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Evidence-based cutoffs for total and adjusted calcium: a major factor in detecting severe hypo- and hypercalcemia

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Abstract

Objectives: Severe hypo- and hypercalcemia are common and urgent treatment is recommended. Free calcium (fCa) is the gold standard but needs blood gas tests with challenging preanalytics. Total calcium (tCa) and calculated adjusted calcium (aCa) are readily available, but their interpretation is hampered by identical tCa and aCa cutoffs, laborious local aCa calculation and difficult comparability of calcium biomarkers.
Methods: Laboratory results from University Medicine Leipzig were evaluated over a five-year period (236,274 patients). A local aCa equation was derived by linear least squares regression, the agreement between fCa, tCa and aCa assessed with Cohen's κ and decision thresholds derived by this indirect method.
Results: The local aCa equation was created from data of 9,756 patients, each with one paired measurement of tCa, fCa and albumin. Derived aCa cutoffs (1.95/3.15 mmol/L) differ markedly from derived tCa cutoffs (1.6/2.9 mmol/L) and severe hypo- and hypercalcemia can be more accurately assessed by aCa (κ=0.489, 0.812) than by tCa (κ=0.445, 0.744). Comparing our approach to standard care (tCa, literature cutoff), a total 3,250 of 3,680 (88.3 %) misclassified measurements were correctly classified when using aCa with evidence-based cutoffs.
Conclusions: Optimized cutoffs for aCa and tCa hold great potential for improved patient care. Locally derived aCa equations differ mostly in the chosen mean normal calcium and provide minimal overall improvement, but entail a close examination of the used cutoffs before application.

Keywords: laboratory medicine; clinical decision support; clinical investigation; calcium; adjustment equation

Introduction

Disorders of calcium (Ca) homeostasis can present as hypocalcemia and hypercalcemia. Plasma Ca concentration depends on bone, kidney, and intestinal organ function, which can be impacted by diseases [1]. Therefore, hypo- and hypercalcemia are common in hospitalized patients (cumulative incidence 28 and 5 %, respectively) [2]. Mild hypocalcemia is associated with symptoms such as muscle spasms, paresthesias, prolonged QT time, while severe forms may present with pronounced symptoms such as laryngospasm, life-threatening arrhythmias, and epileptic seizures [3]. Patients with hypercalcemia show symptoms of muscle weakness, atrioventricular block or coma, and in the long term nephrocalcinosis and coronary heart disease [4]. According to the European Society of Endocrinology (ESE) guidelines, patients with severe hypo- and hypercalcemia should be treated as an urgent medical emergency [5, 6]. However, the symptoms are relatively unspecific and laboratory tests are essential for diagnosis.

Total calcium (tCa) combines three different forms: 1. free Ca (about 45 %, fCa), 2. albumin bound Ca (aCa), similar to protein and immunoglobulin bound (about 45 %) and 3. anion-bound Ca, bound to phosphate, lactate, citrate, sulfate, bicarbonate, and free fatty acids (about 10 %) [7]. The fCa may change rapidly with hyperventilation, causing alkalosis and calcium drop. The rapid variability of fCa has led to the development of point-of-care test (POCT) devices that allow the physician to measure fCa at the ward. With proper preanalytical handling, fCa measured by a POCT device represents

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the accepted standard in the diagnosis of calcium disorders and is established in both emergency and laboratory medicine. Despite the widespread measurability of fCa, cumbersome preanalytical handling and higher costs argue against its frequent screening for severe hypo- and hypercalcemia [8]. A common practice is estimating fCa by aCa, which can be calculated from tCa and albumin. While some hospitals routinely calculate aCa, others use tCa directly as a screening parameter and calculate aCa only when clinically indicated.

The most commonly used aCa equation was published in 1973 by Payne et al. [9] based on a small sample size (n=200) and without a clinical validation cohort. The most recent study to date (Smith et al. [10]) excluded samples with albumin <30 g/L, selected samples (n=13,604) with pH 7.35–7.45 and one patient could contribute multiple measurements [10]. Again, no clinical application cohort was reported. The literature suggests individual equations for each hospital [10–14]. However, this does not happen and Payne's equation has become generally accepted.

