BMJ Open

Serum Ionized Calcium and the Risk of Acute Respiratory Failure in Hospitalized Patients: A Cohort Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034325
Article Type:	Original research
Date Submitted by the Author:	16-Sep-2019
Complete List of Authors:	Thongprayoon, Charat; Mayo Clinic Rochester, Nephrology and Hypertension Chewcharat, Api; Mayo Clinic Rochester, Nephrology and Hypertension Cheungpasitporn, Wisit; University of Mississippi Medical Center, Nephrology Mao, Michael; Mayo Clinic Florida, Nephrology and Hypertension Kianoush, K; Mayo Clinic Rochester, Nephrology and Hypertension
Keywords:	ionized calcium, respiratory failure, mechanical ventilation, hospitalization

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Serum Ionized Calcium and the Risk of Acute Respiratory Failure in Hospitalized Patients: A Cohort Study

Running title: Admission Ionized Calcium Levels and Respiratory Failure

Authors: Charat Thongprayoon, MD.[1], Api Chewcharat MD, MPH[1], Wisit Cheungpasitporn, MD.[2], Michael A Mao, MD.[3] and Kianoush B. Kashani, MD, MS[1,4]

Institutional affiliations:

1. Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, MN
2. Division of Nephrology, Department of Internal Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA
3. Division of Nephrology and Hypertension, Mayo Clinic, Jacksonville, FL 32224, USA;
4. Division of Pulmonary and Critical Care Medicine, Department of Medicine, Mayo Clinic, Rochester, MN

Institution: This work was performed at Mayo Clinic in Rochester, MN.

Corresponding author:

Kianoush B. Kashani, MD, MS, Division of Nephrology and Hypertension, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (kashani.kianoush@mayo.edu).

Conflicts of interest: None

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Authors' contributions

CT, WC and KBK originated the idea, and designed study. CT, AC, WC, and MAM collected data. CT analyzed the data. CT and AC were responsible for writing the manuscript. WC, MAM, KBK supported the editing of the manuscript and added important comments to the manuscript. KBK supervised the study. All authors had access to the data, read, and approved the final manuscript.

Word Count: 1788

Keywords: serum ionized calcium, respiratory failure, mechanical ventilation, hospitalization

ABSTRACT

Objectives: The objective of this study was to evaluate the risk of acute respiratory failure (ARF) in all hospitalized patients based on admission serum ionized calcium

Design: A retrospective cohort study

Setting: a tertiary referral hospital in Rochester, Minnesota, USA

Participants: All hospitalized patients who had serum ionized calcium measurement within 24 hours of hospital admission from January 2009 to December 2013. Patients who were mechanically ventilated at admission were excluded.

Predictors: Admission serum ionized calcium levels was stratified into six groups; ≤4.39, 4.40 to 4.59, 4.60 to 4.79, 4.80 to 4.99, 5.00 to 5.19, and ≥5.20 mg/dL.

Primary outcome measure: The outcome of interest was the development of ARF requiring mechanical ventilation during hospitalization. Logistic regression analysis was performed to assess the independent risk of ARF based on various admission serum ionized calcium, using serum ionized calcium of 5.00 to 5.19 mg/dL as the reference group.

Results: Of 25,709 eligible patients, with the mean serum ionized calcium of 4.8±0.4 mg/dL, ARF requiring mechanical ventilation occurred in 2,563 patients (10%). The incidence of ARF was lowest when admission serum ionized calcium was 5.00-5.19 mg/dL, with progressively increased risk of ARF with decreased serum ionized calcium. In multivariate analysis adjusting for potential confounders, increased risk of ARF requiring mechanical ventilation was significantly associated with admission serum ionized calcium of ≤4.39 (OR 2.49; 95% CI 2.10-2.97), 4.40 to 4.59 (OR 1.75; 95% CI 1.48-2.06), and 4.60 to 4.79 mg/dL (OR 1.47; 95% CI 1.27-1.71), compared with serum ionized calcium of 5.00-5.19 mg/dL. The risk of ARF was not significantly increased when serum ionized calcium was at least 4.80 mg/dL.

Conclusion: Increased risk of ARF requiring mechanical ventilation was observed when admission serum ionized calcium was lower than 4.80 mg/dL in hospitalized patients.

Strength and limitation of this study

- This study is a large cohort study that investigate the association between serum ionized calcium and the risk of respiratory failure requiring mechanical ventilation in all hospitalized patients
- This study extensively adjusted for several potential confounders to assess the independent association
- Due to retrospective observational study design, the causal relationship between admission serum ionized calcium cannot be firmly established.

INTRODUCTION

Serum calcium consists of three portions. Approximately 15% is bound to organic and inorganic anions, 40% is bound to proteins, particularly albumin, and 45% circulates as active ionized calcium.[1] This last portion is strictly controlled by parathyroid hormone (PTH) and vitamin D.[2] Total serum calcium concentration substantially varies upon the serum concentration of albumin and hydration status without any alteration in the concentration of ionized calcium. Hence, measuring only total serum calcium might be misleading. The gold standard for assessing calcium status is to measure ionized calcium.[3 4]

On the other hand, some conditions such as acid-base disorder might cause alteration in ionized calcium without any change in total serum calcium concentration. For instance, during acute respiratory alkalosis episode, there is a decrease in ionized calcium at approximately 0.16 mg/dl per 0.1 unit increase in serum pH.[5] As a result, acute respiratory alkalosis can cause symptoms of hypocalcemia, including tetany, seizure, paresthesia, muscle weakness, cramping and respiratory failure and arrest, which requires mechanical ventilation.[6] However, there is no obvious evidence demonstrating the association between serum ionized calcium and the risk of acute respiratory failure (ARF) requiring mechanical ventilation.

Our study aimed to assess the association between initial serum ionized calcium level at the admission and the risk of ARF requiring mechanical ventilation during hospitalization as we hypothesized that serum ionized calcium might be an early predictor of ARF requiring mechanical ventilation.

