single-centered cross-sectional study conducted on all patients aged between 18 and 80 years admitted to Hospital Selayang main ICU between June and December 2017. Only patients in their first 48 hours of admission to the ICU were included. Exclusion criteria include subjects whose ionised calcium was measured by analysers other than the ICU, duplicate subjects, non-consented participation and subjects who have had therapy known to alter calcium levels administered between the time of ionised calcium and total calcium samplings. Ethical approval for this study was obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia with reference number NMRR-16-2375-33343.

Instruments

Instruments involved in this study included one unit of ABL-800 (Radiometer Medical, Copenhagen, Denmark) in the ICU and 2 units of Olympus AU-2700 (Beckman Coulter, Tokyo, Japan) in the laboratory. The former measures ionised calcium by direct ISE and the latter measures total plasma calcium (o-Cresolphthalein-complexone), plasma albumin (Bromocresol green) and plasma creatinine (enzymatic) by automated spectrophotometry. Method comparison between the two AU-2700 analysers was performed and approved for clinical use before operation. Scheduled quality checks and calibrations for all instruments were met during the study period to ensure accuracy of results.

Sample size

Sample size was estimated using the Bland-Altman method on MedCalc version 19.03. Using a preliminary data set of 20 samples comparing measured with mean calculated ionised calcium, the mean difference and standard deviation of differences were calculated to be 0.2616 mmol/L and 0.3090 mmol/L respectively. Setting the power (β) to 80%, alpha error (α) to 5% and maximum allowable difference (δ) to 1.00 mmol/L, a minimum sample size of 130 was obtained from the software based on the following equation by Lu MJ *et al*.[14]:

$$n = \frac{(2 + z^2_{1-\gamma/2})\,[t_{inv}(1-\beta/2,n-1,\, t_{1-\alpha/2,n-1})]^2 SD^2}{2(z_{1-\gamma/2}SD-\delta)^2}$$

Data collection

Two blood samples were drawn from each subject. The first sample went into a heparinised 1 ml syringe for ionised calcium analysis and the second into a lithium heparin tube for plasma total calcium, albumin and creatinine analyses. Samples were drawn from indwelling arterial catheters or the vein within 10 minutes of tourniquet application without fist-clenching to avoid spurious calcium and albumin elevations. Ionised calcium was measured within 10 minutes of draw to avoid pH changes that may affect measurement. The lithium heparin tube sample was sent to the laboratory for analysis of total calcium albumin and creatinine. All laboratory sample values were then traced within the same day and documented in an Excel spreadsheet. To blind assessment of prediction outcome, estimated ionised calcium was only calculated at the end of data collection using the four equations in Table 1. Other parameters collected for each patient include gender, age, blood pH, ventilation status, haemodialysis therapy and transfusion status.

TABLE 1: Published equations for calculating ionised calcium in critically ill patients

Equation	Year	Formula	Study characteristics	Ref.
Forster*	1985	0.225 + (0.55 × Total Calcium) – (0.007 × albumin)	Derived from 389 patients; no validation cohort	13
Antonio	2015	0.815 × Total Calcium$^{0.5}$		12
Antonio	2015	0.826 × Total Calcium$^{0.5}$ – 0.023 × RF†	Derived from 269 patients (36 critical); validated with cohort of 146 patients (12 critical)	12
Antonio	2015	0.813 × Total Calcium$^{0.5}$ – 0.005 × Albumin$^{0.75}$ + 0.079		12

*original formula transformed to SI units
†RF = 0 for eGFR>60; RF = 1 for eGFR 30-59; RF = 2 for eGFR<30 (eGFR in ml/min/1.73m^2 by CKD-EPI)

Total calcium values compounded by abnormal albumin levels were adjusted using the following formula:

Adjusted calcium, mmol/L = [(40 – albumin, g/L) × 0.02] + total calcium, mmol/L

Data analysis

Statistical analyses were performed on IBM SPSS version 23 and MedCalc version 19.03. Data distribution was analysed for normality using the Shapiro-Wilk test and non-parametric data presented in median with interquartile range (IQR). Group medians were compared using the Mann-Whitney U test and ordinal parameters between groups were compared using the Pearson Chi-Square test. A significance level of 0.05 was taken. Both calculated and measured ionised calcium values were compared by Bland-Altman plot as well as a Passing Bablok regression analysis. Accuracy of each equation in classifying patients into their calcium states was determined following cross-tabulation. In all analyses, measured ionised calcium was taken as the reference value. Method verification, quality checks and maintenance were adequately met to ensure accuracy and reliability of measured ionised calcium values.