The discussion in the literature on how to find the best equation is lively [9–12, 14–16], but the aCa cutoffs to be used for severe calcium disorder detection are not reported. On the other hand, a wide range of cutoffs for tCa have been shown for severe hypo- (<1.4 mmol/L [17] and <2.12 mmol/L [18]) and hypercalcemia (>2.5 mmol/L [19] and >4.0 mmol/L [20]). The ESE suggested tCa and aCa cutoffs for severe hypo- (<1.9 mmol/L) [6] and hypercalcemia (>3.5 mmol/L) [5]. Similar cutoffs for aCa but not for tCa have been supported by the U.S. National Health Institute (USNIH) [21]. Though the values must be different for methodological reasons, it is common practice to use the same cutoffs for tCa and aCa.

In order to increase patient safety, our ultimate aim is to assist physicians with clinical decision support on severe hypo- and hypercalcemia [22]. In this study, based on a large dataset of hospitalized patients, we calculate and report evidence-based cutoffs for tCa and aCa by an indirect method, i.e. derived from optimized concordance with fCa.

Materials and methods

A total of 236,374 patients from two medical centers were included in this retrospective study of 2,598,537 measurements. The University Medicine Leipzig (UML) is a tertiary care hospital in Saxony, Germany (1,451 beds). The Muldentalkliniken (MTK) are two primary care hospitals in Saxony, located in Grimma (177 beds) and Wurzen (178 beds). As shown in Figure 1, data from UML and MTK were processed to create three cohorts: UML-aCa, UML-app and MTK-app.

Analysis of biomarkers

Samples of UML patients are being analyzed 24/7 at the Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics (ILM, ISO 15189, ISO 17025, EU Directive 98/79/EG, EN 13612). All serum total calcium (tCa) measurements were performed on the Cobas® system (Roche Diagnostics GmbH, Mannheim, Germany). The colourimetric method using 5-nitro-5′-methyl-1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′--tetraacetic acid (NMBAPTA) was used [23]. Albumin was measured in serum on the Cobas® system using the bromocresol green (BCG) dye binding method [24]. Measurements of tCa and albumin were performed at admissions of most patients. Free calcium (fCa) was measured in whole blood using ABL 800 Flex® point-of-care (POCT) devices (Radiometer GmbH, Krefeld, Germany), which use a direct calcium ion-selective electrode. Capillary, venous and arterial blood samples were included in the study. Electrolyte-balanced heparin was used for anticoagulation in capillaries or syringes. Analyses were performed either on wards, at intensive care units (ICU) or at ILM (Supplementary Material 1). Simultaneous measurements of tCa and albumin are standard care at UML.

At MTK laboratory samples were collected once a day and analyzed in a contract laboratory. Albumin is only measured upon clinical indication. POCT measurements were performed as well. The same methodology and analyzers seen an UML were used (see above). During the study period, all measurement methods remained unchanged.

Data analysis

Laboratory data were cleaned using just necessary exclusion criteria (Figure 1). Data cleaning, statistical analysis and plotting were performed using base R 4.0.2 [25]. Additional packages included DescTools [26] for Cohen's κ calculation and reshape2 [27] for data wrangling.

Calcium adjustment equations (UML-aCa)

We developed an aCa equation using the UML-aCa cohort, which includes laboratory data from UML patients (2014–2019). The dataset was created by coupling each simultaneously measured albumin and tCa pair with exactly one fCa measurement within ±3 h of the tCa-albumin timestamp, resulting in tCa-albumin-fCa data trios for further processing. When more than one fCa measurement was available, the measurement closest in time was chosen.

Using just one randomly selected data trio per patient from the UML-aCa cohort (resulting in n=9,756), we then performed a linear least squares regression of tCa on albumin according to Barth et al. [12] (also used in Payne et al. [9] and Smith et al. [10]). This analysis was performed collectively, including both sexes and all ages. The resulting intercept and slope were used to create an in-house aCa equation as follows: aCa=tCa (mmol/L) − slope * albumin (g/L) + (mean normal total calcium − intercept), with a mean normal tCa of 2.365 mmol/L.

aCa and tCa cutoffs for severe calcemia (UML-aCa)

Adjusted Ca was calculated using the in-house equation established for all available albumin-tCa-fCa trios in the UML-aCa cohort (n=25,658). Each trio was reference grouped as severely hypo- or hypercalcemic by assessing whether the fCa, as the most unbiased measurement method for investigating effective calcium, fell below 0.9 mmol/L or exceeded 1.6 mmol/L [28, 29]. These fCa cutoffs were agreed based on literature, UML laboratory physician and endocrinologist expertise.