MATERIALS AND METHODS

Study Population

This is a single-center cohort study. All adult patients, admitted to Mayo Clinic Rochester from January 1st, 2009, to December 31st, 2013, with available admission serum ionized calcium, were included. Patients who had ARF requiring mechanical ventilation at the time of admission were excluded. This study was approved by the Mayo Institutional Review Board (IRB number 15-0000024). All included patients provided authorization of their data use for research purpose.

Data Collection and Clinical Outcomes

Clinical characteristics and laboratory data were obtained using automated retrieval from the institutional electronic medical record system. The predictor of interest was the admission serum ionized calcium, defined as the first serum ionized calcium value measured within 24 hours of hospital admission. Estimated GFR was calculated based on age, sex, race, and serum creatinine, using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[7] The Charlson Comorbidity Index was calculated to assess comorbidities at the time of admission.[8] Principal diagnoses were grouped based on ICD-9 codes. Acute kidney injury was defined as an increase in serum creatinine of ≥ 0.3 mg/dL or ≥ 1.5 times from most recent outpatient serum creatinine before hospital admission. The outcome of interest was ARF requiring mechanical ventilation during hospitalization. The use of mechanical ventilation during the procedure was not included as the outcome.

Patient and Public Involvement

Patients or the public were no involved in the design, or conduct, or reporting, or dissemination of this study.

Statistical Analysis

Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were presented as count with percentage. Continuous and categorical variables were compared

among admission serum ionized calcium group, using ANOVA and the Chi-square test, respectively. Admission serum ionized calcium was categorized into 6 groups based on the percentile distribution (10% | 25% | 50% | 75% | 90%): ≤4.39, 4.40 to 4.59, 4.60 to 4.79, 4.80 to 4.99, 5.00 to 5.19, and ≥5.20 mg/dL. The admission serum ionized calcium level of 5.00 to 5.19 mg/dL was selected as the reference group for outcome comparison since it was associated with the lowest incidence of ARF requiring mechanical ventilation. Multivariate logistic regression analysis was performed to assess the independent association between admission serum ionized calcium levels and ARF requiring mechanical ventilation. Odds ratio (OR) with 95% confidence interval (CI) were reported. The a priori-defined adjusting variables included age, sex, race, baseline GFR, Charlson Comorbidity Index, history of coronary artery disease, hypertension, diabetes mellitus, congestive heart failure, chronic obstructive pulmonary disease, asthma, dementia, stroke, cirrhosis, principal diagnosis, acute kidney injury and the use of vasopressor at hospital admission. There was no missing data in this study. A two-tailed p-value of less than 0.05 was considered statistically significant. All analyses were performed using JMP statistical software (version 10, SAS Institute, Cary, NC).

RESULTS

Clinical characteristics

A total of 33,255 patients with available serum ionized calcium measurement within 24 hours of hospital admission were identified. After 7,546 patients who were on mechanical ventilation at admission were excluded, 25,709 patients were analyzed. Fifty-four percent of enrolled patients were male. The mean age was 63±17 years. The mean admission serum ionized calcium was 4.8 mg/dL. 9%, 14%, 28%, 28%, 14%, 8% had admission serum ionized calcium of ≤4.39, 4.40 to 4.59, 4.60 to 4.79, 4.80 to 4.99, 5.00 to 5.19, and ≥5.20 mg/dL. The clinical characteristics of patients based on admission serum ionized calcium levels were shown in **Table 1**.

Admission serum ionized calcium and risk of in-hospital acute respiratory failure

Of 25,709 patients, the overall incidence of ARF requiring mechanical ventilation during hospitalization was 10%. The lowest incidence of ARF requiring mechanical ventilation was when admission serum ionized calcium of 5.00 to 5.19 mg/dL (7.3%), while the highest incidence was when admission serum ionized calcium of ≤4.39 mg/dL (16.6%) (**Figure 1** and **Table 2**). Progressively increased incidence of ARF requiring mechanical ventilation was observed with decreased admission serum ionized calcium. Multivariable analysis adjusting for potential confounders showed that increased risk of ARF requiring mechanical ventilation was significantly associated with admission serum ionized calcium of ≤4.39 (OR 2.49; 95% CI 2.10-2.97), 4.40 to 4.59 (OR 1.75; 95% CI 1.48-2.06), and 4.60 to 4.79 mg/dL (OR 1.47; 95% CI 1.27-1.71), compared with serum ionized calcium of 5.00-5.09 mg/dL. There was no significant difference in risk of ARF requiring mechanical ventilation when admission serum ionized calcium ≥4.8 mg/dL.

DISCUSSION

The results of our study revealed that admission serum ionized calcium ≤ 4.79 mg/dL was significantly associated with higher odds of ARF requiring mechanical ventilation compared to admission serum ionized calcium of 5.00-5.19 mg/dL. While either admission serum ionized calcium of 4.80-4.99 mg/dL or ≥5.20 mg/dL suggested higher odds of ARF requiring mechanical ventilation. Nonetheless, these associations were not significant.

Our study is the first observational study that assessed the serum ionized calcium level as a predictor of ARF requiring mechanical ventilation among hospitalized patients. Calcium is known to inhibit sodium channels and represses depolarization of nerve and muscle fibers. Hence, hypocalcemia decreases the threshold for depolarization to fire an action potential[9]. Patients with hypocalcemia may present with various clinical signs and symptoms that correlate

with neuromuscular irritability, such as neurological, cardiovascular, psychiatric, and respiratory manifestations.[10 11] In addition to muscle weakness and tetany causing ARF,[10 12 13] Laryngeal spasm and bronchospasm leading to ARF have also been reported in patients with severe hypocalcemia.[14] Low ionized serum calcium may also causes cardiac dysfunction including QTc interval prolongation and reduced left ventricular systolic function, resulting in acute pulmonary edema.[15] In addition, lower serum ionized calcium may also reflect the severity of respiratory alkalosis which eventually leads to diaphragm fatigue and weakness due to hypocalcemia as well as the heavy workload of the diaphragm from principal disease per se.[16 17] Our findings suggested a role of ionized serum calcium level as an early predictor of ARF requiring mechanical ventilation.