RESULTS

A total of 281 patients were recruited following two exclusions due to sample rejection (inadequate volume and gross haemolysis) with males forming the majority (62.3%). A discrepancy in calcium states was found between adjusted total calcium and measured ionised calcium where adjusted total calcium showed a normocalcaemic majority (60.9%) whilst measured ionised calcium showed a hypocalcaemic majority (92.2%) (Table 2).

In terms of clinical states at the point of sample collection, 65.1% of subjects were mechanically ventilated, 18.1% were on haemodialysis and over a third were transfused with blood products within 24 hours prior to blood sampling (Table 2). Pearson Chi-Square test showed no significant associations between all three clinical states with measured ionised calcium status (Table 3). The top two reasons for ICU admission were post-elective surgery observation (18.9%) and sepsis of various infective sources (17.8%).

When classified to groups of hypocalcaemia and non-hypocalcaemia based on measured ionised calcium, the adjusted total calcium, all four equations' calculated ionised calcium and pH showed significant median differences across groups (Table 3). Other parameters such as age, creatinine, eGFR and albumin showed no significant difference between group medians.

Comparison between calculated and measured ionised calcium

From the Bland-Altman plot (Figure 1), all four equations overestimated ionised calcium values with positive mean differences far exceeding the allowable performance limit of 0.05 mmol/L set by the Royal College of Pathologists of Australasia.[15] The absolute difference between calculated and measured ionised calcium reduces with increasing measured ionised calcium values. Passing Bablok regression analysis in Figure 2 shows significant constant and proportional biases for all four equations. While Equations 1, 2 and 3 demonstrated lower random differences as compared to Forster's Equation, the significant positive bias at the medical

TABLE 2: Baseline characteristics of overall cohort

Parameter, N=281	***n (%)****	***Median [IQR]***	***Reference Interval***
Age, years		53 [36-64]	
Gender			
Male	175 (62.3)		
Female	106 (37.7)		
Adjusted calcium, mmol/L		2.29 [2.16-2.42]	2.10 - 2.60
Hypocalcaemia	90 (32.0)		
Normocalcaemia	171 (60.9)		
Hypercalcaemia	20 (7.1)		
Measured ionised calcium, mmol/L		0.91 [0.79-0.98]	1.10 - 1.35
Hypocalcaemia	259 (92.2)		
Normocalcaemia	21 (7.5)		
Hypercalcaemia	1 (0.4)		
Equation 1 ionised calcium, mmol/L		1.15 [1.11-1.19]	1.10 - 1.35
Hypocalcaemia	44 (15.7)		
Normocalcaemia	237 (84.3)		
Hypercalcaemia	0 (0)		
Equation 2 ionised calcium, mmol/L		1.16 [1.11-1.19]	1.10 - 1.35
Hypocalcaemia	51 (18.1)		
Normocalcaemia	230 (81.9)		
Hypercalcaemia	0 (0)		
Equation 3 ionised calcium, mmol/L		1.16 [1.12-1.20]	1.10 - 1.35
Hypocalcaemia	36 (12.8)		
Normocalcaemia	244 (86.8)		
Hypercalcaemia	1 (0.4)		
Forster ionised calcium, mmol/L		1.15 [1.07-1.21]	1.10 - 1.35
Hypocalcaemia	86 (30.6)		
Normocalcaemia	183 (65.1)		
Hypercalcaemia	12 (4.3)		
Albumin, g/L		26 [22-30]	35 – 50
Acid-base status, pH		7.40 [7.34-7.44]	7.35 - 7.45
Normal	157 (55.9)		
Acidotic	71 (25.3)		
Alkalotic	53 (18.9)		
Creatinine, umol/L		108 [68-253]	44 – 88
eGFR, ml/min/1.73m^2		62 [22-102]	>90
>60	146 (52.0)		
30-60	45 (16.0)		
<30	90 (32.0)		
Ventilatory status			
Ventilated	202 (71.9)		
Not ventilated	79 (28.1)		
Dialysis status			
Dialysed	51 (18.1)		
Not dialysed	230 (81.9)		
Transfusion status			
Transfused	105 (37.4)		
Not transfused	176 (62.6)		

*expressed in percentage of overall cohort of N=281

TABLE 3: **Baseline characteristics of cohort as classified according to calcium status**