In order to derive optimized cutoffs (by an indirect method), the concordance of fCa and other Ca decision criteria was analyzed as Cohen's κ [30] within the UML-aCa cohort. While values ≤0 indicate no agreement or even disagreement, a value of one indicates a perfect agreement [31]. For a range of cutoffs (hypocalcemia: 1.0–2.5 mmol/L, hypercalcemia: 2.0–5.0 mmol/L, increments of 0.025 mmol/L), the

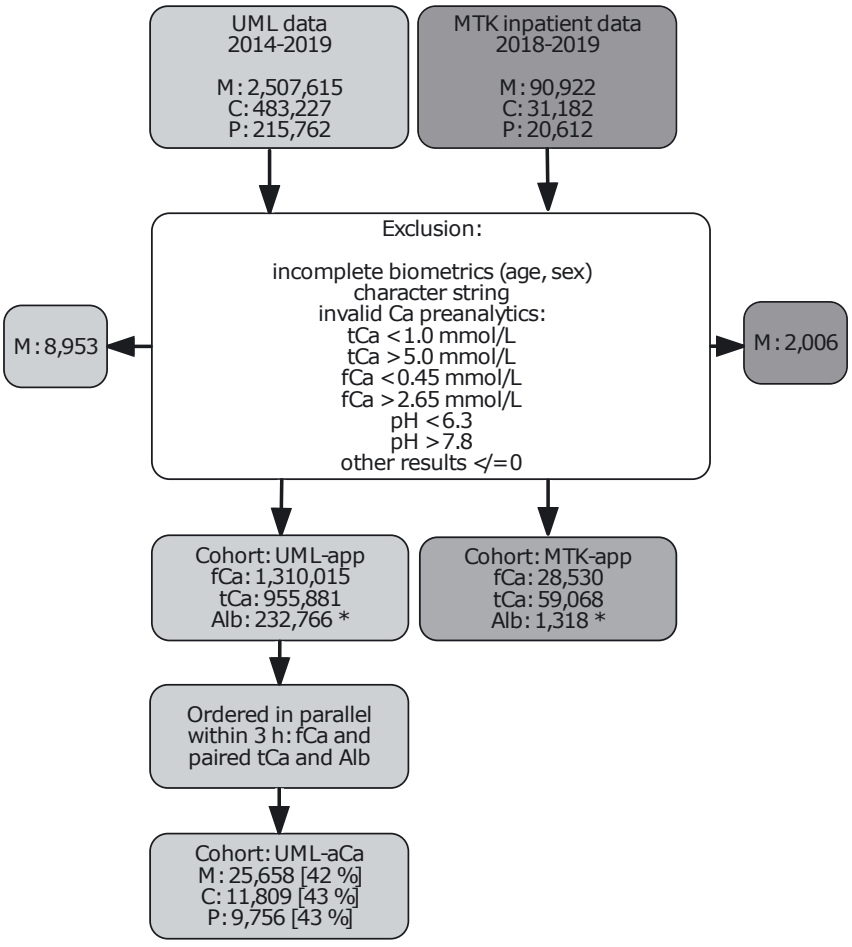

Figure 1: Raw data from University Medicine Leipzig (UML) and Muldentalkliniken (MTK) and data processing. Incomplete, preanalytical or unplausible altered measurements were excluded. Albumin measurements only considered if ordered together with tCa (*). Female data fraction given in square brackets. Ca, calcium; tCa, total calcium; fCa, free calcium; aCa, adjusted calcium; Alb, albumin; M, measurements; C, cases; P, patients.

agreement of the fCa grouping was compared with the grouping according to the tCa and aCa (UML).

Cutoff application and transfer (UML-app, MTK-app)

The cohorts UML-app and MTK-app (Figure 1) were used to apply the derived aCa equation and cutoffs as well as established decision thresholds to two distinct hospital cohorts and assess the resulting number of detected severely calcemic measurements.

Results

Cohort description

In our retrospective study we investigated 215,762 patients at the UML between 2014 and 2019. The MTK contributed 20,612 patients between 2018 and 2019. After excluding preanalytical outliers and focusing on fCa, tCa and albumin measured simultaneously, 9,735 patients (25,658 measurements) remained in the UML-aCa cohort for aCa equation and cutoff creation. The UML-app and MTK-app cohorts were used for cutoff evaluation. Patient characteristics of UML-aCa, UML-app, MTK-app are described in Table 1.