Our study had several strengths. We included a large cohort of 25,709 patients. Hence, we could use multiple logistic regressions to extensively adjust for several potential confounders without causing any overfitting problems. Moreover, we categorized serum ionized calcium based on percentile to explore the possibility of non-linear association, which might not be seen if we modeled serum ionized calcium as a continuous variable.

There are limitations to our study. Firstly, arterial blood gas at admission is lacking. Hence, we could not fully adjust metabolic/respiratory derangements. The type of respiratory failure was not investigated in this study. Secondly, due to retrospective observational study design, causality might not be well-established. It is possible that lower ionized calcium is a marker of the initial severity of ARF, which predicted the requirement for mechanical ventilation or the lower ionized calcium plays a pivotal role in diaphragm weakness which leads to ARF requiring mechanical ventilation. However, regardless of which mechanistic pathway it is, we raise the importance of serum ionized calcium as the main predictor of ARF requiring mechanical ventilation.

In conclusion, there is an association between admission serum ionized calcium lower than 4.80 mg/dL and higher odds of ARF requiring mechanical ventilation during hospitalization. Hence, a serum ionized calcium level might be a good predictor of ARF requiring mechanical ventilation. The impact of calcium replacement among patients with hypocalcemia on development of ARF needs to be investigated in prospective investigations.

Conflict of interest statement for all authors:

We do not have any financial or non-financial potential conflicts of interest.

References

1. Pekar JD, Grzych G, Durand G, Haas J, Lionet A, Brousseau T, et al. Calcium state estimation by total calcium: the evidence to end the never-ending story. *Clin Chem Lab Med* 2019.

2. Bushinsky DA, Monk RD. Electrolyte quintet: Calcium. *Lancet* 1998;352(9124):306-11.

3. Ladenson JH, Lewis JW, Boyd JC. Failure of total calcium corrected for protein, albumin, and pH to correctly assess free calcium status. *J Clin Endocrinol Metab* 1978;46(6):986-93.

4. Gauci C, Moranne O, Fouqueray B, de la Faille R, Maruani G, Haymann JP, et al. Pitfalls of measuring total blood calcium in patients with CKD. *J Am Soc Nephrol* 2008;19(8):1592-8.

5. Oberleithner H, Greger R, Lang F. The effect of respiratory and metabolic acid-base changes on ionized calcium concentration: in vivo and in vitro experiments in man and rat. *Eur J Clin Invest* 1982;12(6):451-5.

6. Wang S, McDonnell EH, Sedor FA, Toffaletti JG. pH effects on measurements of ionized calcium and ionized magnesium in blood. *Arch Pathol Lab Med* 2002;126(8):947-50.

7. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150(9):604-12.

8. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. *J Clin Epidemiol* 1994;47(11):1245-51.

9. Armstrong C, Cota G. Calcium block of Na+ channels and its effect on closing rate. *Proceedings of the National Academy of Sciences* 1999;96(7):4154-57.

10. Zaloga GP. Hypocalcemia in critically ill patients. *Critical care medicine* 1992;20(2):251-62.

11. Thongprayoon C, Cheungpasitporn W, Mao MA, Sakhuja A, Erickson SB. Admission calcium levels and risk of acute kidney injury in hospitalised patients. *International journal of clinical practice* 2018;72(4):e13057.

12. Chernow B, Zaloga G, McFADDEN E, Clapper M, Kotler M, Barton M, et al. Hypocalcemia in critically ill patients. *Critical care medicine* 1982;10(12):848-51.

13. Zaloga GP, Chernow B. Hypocalcemia in critical illness. *JAMA* 1986;256(14):1924-29.

14. Aguilera I, Vaughan R. Calcium and the anaesthetist. *Anaesthesia* 2000;55(8):779-90.

15. Newman DB, Fidahussein SS, Kashiwagi DT, Kennel KA, Kashani KB, Wang Z, et al. Reversible cardiac dysfunction associated with hypocalcemia: a systematic review and meta-analysis of individual patient data. *Heart Fail Rev* 2014;19(2):199-205.

16. Moe SM. Disorders involving calcium, phosphorus, and magnesium. *Primary Care: Clinics in Office Practice* 2008;35(2):215-37.

17. Baird GS. Ionized calcium. *Clinica chimica acta* 2011;412(9-10):696-701.

Table

Table 1 Baseline clinical characteristics

Table 2 The association between admission serum ionized calcium levels and in-hospital acute respiratory failure requiring mechanical ventilation

Figure legend

Figure 1 The rate of mechanical ventilation use in hospital based on admission serum ionize calcium level