Parameter, N=281	Hypocalcaemia, N=259		Non-hypocalcaemia, N=22		$X^2(df)^{\dagger}$	Sig.
	*n (%)**	*Median [IQR]*	*n (%)**	*Median [IQR]*		
Age, years		53 [36-64]		51 [36-66]		0.843‡
Gender					1.529(2)	0.466§
Male	163 (58.0)		12 (3.2)			
Female	96 (34.2)		10 (3.6)			
Adjusted calcium, mmol/L		2.28 [2.14-2.40]		2.55 [2.33-2.67]		<0.001‡
Measured ionised calcium, mmol/L		0.9 [0.78-0.96]		1.12 [1.11-1.14]		<0.001‡
Equation 1 ionised calcium, mmol/L		1.15 [1.11-1.19]		1.23 [1.18-1.27]		<0.001‡
Equation 2 ionised calcium, mmol/L		1.15 [1.11-1.19]		1.21 [1.18-1.26]		<0.001‡
Equation 3 ionised calcium, mmol/L		1.16 [1.12-1.19]		1.23 [1.20-1.27]		<0.001‡
Forster ionised calcium, mmol/L		1.14 [1.07-1.20]		1.28 [1.20-1.36]		<0.001‡
Albumin, g/L		26 [22-30]		25 [22-28]		0.846‡
Acid-base status, pH		7.40 [7.34-7.45]		7.35 [7.28-7.40]		0.001‡
Normal	145 (51.6)		12 (4.3)		9.603(4)	0.048§
Acidotic	61 (21.7)		10 (3.6)			
Alkalotic	53 (18.9)		0 (0)			
Creatinine, umol/L		108 [68-257]		95 [59-238]		0.583‡
eGFR, ml/min/1.73m^2		61 [22-102]		75 [19-107]	0.985(4)	0.912§
>60	134 (47.7)		12 (4.3)			
30-60	42 (14.9)		3 (1.1)			
<30	83 (29.5)		7 (2.5)			
Ventilatory status					2.572(2)	0.276§
Ventilated	187 (66.5)		15 (5.4)			
Not ventilated	72 (25.6)		7 (2.5)			
Dialysis status					0.458(2)	0.795§
Dialysed	48 (17.1)		3 (1.1)			
Not dialysed	211 (75.1)		19 (6.8)			
Transfusion status					1.691(2)	0.429§
Transfused	96 (34.2)		9 (3.2)			
Not transfused	163 (58.0)		13 (4.6)			

*expressed in percentage of overall cohort of N=281
†X^2 = Pearson Chi-Square value; df = degrees of freedom
‡ significance of median difference between hypocalcaemic and non-hypocalcaemic groups by Mann-Whitney U test; significance level is 0.05
§ significance of association between hypocalcaemic and non-hypocalcaemic groups by Pearson Chi-Square test; significance level is 0.05

decision limit (MDL) proved that none of the four equations were in agreement to measured ionised calcium.

Classification accuracy into calcium states
From Table 4, adjusted total calcium misclassified 66.4% of hypocalcaemic patients as normal or high calcium with an overall accuracy of 37.7% (P<0.001). All four published equations had poorer accuracies ranging between 19.9-37.0% with Forster's Equation giving the best accuracy of the four.