Calculation of an in-house equation for adjusted calcium (aCa)

The derived aCa (UML) in-house equation was highly similar to Smith's equation [10], whereas the difference to Payne's equation [9] was more pronounced. We found a slope of 0.0174 and an intercept of 1.5769 for the linear regression of

Table 1: Description of cohort characteristics for UML-aCa and the application cohorts MTK-app and UML-app.

Cohort	Patients, n	Age of patients, years Median (IQR)	tCa, n	tCa, mmol/L Median (IQR)	fCa, n	fCa, mmol/L Median (IQR)	Alb, n	Alb, g/L Median (IQR)
UML-aCa	9,735	64.6 (49.1–76.7)	25,658	2.2 (2–2.3)	25,658	1.2 (1.1–1.3)	25,658	30.8 (25.3–37.2)
MTK-app	20,547	65 (43.9–79.1)	59,052	2.2 (2.1–2.3)	28,522	1.2 (1.1–1.2)	1,318	25.2 (21.1–29.4)
UML-app	215,459	52.8 (30.4–70.7)	955,881	2.3 (2.2–2.4)	1,310,015	1.2 (1.1–1.2)	232,766	40.4 (33.1–44.5)

UML, University Medicine Leipzig; MTK, Muldentalkliniken; tCa, total calcium; fCa, free calcium; aCa, adjusted calcium; Alb, albumin; IQR, interquartile range.

tCa on albumin, and used the local mean normal calcium of 2.365 for equation establishment.

$$\text{aCa (UML)} = \text{tCa}\left(\frac{\text{mmol}}{\text{L}}\right) - 0.0174 * \text{Albumin}\left(\frac{\text{g}}{\text{L}}\right) + 0.7881$$

$$\text{aCa (Smith)} = \text{tCa}\left(\frac{\text{mmol}}{\text{L}}\right) - 0.018 * \text{Albumin}\left(\frac{\text{g}}{\text{L}}\right) + 0.72$$

$$\text{aCa (Payne)} = \text{tCa}\left(\frac{\text{mmol}}{\text{L}}\right) - 0.025 * \text{Albumin}\left(\frac{\text{g}}{\text{L}}\right) + 1$$

Novel aCa and tCa cutoffs on fCa concordance

Figure 2 shows the concordance results for UML patients for severe hypo- (Figure 2A) and hypercalcemia (Figure 2B). Generally, the highest reached concordance of tCa and aCa (UML) was markedly lower for hypocalcemia (κ=0.45, 0.49) than hypercalcemia (κ=0.74, 0.81) (Table 2). Between the decision criteria, differences were most pronounced in Ca cutoffs at which the maximum κ was reached. For comparable κ in severe hypocalcemia, tCa cutoffs (1.6 mmol/L) were 0.3 mmol/L lower compared to aCa cutoffs (1.9 mmol/L) and for severe hypercalcemia 0.225 mmol/L higher compared to aCa. Overall, maximum concordance for detecting severe hypo- and hypercalcemia was accomplished by using the UML in-house aCa equation. Total calcium alone achieved the least concordance.

The cutoff for hypocalcemia recommended in literature failed to meet the maximized concordance for fCa with tCa (old: 1.9 mmol/L, κ=0.101; new: 1.6 mmol/L, κ=0.445) but was almost perfectly fitting for aCa (UML) (old: 1.9 mmol/L, κ=0.479; new: 1.95, κ=0.489). Furthermore, literature-recommended cutoffs for severe hypercalcemia could not be transferred for tCa (old: 3.5 mmol/L, κ=0.27; new: 2.925 mmol/L, κ=0.744) and aCa (old: 3.5 mmol/L, κ=0.592; new: 3.15 mmol/L, κ=0.812) (Table 2). aCa (UML) is superior to tCa for the detection of severe hypo- and hypercalcemia, as the cutoffs optimized according to Cohen's κ show higher agreement with fCa.