Table 1 baseline clinical characteristics

variables	All	Serum ionized calcium level at hospital admission (mg/dl)						
		≤4.39	4.40-4.59	4.60-4.79	4.80-4.99	5.00-5.19	≥5.20	p
N	25709	2336	3539	7108	7137	3589	2000	
Age (year)	63±17	60±17	63±17	64±17	64±17	63±18	65±17	<0.001
Male	13829 (54)	1207 (52)	1977 (56)	3974 (56)	3888 (54)	1853 (52)	930 (47)	<0.001
Caucasian	23712 (92)	2096 (90)	3269 (92)	6598 (93)	6596 (92)	3311 (92)	1842 (92)	<0.001
GFR (ml/min/1.73m2)	73±32	62±39	73±33	76±29	76±29	75±31	63±33	<0.001
Charlson score	2.3±2.6	2.4±2.7	2.4±2.7	2.2±2.6	2.1±2.5	2.1±2.5	2.6±2.8	<0.001
Comorbidities								
- CAD	5516 (21)	410 (18)	678 (19)	1498 (21)	1614 (23)	839 (23)	477 (24)	<0.001
- HTN	14439 (56)	1266 (54)	1906 (54)	3906 (55)	4039 (57)	2069 (58)	1253 (63)	<0.001
- DM	5884 (23)	545 (23)	787 (22)	1492 (21)	1649 (23)	894 (25)	517 (26)	<0.001
- CHF	2051 (8)	170 (7)	319 (9)	547 (8)	538 (8)	289 (8)	188 (9)	0.01
- COPD	2663 (10)	209 (9)	353 (10)	728 (10)	764 (11)	396 (11)	213 (11)	0.13
- Asthma	2047 (8)	174 (7)	275 (8)	558 (8)	592 (8)	312 (9)	136 (7)	0.13
- Dementia	491 (2)	20 (0.8)	43 (1)	131 (2)	154 (2)	84 (2)	59 (3)	<0.001
- Stroke	2326 (9)	165 (7)	264 (7)	579 (8)	679 (10)	403 (11)	236 (12)	<0.001
- Cirrhosis	759 (3)	121 (5)	145 (4)	208 (3)	156 (2)	73 (2)	56 (3)	<0.001
Principal diagnosis								<0.001
- Cardiovascular	5554 (22)	295 (13)	566 (16)	1549 (22)	1832 (26)	908 (25)	404 (20)	
- Hematology/Oncology	5526 (21)	415 (18)	932 (26)	1804 (25)	1402 (20)	599 (17)	374 (19)	
- Infectious disease	1044 (4)	236 (10)	204 (6)	248 (3)	180 (3)	97 (3)	79 (4)	
- Endocrine/metabolic	1078 (4)	136 (6)	149 (4)	252 (4)	253 (4)	128 (4)	160 (8)	
- Respiratory	1104 (4)	94 (4)	144 (4)	294 (4)	346 (5)	140 (4)	86 (4)	
- Gastrointestinal	2774 (11)	336 (14)	436 (12)	739 (10)	720 (10)	381 (11)	162 (8)	
- Genitourinary	1104 (4)	223 (10)	165 (5)	228 (3)	210 (3)	117 (3)	161 (8)	
- Injury and poisoning	3419 (13)	330 (14)	490 (14)	957 (13)	937 (13)	487 (14)	218 (11)	
- Other	4106 (16)	271 (12)	453 (13)	1037 (15)	1257 (18)	732 (20)	356 (18)	
Acute kidney injury at admission	5671 (22)	869 (37)	850 (24)	1287 (18)	1255 (18)	707 (20)	703 (35)	<0.001
Vasopressor use at admission	1306 (5)	183 (8)	211 (6)	392 (6)	345 (5)	109 (3)	66 (3)	<0.001

Continuous data are presented as mean ± SD; categorical data are presented as count (percentage)

Table 2 The association between admission serum ionized calcium levels and in-hospital acute respiratory failure requiring mechanical ventilation

Serum ionized calcium level at hospital admission (mg/dl)	Mechanical ventilator in hospital	Univariate analysis		Multivariate analysis	
		OR (95% CI)	p	Adjusted OR (95 % CI)	P
≤4.39	388 (16.6)	2.53 (2.14-2.99)	<0.001	2.49 (2.10-2.97)	<0.001
4.40-4.59	429 (12.1)	1.75 (1.49-2.06)	<0.001	1.75 (1.48-2.06)	<0.001
4.60-4.79	742 (10.4)	1.48 (1.28-1.71)	<0.001	1.47 (1.27-1.71)	<0.001
4.80-4.99	590 (8.3)	1.14 (0.98-1.33)	0.08	1.13 (0.97-1.32)	0.11
5.00-5.19	262 (7.3)	1 (ref)	-	1 (ref)	-
≥5.20	152 (7.6)	1.04 (0.85-1.29)	0.68	1.15 (0.93-1.42)	0.54

Adjusted for age, sex, race, eGFR, Charlson Comorbidity Score, history of coronary artery disease, hypertension, diabetes mellitus, congestive heart failure, chronic obstructive pulmonary disease, asthma, dementia, stroke, cirrhosis, principal diagnosis, acute kidney injury and vasopressor use at hospital admission

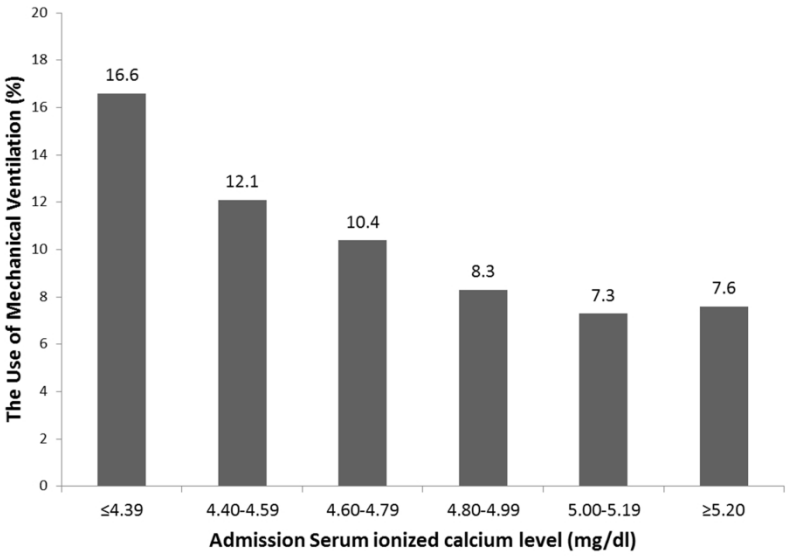


Figure 1 The rate of mechanical ventilation use in hospital based on admission serum ionize calcium level