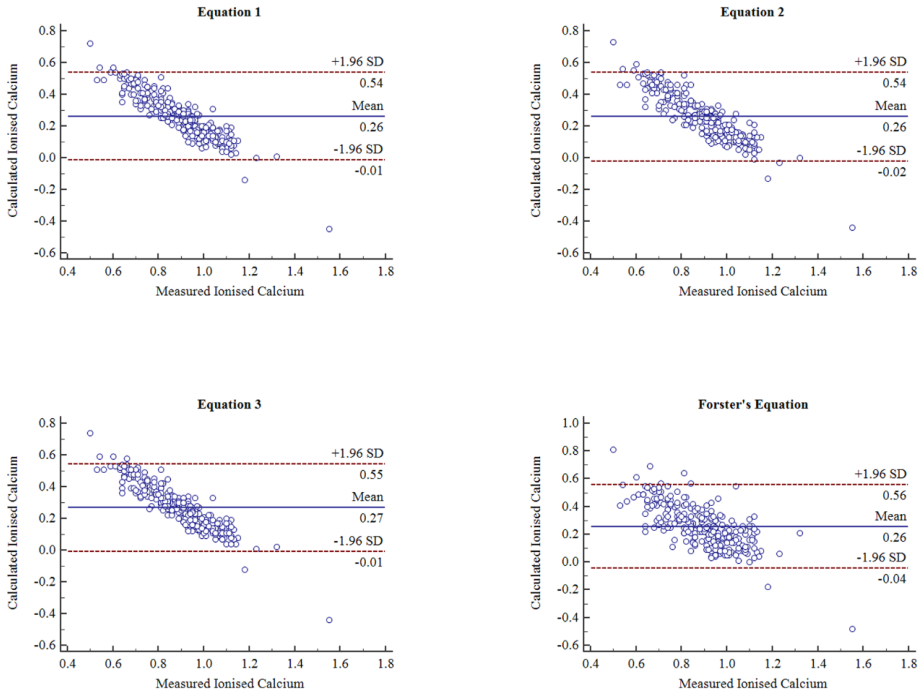

FIG. 1: Bland Altman difference plot between calculated and measured ionised calcium.

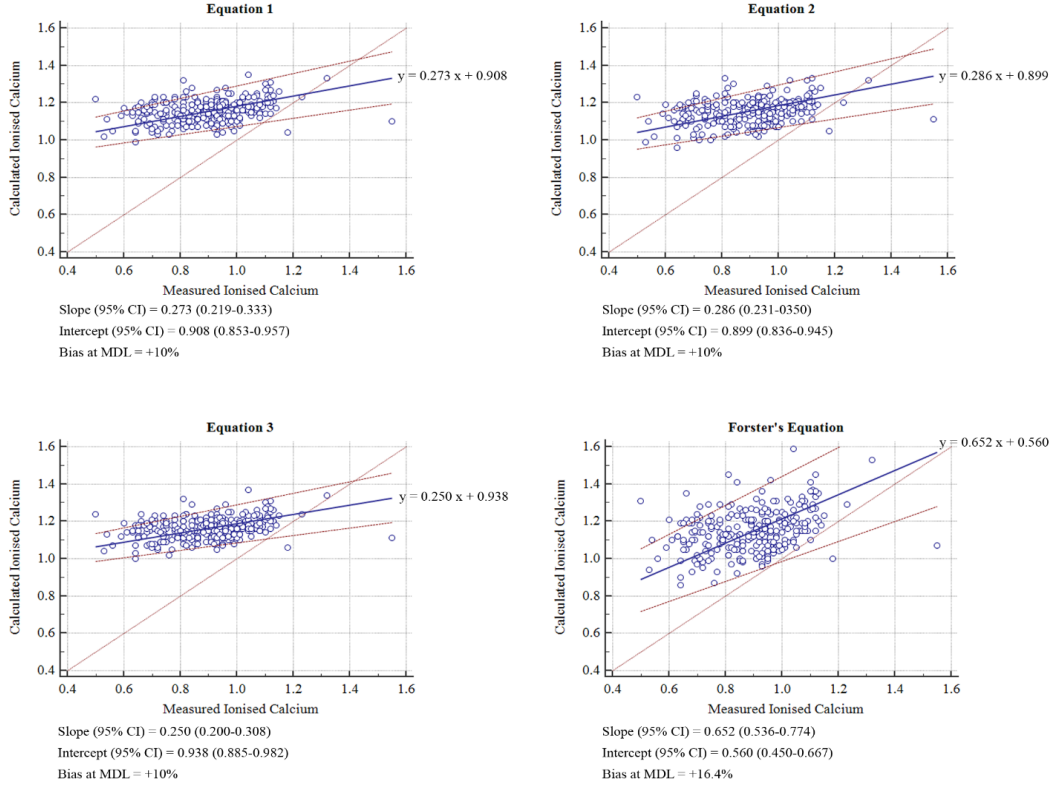

FIG. 2: Passing Bablok graph of calculated against measured ionised calcium.

TABLE 4: Accuracy of different variables in classifying patients into their calcium states according to measured ionised calcium

Variable	Correct classification of calcium state		Wrong classification of calcium state		Overall accuracy (TP+TN)/n
	Hypocalcaemia (True positive) n (%)*	**No hypocalcaemia** (True negative) n (%)†	**Hypocalcaemia** (False positive) n (%)‡	**No hypocalcaemia** (False negative) n (%)§	
Adjusted calcium	87 (31.0)	19 (6.8)	3 (1.1)	172 (61.2)	37.7%
Equation 1	43 (15.3)	21 (7.5)	1 (0.4)	216 (76.9)	22.8%
Equation 2	50 (17.8)	21 (7.5)	1 (0.4)	209 (74.4)	25.3%
Equation 3	35 (12.5)	21 (7.5)	1 (0.4)	224 (79.8)	19.9%
Forster	84 (29.9)	20 (7.1)	2 (0.7)	175 (62.3)	37.0%