Without optimized cutoff for tCa 3,562 measurements would be misclassified as hypocalcemic, which can be reduced by 89 % by using the optimized cutoff. For hypercalcemia, a reduction in misclassification of 41 % can be achieved. Optimized hypocalcemia cutoffs for aCa are almost equivalent to the current one and don't reduce misclassification. For

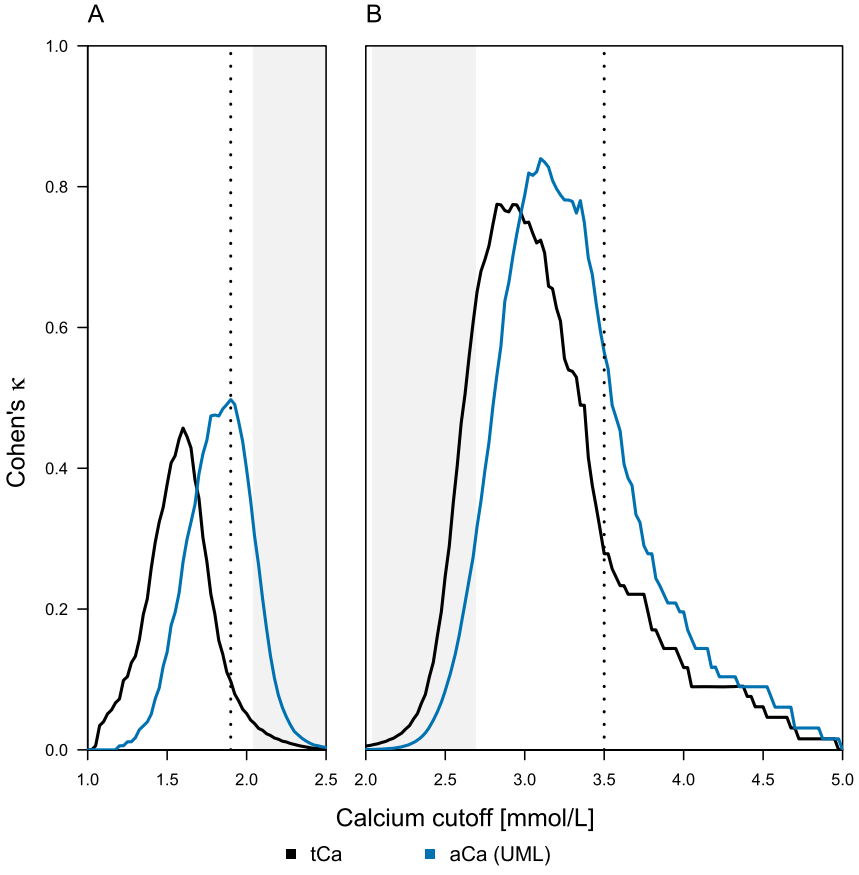

Figure 2: Concordance (given as Cohen's *κ*) of severe calcium disorder detection using fCa compared to tCa or aCa for hypo- (A) and hypercalcemia (B), using the UML equation. Dotted lines: literature suggested cutoffs. Grey area: tCa reference interval (2.04). UML, University Medicine Leipzig; tCa, total calcium; aCa, adjusted calcium; fCa, free calcium.

Table 2: Evaluation of tCa and aCa cutoffs detecting severe hypo- (A) and hypercalcemia (B) based on classification agreement with fCa using the aCa (UML) equation. Cutoffs for maximized Cohen's κ and from literature (*). False negatives and positives combined into false detection.

	Cohort	Adjustment	Cutoff, mmol/L	fCa Positive, n	True positive	False detection	Specificity	Sensitivity	Cohen's κ
A	UML-aCa	tCa (none)	1.6	351	156	377	0.993	0.444	0.445
	UML-aCa	tCa (none)	1.9*	351	250	3,562	0.863	0.712	0.101
	UML-aCa	aCa (UML)	1.95	351	186	378	0.992	0.530	0.489
	UML-aCa	aCa (UML)	1.9*	351	156	331	0.995	0.444	0.479
B	UML-aCa	tCa (none)	2.925	136	101	69	0.999	0.743	0.744
	UML-aCa	tCa (none)	3.5*	136	22	118	1	0.162	0.270
	UML-aCa	aCa (UML)	3.15	136	113	52	0.999	0.831	0.812
	UML-aCa	aCa (UML)	3.5*	136	59	81	1	0.434	0.592

UML, University Medicine Leipzig; tCa, total calcium; aCa, adjusted calcium; fCa, free calcium.