254x190mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7, 8
Study size	10	Explain how the study size was arrived at	7, 8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7, 8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7, 8
		(*b*) Describe any methods used to examine subgroups and interactions	7, 8
		(*c*) Explain how missing data were addressed	7, 8
		(*d*) If applicable, explain how loss to follow-up was addressed	7, 8
		(*e*) Describe any sensitivity analyses	7, 8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8, Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear	8, 9, Table 2

		which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9, 10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Serum Ionized Calcium and the Risk of Acute Respiratory Failure in Hospitalized Patients: A Single-Center Cohort Study in the United States

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034325.R1
Article Type:	Original research
Date Submitted by the Author:	05-Feb-2020
Complete List of Authors:	Thongprayoon, Charat; Mayo Clinic Rochester, Nephrology and Hypertension Cheungpasitporn, Wisit; University of Mississippi Medical Center, Nephrology Chewcharat, Api; Mayo Clinic Rochester, Nephrology and Hypertension Mao, Michael; Mayo Clinic Florida, Nephrology and Hypertension Kianoush, K; Mayo Clinic Rochester, Nephrology and Hypertension
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Respiratory medicine
Keywords:	ionized calcium, respiratory failure, mechanical ventilation, hospitalization

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Serum Ionized Calcium and the Risk of Acute Respiratory Failure in Hospitalized Patients: A Single-Center Cohort Study in the United States

Running title: Admission Ionized Calcium Levels and Respiratory Failure

Authors: Charat Thongprayoon, MD.[1], Wisit Cheungpasitporn, MD.2, Api Chewcharat MD, MPH[1], Michael A Mao, MD.[3] and Kianoush B. Kashani, MD, MS[1,4]

Institutional affiliations:

1. Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, MN
2. Division of Nephrology, Department of Internal Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA
3. Division of Nephrology and Hypertension, Mayo Clinic, Jacksonville, FL 32224, USA;
4. Division of Pulmonary and Critical Care Medicine, Department of Medicine, Mayo Clinic, Rochester, MN

Institution: This work was performed at Mayo Clinic in Rochester, MN.

Corresponding author:

Kianoush B. Kashani, MD, MS, Division of Nephrology and Hypertension, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (kashani.kianoush@mayo.edu).

Conflicts of interest: None

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Authors' contributions

CT, WC and KBK originated the idea, and designed study. CT, AC, WC, and MAM collected data. CT analyzed the data. CT and AC were responsible for writing the manuscript. WC, MAM, KBK supported the editing of the manuscript and added important comments to the manuscript. KBK supervised the study. All authors had access to the data, read, and approved the final manuscript.

Word Count: 2137

Keywords: serum ionized calcium, respiratory failure, mechanical ventilation, hospitalization

ABSTRACT

Objectives: The objective of this study was to evaluate the risk of acute respiratory failure in all hospitalized patients based on admission serum ionized calcium.

Design: A retrospective cohort study

Setting: a tertiary referral hospital in Rochester, Minnesota, USA

Participants: All hospitalized patients who had serum ionized calcium measurement within 24 hours of hospital admission from January 2009 to December 2013. Patients who were mechanically ventilated at admission were excluded.

Predictors: Admission serum ionized calcium levels was stratified into six groups; ≤4.39, 4.40 to 4.59, 4.60 to 4.79, 4.80 to 4.99, 5.00 to 5.19, and ≥5.20 mg/dL.

Primary outcome measure: The primary outcome was the development of acute respiratory failure requiring mechanical ventilation during hospitalization. Logistic regression analysis was fit to assess the independent risk of acute respiratory failure based on various admission serum ionized calcium, using serum ionized calcium of 5.00 to 5.19 mg/dL as the reference group.

Results: Of 25,709 eligible patients, with the mean serum ionized calcium of 4.8±0.4 mg/dL, acute respiratory failure requiring mechanical ventilation occurred in 2,563 patients (10%). The incidence of acute respiratory failure was lowest when admission serum ionized calcium was 5.00-5.19 mg/dL, with the progressively increased risk of acute respiratory failure with decreased serum ionized calcium. In multivariate analysis with adjustment for potential confounders, the increased risk of acute respiratory failure requiring mechanical ventilation was significantly associated with admission serum ionized calcium of ≤4.39 (OR 2.52; 95% CI 2.12-3.00), 4.40 to 4.59 (OR 1.76; 95% CI 1.49-2.07), and 4.60 to 4.79 mg/dL (OR 1.48; 95% CI 1.27-1.72), compared with serum ionized calcium of 5.00-5.19 mg/dL. The risk of acute

respiratory failure was not significantly increased when serum ionized calcium was at least 4.80 mg/dL.

Conclusion: The increased risk of acute respiratory failure requiring mechanical ventilation was observed when admission serum ionized calcium was lower than 4.80 mg/dL in hospitalized patients.

Strength and limitation of this study

- This study is a large cohort study that investigate the association between serum ionized calcium and the risk of respiratory failure requiring mechanical ventilation in all hospitalized patients
- This study extensively adjusted for several potential confounders to assess the independent association
- Due to retrospective observational study design, the causal relationship between admission serum ionized calcium cannot be firmly established.