*number & percentage of patients with hypocalcaemia correctly classified as having hypocalcaemia = specificity
†number & percentage of patients without hypocalcaemia correctly classified as not having hypocalcaemia = sensitivity
‡number & percentage of patients without hypocalcaemia classified as having hypocalcaemia
§number & percentage of patients with hypocalcaemia classified as not having hypocalcaemia
Abbreviations: TP, true positive; TN, true negative

DISCUSSION

Hypocalcaemia is a common finding in critically ill patients with a reported frequency ranging between 65 to 88%.[16] This study supports this finding where 92.2% of recruits were found to have a measured ionised calcium of less than 1.1 mmol/L within the first 48 hours of admission to the ICU. Explanations as to how hypocalcaemia reigns in critical illness include increased faecal and/or urinary excretion of calcium, catecholamine-induced shift of calcium into tissues, insufficient dietary intake of calcium as well as vitamin D deficiency.[3,5]

The 48-hour period was selected based on the physiological kinetics that occur in a critically ill patient during this time frame. Sequential assessment models such as the Mortality Probability Model 48 (MPM48) are often used to estimate mortality at 48 hours of ICU admission.[17,18] ICU-acquired infections – defined as infections occurring after 48 hours of ICU admission – independently increases mortality risk and prolongs hospital stay.[19] Calcium metabolism may therefore be compounded by the septic reaction if taken beyond 48 hours. Furthermore, many studies in critical care use the first 48 hours of admission as an inclusion criterion.[20,21]

Over a quarter of recruits were acidotic and data supports the association between pH and ionised calcium. pH is known to affect ionised calcium level where the competition between hydrogen ions and ionised calcium for albumin and other ligands' binding sites cause an increase in ionised calcium in acidotic states.[22] With every 0.1 unit decrease in pH, ionised calcium increases by approximately 0.05 mmol/L.[23] Therefore, to minimise the effect of pH on ionised calcium measurement in this study, whole blood ionised calcium was analysed within 10 minutes of draw and ionised calcium interpreted alongside pH.

Hypocalcaemia is a known complication of end stage renal disease due to reduced hydroxylation of 25-hydroxy-cholecalciferol to 1,25-dihydroxy-cholecalciferol by the kidneys.[1] Additionally, citrate – a calcium chelator – is commonly used as an anticoagulant to keep the extracorporeal circuit of continuous renal replacement therapy (CRRT) patent. However, due to extracorporeal calcium replenishment in regional citrate anticoagulation, haemodialysis is rarely an independent cause of hypocalcaemia as this study has shown.[24]

One unit of whole blood product contains about 3 grams of citrate with the majority found in fresh frozen plasma.[25,26] Ionised hypocalcaemia is a known complication of citrate toxicity which is frequently seen in massive blood transfusions and patients with impaired citrate clearance due to renal or hepatic derangements.[26] This complication is not seen in this cohort.

Hypoalbuminemia was seen in 79% (data not shown) of subjects, supporting its role as a negative acute phase reactant. Contrary to current understanding of the calcium-albumin relationship, the correlation between measured ionised calcium and plasma albumin was very weak and insignificant (Spearman correlation -0.015, P=0.804) (data not shown). This suggests that the degree of calcium-albumin binding during illness is individualised.[27]

Having established the lack of significant correlation between plasma albumin and measured ionised calcium, it is not surprising to see how poorly adjusted calcium agreed with measured ionised calcium values. When comparing adjusted with measured ionised calcium values, only 37.7% of values tallied according to the respective groups of hypocalcaemia and non-hypocalcaemia (Table 4). Adjusted calcium has been shown to be overestimated in ill patients[11,27,28] with one study suggesting that adjusted calcium should not be reported in patients with albumin levels below 30 g/L to reduce misclassification.[28] Our findings support this notion where adjusted calcium misclassified 66.4% (172/259) of low ionised hypocalcaemics as having normal/high calcium (Table 4). It is important to note that the adjusted calcium formula was derived from a stable outpatient population[29] hence adjusting total calcium for plasma albumin is not recommended in the critically ill. Furthermore, the formula does not take into account other calcium-binding ligands – the physiological responses of which are uncertain in critical illness.