Hypercalcemia, up to 45 % of misclassifications could be prevented by using the new aCa cutoffs. At the same time, using optimized cutoffs for hypercalcemia detection always increases the fraction of correctly classified samples (true positives), e.g. from 16 to 74 % for tCa or 37–83 % for aCa (UML) (Table 2).

Application of the novel and literature recommended cutoffs at two different hospitals

Our novel cutoffs with maximized Cohen's κ (Table 2) and the cutoffs currently recommended in the literature for severe hypo- and hypercalcemia were applied to two patient cohorts (UML-app and MTK-app, Figure 1). The resulting quantity of severe hypo- and hypercalcemia was further visualized in Figure 3, depicting the effect of optimized cutoffs and different equations on clinical routine, i.e. increase or decrease of conspicuous measurements requiring further investigation.

At UML the most pronounced shift in measurements above the cutoffs was observed in tCa. All calculations of aCa are right-shifted compared to tCa. For both tCa and aCa (UML), the total number of measurements and calculations exceeding the new cutoffs was lower (n=5,825) compared with the cutoffs recommended in the literature (n=28,090). This was mostly due to the altered tCa cutoff (n=26,070). In severe hypocalcemia, tCa gains specificity (0.993), while in severe hypercalcemia, the maximum specificity is already achieved with the new cutoffs (0.999). In terms of sensitivity, aCa (0.83) outperforms tCa (0.74). The new cutoffs lay well outside the central 95 % of the data.

At MTK, overall fewer fCa, tCa, and aCa measurements were available, making interpretation less robust. In general, the same shift as for UML was observed in tCa measurements exceeding the cutoffs. The relative proportions of tCa exceeding the cutoffs for severe hypo- (MTK: 0.21 %, UML: 0.2 %) and hypercalcemia (MTK: 0.38 %, UML: 0.34 %) were comparable between the two hospitals. For aCa, a wider distribution of the central 95 % measurements was observed, as well as a shift to the right compared to the calculations for UML. While the new UML cutoff for hypocalcemia was well outside the central 95 % of the data, the cutoff for hypercalcemia lay within it.

Effect of age and sex on the aCa (UML) equation

The reference intervals for calcium and albumin are age-dependent and might contribute to the equation. We investigated the influence of age and sex on slope and intercept of the Calcium-Albumin regression by a rolling window analysis (Supplementary Material 2). The 95 % confidence interval of the slope for girls younger than five years was found to deviate from the overall slope for the complete cohort. This was not observed for boys younger than five years. Creating and using two different equations (for <5 years and ≥5 years of age) had minimal effects on the assessment of samples as hypo- or hypercalcemic (Supplementary Material 3–6). The lack of data in UML-aCa (<5 years) prevents a detailed comparison of both subcohorts (Supplementary Material 6).

Discussion

We present the first work developing and applying optimized cutoffs for severe hypo- and hypercalcemia for aCa and tCa based on fCa. The study uses an indirect method for