INTRODUCTION

Calcium has many essential functions including intracellular signaling, muscle function, nerve transmission, and mediating vascular contraction and vasodilatation.[1] Serum calcium consists of three portions.[1] Approximately 15% is bound to organic and inorganic anions, 40% is bound to proteins, particularly albumin, and 45% circulates as active ionized calcium.[2] This last portion is strictly controlled by parathyroid hormone and vitamin D.[3] Total serum calcium concentration substantially varies upon the serum concentration of albumin and hydration status without any alteration in the concentration of ionized calcium.[4-7] Hence, measuring only total serum calcium might be misleading. The gold standard for assessing calcium status is to measure ionized calcium.[8-10]

Recently, we demonstrated that both decreased and elevated serum calcium levels were related to higher short-term and long-term mortality in hospitalized patients.[11 12] However, the cause of death was not investigated in the previous study. Acute respiratory failure (ARF) is a common and life-threatening condition among hospitalized patients,[13-15] and is associated with high morbidity and mortality worldwide.[16-22] Previously, we identified several electrolyte derangements at the time of hospital admission as risk factors for ARF in hospitalized patients. These risk factors included hypo- or hyperphosphatemia,[20] low serum creatinine,[21] hypoalbuminemia,[23] hypo- or hyperkalemia,[24] and hypo- or hypermagnesemia.[25] Alterations of serum calcium have been linked to the development of ARF in several case reports.[26-30] While hypocalcemia can lead to ARF due to muscle weakness, tetany, laryngeal and bronchospasm, patients with severe hypercalcemia can present with lethargy, confusion, and coma, resulting in ARF.[26-30] However, previously described cases focused on the total serum calcium concentration, and the risk of in-hospital ARF among patients with various serum ionized calcium levels is not elucidated in a large clinical study.

Our study aimed to assess the association between serum ionized calcium level, measured at the admission, and the risk of ARF requiring mechanical ventilation during hospitalization as we hypothesized that serum ionized calcium is an early predictor of ARF requiring mechanical ventilation.

MATERIALS AND METHODS

Study Population

This is a single-center cohort study. We used our previous cohort of all adult hospitalized patients with available admission serum ionized calcium from years 2009 through 2013 in the analysis. Because we aimed to assess the risk of ARF as the primary outcome measure, we further excluded mechanically-ventilated patients at the time of admission. This study was approved by the Mayo Institutional Review Board (IRB number 15-000024). The need for informed consent was exempted due to the minimal risk nature of the study but all included patients provided authorization of their data use for research purpose.

Data Collection and Outcomes

Automated retrieval from the institutional electronic medical record system was utilized to obtain clinical characteristics and laboratory data of patients in this study. The primary predictor was the admission serum ionized calcium, defined as the initial serum ionized calcium value measured within 24 hours of hospital admission. CKD-EPI equation was used to estimate glomerular filtration rate (eGFR).[31] Comorbidity burden of an individual patient was assessed using Charlson Comorbidity Index.[32] Acute kidney injury was defined based on KDIGO criteria. The primary outcome measure was in-hospital ARF requiring invasive mechanical ventilation, as previously described in our published studies.[20 21 23-25] The use of invasive mechanical ventilation was abstracted from our intensive care unit (ICU) DataMart which documented all mechanical ventilation use in the ICU, including the start and end time of mechanical ventilation.

The use of mechanical ventilation during the procedure and non-invasive ventilation support were not considered as the outcome. ARF was considered hypercapnic if pCO2 from arterial blood gas before mechanical ventilation was at least 50 mmHg.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination of this study.

Statistical Analysis

The difference in continuous and categorical variables among admission serum ionized calcium group was tested, using ANOVA and the Chi-square test, respectively. The restricted cubic spline with 5 knots was constructed to depict the potential non-linear association between admission serum ionized calcium and the risk of ARF requiring mechanical ventilation. Admission serum ionized calcium was categorized into 6 groups based on the percentile distribution (10% | 25% | 50% | 75% | 90%): ≤4.39, 4.40 to 4.59, 4.60 to 4.79, 4.80 to 4.99, 5.00 to 5.19, and ≥5.20 mg/dL. The admission serum ionized calcium level of 5.00 to 5.19 mg/dL was the reference group because it was considered within normal reference range of 4.57-5.43 mg/dL, based on our institutional laboratory test, and it had the lowest incidence of ARF requiring mechanical ventilation. Multivariable logistic regression was conducted to evaluate admission serum ionized calcium level that was independently associated with the risk of ARF requiring mechanical ventilation. The odds ratio (OR) was adjusted for a pre-specified variables reported in Table 1. Two sensitivity analyses was performed to 1) assess the association between admission serum ionized calcium and the risk of hypercapnic ARF requiring mechanical ventilation and 2) assess the association between albumin-corrected total serum calcium and the risk of ARF requiring mechanical ventilation. Missing data were not imputed. All

analyses were two tailed. Statistical significance was achieved when p-value less than 0.05. JMP statistical software (version 10, SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Clinical characteristics

We screened 288,120 hospital admissions during the study period. We excluded patients with no research authorization (n=1,701), pediatric patients (n=32,139), those with no admission serum ionized calcium measurement (n=184,241), those on mechanical ventilation at hospital admission (n=7,546), and re-admission (n=36,784) were excluded. A total of 25,709 eligible patients with available admission serum ionized calcium were analyzed (Figure S1). Fifty-four percent of enrolled patients were male. The mean age was 63±17 years. The mean admission serum ionized calcium was 4.8±0.4 mg/dL. 9%, 14%, 28%, 28%, 14%, 8% had admission serum ionized calcium of ≤4.39, 4.40 to 4.59, 4.60 to 4.79, 4.80 to 4.99, 5.00 to 5.19, and ≥5.20 mg/dL. Table 1 showed the clinical characteristics of patients based on admission serum ionized calcium levels.

Admission serum ionized calcium and the risk of in-hospital acute respiratory failure

Of 25,709 patients, 2563 (10.0%) had in-hospital ARF requiring mechanical ventilation. Patients with admission serum ionized calcium of 5.00 to 5.19 mg/dL had the lowest incidence of in-hospital ARF, whereas patients with admission serum ionized calcium of ≤4.39 mg/dL had the highest incidence (**Table 2**). Higher incidence of in-hospital ARF was noted with decreased admission serum ionized calcium (**Figure 1**) below 5.00 mg/dL. In multivariable analysis with adjustment for potential confounders, the higher risk of in-hospital ARF was significantly associated with admission serum ionized calcium of ≤4.39 (OR 2.52; 95% CI 2.12-3.00), 4.40 to 4.59 (OR 1.76; 95% CI 1.49-2.07), and 4.60 to 4.79 mg/dL (OR 1.48; 95% CI 1.27-1.72), compared with admission serum ionized calcium of 5.00-5.19 mg/dL. There was no significant

difference in the risk of in-hospital ARF when admission serum ionized calcium was at least 4.8 mg/dL. There was a higher risk of in-hospital ARF with an adjusted OR of 1.85 (95% CI 1.66-2.09) when admission serum ionized decreased by 1 mg/dL.