To the authors' knowledge, only four equations to estimate ionised calcium particularly in critically ill patients are available in current literature. In Antonio JM's study, Forster's equation and Equations 1, 2 and 3 only showed moderate overestimation of ionised calcium by up to 0.05 mmol/L.[12] However, our study shows a higher degree of overestimation by 0.24-0.25 mmol/L for all four equations (calculated by median differences in Table 2). This suggests that: (1) no formula derived from another facility can be transferred across to another considering the differences in patient population and laboratory settings; (2) the population in this study is heterogeneous and should be partitioned according to clinical states; and (3) the small sample size of 12 critical patients in Antonio JM's study undermined the statistical power.

Realising the weak association between calculated and measured ionised calcium, attempts on deriving a novel estimation equation were abandoned. However, the discrepancy between measured ionised calcium and adjusted total calcium states could not be ignored. This gap between measured ionised and adjusted total calcium could be due to the altered calcium-albumin relationship in illness, pH fluctuations, presence of free fatty acids, parenteral nutrition and the use of calcium-binding citrate in blood products and renal replacement therapy.[5,23,27] Some causes of the gap between total and ionised calcium are explained in Table 5.[22,30-33]

Direct measurement of ionised calcium is undoubtedly superior to calculated calcium values in assessing calcium status in the critically ill. Even from a practical point of view, it has a significantly shorter turnaround time than total calcium analysis in the laboratory as the latter is affected by log-in processes, sample preparation, analytical errors and result verification. This does not include time wasted due to difficult phlebotomy or delayed transportation to the laboratory.

Nonetheless, to place a point-of-care testing (POCT) analyser in each non-ICU unit that houses critical patients is limited due to issues of cost, maintenance, monitoring, pre-analytical interferences and susceptibility to result errors. Thorough assessment and discussion with the hospital's POCT team are therefore needed prior to starting a POCT service for ionised calcium in other peripheral units.

Even though cost of analysis of total calcium including albumin is low, the inaccurate measurement leads to misclassifications of calcium status. Hence ionised calcium measurement is the preferred method for assessing calcium states in critically ill patients.

Being a single-centered study involving a heterogeneous group of critical patients are limitations of this study. Further research looking into ionised calcium of specific sub-populations in the ICU is therefore required. A follow up of critical patients with serial plasma calcium, albumin and pH throughout admission may be more beneficial instead of a single sample on admission to study the physiological changes

TABLE 5: Causes and explanation of the calcium gap

Total calcium	Ionised calcium	Causes	Explanation	Ref.
↓	Normal	Hypoalbuminaemia e.g. liver failure, nephrotic syndrome	Albumin-bound calcium fraction decreases	
↑	Normal	Hyperalbuminaemia e.g. prolonged tourniquet application, high-protein diet, severe dehydration	Albumin-bound calcium fraction increases	
		Multiple myeloma	Rarely, monoclonal globulins bind calcium, causing elevated total calcium. In most cases, true hypercalcaemia (↑ total and ↑ ionised) prevails in multiple myeloma.	31
Normal	↓	Chronic kidney disease	Concomitant metabolic acidosis and renal failure	
		Acute respiratory alkalosis	Ionised calcium binds to albumin in place of hydrogen ions	22
		Chronic respiratory alkalosis	Renal resistance to PTH causes hypercalciuria	30
↑/normal	↓	Citrate chelation e.g. dialysis	Concomitant ionised calcium chelation and calcium-citrate complexing	32
↑	↑↑	Primary hyperparathyroidism	Decreased binding of ionised calcium to albumin causes raised ionised-to-total-calcium ratio	33

and relationship between the analytes in critical illness.

CONCLUSION

Calculated ionised calcium using the published four equations specific to the critically ill significantly overestimates calcium levels and together with adjusted total calcium tend to misclassify hypocalcaemic patients as having normal/high calcium. It is therefore not recommended to calculate ionised calcium or adjust total calcium for albumin in critically ill patients as overestimated calcium may lead to suboptimal management of these patients. Measured ionised calcium should also be interpreted alongside pH as alkalosis causes significant reductions.

Acknowledgement: The authors would like to thank the ICU staff of Hospital Selayang, Ministry of Health Malaysia for welcoming data collection in the unit. Also thanking the Director General of Health Malaysia for his permission to publish this article.

Authors' contributions: NSZ conceptualised the study and was involved in data curation, data analysis and manuscript writing. FAJ supervised the study, revised intellectual content and participated in manuscript editing. TN supervised the study design and provided access to research components e.g. site, equipment, samples and data.

Conflict of interest: The authors declare they have no conflict of interest.

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