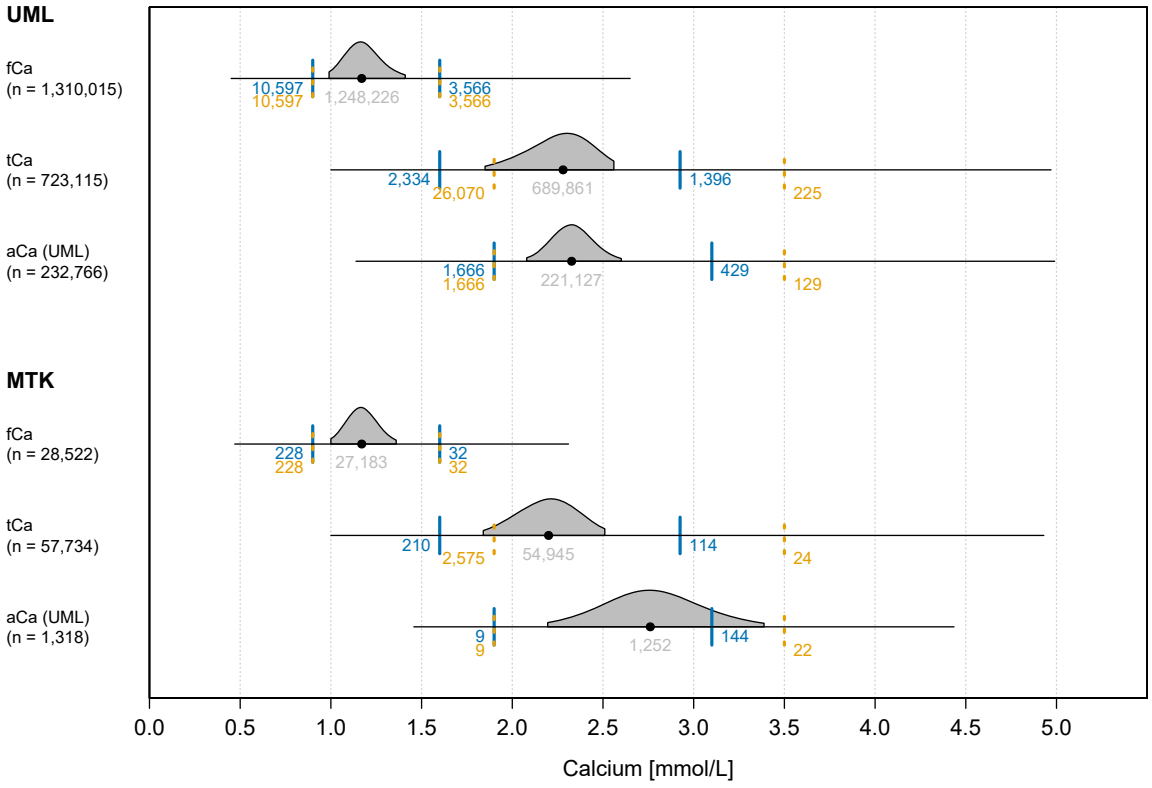

Figure 3: Assessment of severe calcemia cutoffs in two hospitals. Numbers represent exceeding measurements per category according to the used cutoff. Grey density plot: 2.5th and 97.5th percentile range. Black dot: median. Blue: optimized aCa (UML)-cutoffs. Orange: literature-suggested cutoffs. UML, University Medicine Leipzig; MTK, Muldentalkliniken; tCa, total calcium; aCa, adjusted calcium; fCa, free calcium.

decision limit detection and is based on the largest cohort to date, the first from continental Europe. Patients could benefit from more accurate calcium level interpretations with the corrections described in this study. Compared to the lowest performing standard screening (tCa), our optimization (aCa (UML)) would allow for the correct classification of 88.3 % of all currently misclassified measurements, potentially impacting the diagnostic and therapeutic follow-up of these patients. The new cutoffs still do not show perfect agreement with fCa, but are a much better fit for fCa status than previously published ones.

Although aCa is calculated and tCa is measured, the same reference intervals and the same cutoffs are usually being applied. Thus, a major benefit of aCa over tCa is not yet being utilized by physicians. Our results for tCa (1.6 mmol/L) showed poor agreement for severe hypocalcemia to recommendations from the ESE (1.9 mmol/L) and USNIH (none). We were surprised that the societies' data for aCa agreed well with our optimized results (1.9 mmol/L, ESE: 1.9 mmol/L, USNIH: 1.8 mmol/L). Interestingly, the Cohen's κ for severe hypocalcemia is generally lower than for severe hypercalcemia, possibly due to increased calcium kinetics in critically ill patients with acute hypocalcemia [32]. For severe hypercalcemia the literature recommended tCa cutoffs (ESE: 3.5 mmol/L, USNIH: none) did not match our optimization (2.925 mmol/L). In our aCa evaluation an almost perfect fit was found at 3.15 mmol/L, which was lower than the literature recommended cutoffs (ESE: 3.5 mmol/L, USNIH: 3.4 mmol/L). So far, the consideration of tCa and aCa as distinct analytes has not been addressed clearly in general guidelines. In summary, the literature recommended cutoffs were not applicable at the UML nor the MTK and the optimized cutoffs should be preferred (Table 2). Considering that the used mean normal calcium greatly influences the aCa equation, the establishment of accompanying decision thresholds seems only reasonable.