In a sensitivity analysis, admission serum ionized calcium level of ≤4.79 mg/dL was significantly associated with the higher risk of in-hospital hypercapnic ARF requiring mechanical ventilation (Table S1). In addition, the analysis in 8,534 patients with available admission albumin-corrected total serum calcium showed that admission serum calcium of ≤8.5 mg/dL was significantly associated with the higher risk of in-hospital ARF (OR 1.45; 95% CI 1.28-1.63), compared with admission serum calcium of 8.6-10.0 mg/dL (the normal reference range in our hospital). In contrast, admission serum calcium of ≥10.1 mg/dL was not significantly associated with the higher risk of in-hospital ARF (OR 1.08; 95% CI 0.85-1.37). There was a higher risk of in-hospital ARF with an adjusted OR of 1.10 (95% CI 1.04-1.17) when admission serum ionized calcium decreased by 1 mg/dL.

DISCUSSION

This study demonstrated a statistically significant inverse relationship between admission serum ionized calcium and the subsequent risk of in-hospital ARF requiring mechanical ventilation in hospitalized patients. Lower admission serum ionized calcium was progressively associated with the higher risk of in-hospital ARF when admission serum calcium was below 4.8 mg/dL. There was no difference in the risk of in-hospital ARF when admission serum ionized calcium was at least 4.8 mg/dL.

Our study is the first observational study that assessed the serum ionized calcium level as a predictor of in-hospital ARF requiring mechanical ventilation among hospitalized patients. Calcium is known to inhibit sodium channels and represses depolarization of nerve and muscle

fibers. Hence, hypocalcemia decreases the threshold for depolarization to fire an action potential[33]. Patients with hypocalcemia may present with various clinical signs and symptoms that correlate with neuromuscular irritability, such as neurological, cardiovascular, psychiatric, and respiratory manifestations.[27 34 35] In addition to muscle weakness and tetany causing ARF,[26-28] Diaphragmatic weakness, laryngeal spasm, and bronchospasm leading to ARF have also been reported in patients with severe hypocalcemia.[29] Low serum ionized calcium may also cause cardiac dysfunction including QTc interval prolongation and reduced left ventricular systolic function, resulting in acute pulmonary edema.[36] In addition, lower serum ionized calcium may also reflect the severity of respiratory alkalosis which eventually leads to diaphragm fatigue and weakness due to hypocalcemia as well as the heavy workload of the diaphragm from principal disease per se.[37 38] The data on the simultaneous assessment of partial pressure of oxygen (PaO2) and fraction of inspired oxygen (FiO2) was limited by inaccuracies of FiO2 documentation. Therefore, the assessment of the correlation between ionized serum calcium level and hypoxemic respiratory failure was challenging. Meanwhile, we were able to assess the association between admission serum ionized calcium and the risk of in-hospital hypercapnic ARF. We demonstrated that low admission serum ionized calcium of ≤4.79 mg/dL was significantly associated with the higher risk of in-hospital hypercapnic ARF. In addition, we demonstrated that hypocalcemia based on total serum calcium level, which is not altered by acid/base status, was also associated with the higher risk of in-hospital ARF. Our findings suggested a role of ionized serum calcium level as an early predictor of in-hospital ARF.

Our study had several strengths. We included a large cohort of 25,709 patients. Hence, we could use multiple logistic regressions to comprehensively adjust for a number of potential confounders without causing any overfitting problems. Moreover, we categorized serum ionized calcium based on percentile to explore the possibility of non-linear association, which might not be seen if we modeled serum ionized calcium as a continuous variable.

There are some limitations in our study. Due to the retrospective nature of our observational study design, causality would not be established. It is possible that lower ionized calcium is a marker of the initial severity of ARF, which predicted the requirement for mechanical ventilation or the lower ionized calcium plays a pivotal role in diaphragm weakness which leads to ARF. However, regardless of which mechanistic pathway, we were able to highlight the significance of serum ionized calcium as a predictor of in-hospital ARF. Although we extensively adjusted for potential confounders, the association between admission serum ionized calcium and the risk of in-hospital ARF might remain confounded by unmeasured or unknown factors. The data from this study were retrieved from the institutional electronic database. Unfortunately, some important clinical information such as the causes of serum ionized calcium derangements, vitamin D levels, the causes and types of in-hospital ARF, underlying neuromuscular disease, and chronic respiratory failure, were not available or incomplete in our database and, therefore, we were not able to report them. There is a large and growing literature implicating inadequate vitamin D status as a risk factor for adverse outcomes including bronchospasm, acute respiratory infections, and chronic obstructive pulmonary disease (COPD) exacerbations.[39-41] Thus, future studies are required to assess whether low vitamin D levels modify the effect of decreased admission serum ionized calcium and the increased risk of ARF. Also, serum ionized calcium is often not measured in hospitalized patients when they were initially admitted to the hospital. A selection bias should be considered when we limited the analysis in patients with available admission serum ionized calcium. Included patients who had available admission serum ionized calcium were older, lower eGFR, had more comorbidity conditions, were more primarily admitted for hematology/oncology or gastrointestinal disease than those who did not have available admission serum ionized calcium and were excluded from the analysis (Table S2). Finally, our study was a single-center study, and most of the included individuals were from the Caucasian race. This might limit the generalizability of the study.