Although measurements with obvious preanalytic errors were excluded but no "healthy" hospitalized population was selected, the resulting aCa (UML) equation was nearly identical compared with equations using differing sets of exclusion criteria [10]. The influence of measurement methods has been discussed in literature [13], but we found no difference regarding aCa equations set up with other laboratory methods than ours [9, 10]. A minor age dependency of the slope, driven mainly by girls up to age five years, was seen in our data but requires confirmation in an

independent cohort. Jassam et al. [33] suggested an age limit for different aCa equations at the age of one year. Nevertheless, in our data the performance impact of the resulting equation was negligible. Although enough measurements were available to set up the aCa equation, the pediatric subcohort did not provide a sufficient number of severe hypo- and hypercalcemia cases in children in the years investigated to validate and report optimal cutoffs (Supplementary Material 6).

The literature agrees on calculating your own local equation [10–14]. However, in daily care, aCa (Payne) is mostly used. We investigated what happens when a local equation is used in another, local hospital. We found noticeably wide and right-shifted distributions of aCa. We included two hospitals with different standard screening procedures, routine determination of aCa (UML) vs. only "on suspicion" (MTK). If large laboratory data sets are not available, as in the latter case, any aCa equation adopting the adjusted cutoffs for aCa and tCa seems sufficient. However, it is strongly recommended that the equation and cutoffs be clinically evaluated in the local cohort. We are currently conducting such a study at UML in the context of a clinical decision support system.

The usefulness of aCa equations has previously been judged by assessing hypo- and hypercalcemia exceeding tCa and fCa reference intervals and comparing totals [10]. However, from a medical point of view, the comparison of how many aCa are within the reference interval of tCa is irrelevant. The patient will have no obvious symptoms and therefore no diagnosis and treatment of hypo- or hypercalcemia. It is more important for patient care to be aware of clinically relevant aCa calculations in severe hypo- and hypercalcemia. However, tCa is still commonly used, and based on our study we suggest that aCa be preferred over tCa, as it improves the accuracy of interpretation. It could be argued that the widespread availability of fCa measurements diminishes the overall need for tCa and aCa. However, aCa does not replace fCa, but rather provides a free screening tool, combined with albumin, to aid in the early screening for severe calcium disorders. In addition, aCa offers other advantages over fCa in daily clinical practice, such as high quality instrument maintenance by laboratory professionals, simple preanalytics, less susceptibility to coagulation errors and, according to patient blood management strategies, additional blood sampling only when indicated by aCa.

Limitations

We assume fCa reflects the patient's active calcium. However, it should be noted that fCa also correlates with albumin, although to a lesser extent than tCa [34]. The cutoffs for fCa were taken from the literature. They were set by experts before assessing tCa and aCa. It would be preferable to have experimentally validated fCa cutoffs for severe hypo- and hypercalcemia in humans. However, this is ethically difficult.

Our reported equation and cutoffs are not applicable to children aged up to five years due to the detected deviation of their tCa~albumin slope. We strongly encourage further investigations to enable robust conclusions for this subcohort also.

No further exclusion criteria than those mentioned in Figure 1 were applied when selecting fCa measurements for equation establishment, although recommendations are available [35]. But we found little differences to the most recently published equation (using restrictive exclusion criteria) and a recent publication corroborates that most inclusion criteria have little influence on equation slope and intercept [36].

We could not calculate the optimal cutoffs for aCa (MTK) because tCa is rarely measured together with albumin. We suspect that albumin was ordered less because one laboratory did not perform the analyses locally, patients were less severely ill, and preselection for albumin measurement was much stricter. However, the cutoffs and equations could be compared for additional cohorts and locations if calculations were performed on the same basis.

Conclusions

The measurement of fCa is standard laboratory care, but only if there is a strong suspicion for a calcium disease. fCa should not be used as a screening tool, mainly for economic, but also medical reasons. The measurement of tCa and aCa is useful to support the clinical suspicion and to guide follow-up diagnostics such as fCa. We showed that aCa and tCa cutoffs differ and that aCa interpretation is generally superior to tCa. Adjusted cutoffs for aCa and tCa have a high potential to improve diagnostics, whereas local adjustment of the equation provided only minimal improvement. This is the first study to report optimized cutoffs for severe hypo- and hypercalcemia, which can be transferred to further hospitals. Consequently, we encourage further clinical evaluation of equation – cutoff pairs in one's local cohort to support the physician with the most reliable screening.

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Research ethics: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty of the University of Leipzig, Germany (No. 214/18-ek).

Informed consent: Written consent was waived because no risk was added to patients during secondary use of existing routine data.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflicts of interest.

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Data availability: Aggregated data is available from the corresponding author upon request.

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