In summary, there is an association between admission serum ionized calcium lower than 4.80 mg/dL and higher risk of in-hospital ARF requiring mechanical ventilation. Hence, a serum ionized calcium level might potentially be a good predictor of ARF requiring mechanical ventilation.

Conflict of interest statement for all authors:

We do not have any financial or non-financial potential conflicts of interest.

Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information. Raw data is available upon a reasonable request.

References

1. Beto JA. The role of calcium in human aging. *Clin Nutr Res* 2015;4(1):1-8. doi: 10.7762/cnr.2015.4.1.1 [published Online First: 2015/02/26]

2. Pekar JD, Grzych G, Durand G, et al. Calcium state estimation by total calcium: the evidence to end the never-ending story. *Clin Chem Lab Med* 2019 doi: 10.1515/cclm-2019-0568 [published Online First: 2019/09/02]

3. Bushinsky DA, Monk RD. Electrolyte quintet: Calcium. *Lancet* 1998;352(9124):306-11. doi: 10.1016/s0140-6736(97)12331-5 [published Online First: 1998/08/05]

4. Michaelsson K, Melhus H, Warensjo Lemming E, et al. Long term calcium intake and rates of all cause and cardiovascular mortality: community based prospective longitudinal cohort study. *BMJ (Clinical research ed)* 2013;346:f228. doi: 10.1136/bmj.f228 [published Online First: 2013/02/14]

5. Collage RD, Howell GM, Zhang X, et al. Calcium supplementation during sepsis exacerbates organ failure and mortality via calcium/calmodulin-dependent protein kinase kinase signaling. *Critical care medicine* 2013;41(11):e352-60. doi: 10.1097/CCM.0b013e31828cf436 [published Online First: 2013/07/28]

6. Yan SD, Liu XJ, Peng Y, et al. Admission Serum Calcium Levels Improve the GRACE Risk Score Prediction of Hospital Mortality in Patients With Acute Coronary Syndrome. *Clinical cardiology* 2016;39(9):516-23. doi: 10.1002/clc.22557 [published Online First: 2016/06/10]

7. Miura S, Yoshihisa A, Takiguchi M, et al. Association of Hypocalcemia With Mortality in Hospitalized Patients With Heart Failure and Chronic Kidney Disease. *Journal of cardiac failure* 2015;21(8):621-7. doi: 10.1016/j.cardfail.2015.04.015 [published Online First: 2015/05/20]

8. Ladenson JH, Lewis JW, Boyd JC. Failure of total calcium corrected for protein, albumin, and pH to correctly assess free calcium status. *J Clin Endocrinol Metab* 1978;46(6):986-93. doi: 10.1210/jcem-46-6-986 [published Online First: 1978/06/01]

9. Gauci C, Moranne O, Fouqueray B, et al. Pitfalls of measuring total blood calcium in patients with CKD. *J Am Soc Nephrol* 2008;19(8):1592-8. doi: 10.1681/asn.2007040449 [published Online First: 2008/04/11]

10. Oberleithner H, Greger R, Lang F. The effect of respiratory and metabolic acid-base changes on ionized calcium concentration: in vivo and in vitro experiments in man and rat. *Eur J Clin Invest* 1982;12(6):451-5. doi: 10.1111/j.1365-2362.1982.tb02223.x [published Online First: 1982/12/01]

11. Thongprayoon C, Cheungpasitporn W, Chewcharat A, et al. Hospital mortality and long-term mortality among hospitalized patients with various admission serum ionized calcium levels. *Postgrad Med* 2020:1-6. doi: 10.1080/00325481.2020.1728980 [published Online First: 2020/02/19]

12. Cheungpasitporn W, Thongprayoon C, Mao MA, et al. Impact of admission serum calcium levels on mortality in hospitalized patients. *Endocr Res* 2018;43(2):116-23. doi: 10.1080/07435800.2018.1433200 [published Online First: 2018/01/31]

13. Chakrabarti B, Calverley PM. Management of acute ventilatory failure. *Postgrad Med J* 2006;82(969):438-45. doi: 10.1136/pgmj.2005.043208 [published Online First: 2006/07/11]

14. Rochwerg B, Brochard L, Elliott MW, et al. Official ERS/ATS clinical practice guidelines: noninvasive ventilation for acute respiratory failure. *Eur Respir J* 2017;50(2) doi: 10.1183/13993003.02426-2016 [published Online First: 2017/09/02]

15. Afshar M, Joyce C, Oakey A, et al. A Computable Phenotype for Acute Respiratory Distress Syndrome Using Natural Language Processing and Machine Learning. *AMIA Annu Symp Proc* 2018;2018:157-65.

16. Behrendt CE. Acute respiratory failure in the United States: incidence and 31-day survival. *Chest* 2000;118(4):1100-5. doi: 10.1378/chest.118.4.1100 [published Online First: 2000/10/18]

17. Stefan MS, Shieh MS, Pekow PS, et al. Epidemiology and outcomes of acute respiratory failure in the United States, 2001 to 2009: a national survey. *J Hosp Med* 2013;8(2):76-82. doi: 10.1002/jhm.2004 [published Online First: 2013/01/22]

18. Hughes M, Grant IS, MacKirdy FN. Incidence and mortality after acute respiratory failure and acute respiratory distress syndrome in Sweden, Denmark, and Iceland. *Am J Respir Crit Care Med* 2000;162(1):332-3. doi: 10.1164/ajrccm.162.1.16213b [published Online First: 2000/07/21]

19. Vincent JL, Akca S, De Mendonca A, et al. The epidemiology of acute respiratory failure in critically ill patients(*). *Chest* 2002;121(5):1602-9. doi: 10.1378/chest.121.5.1602 [published Online First: 2002/05/15]

20. Thongprayoon C, Cheungpasitporn W, Chewcharat A, et al. Admission serum phosphate levels and the risk of respiratory failure. *Int J Clin Pract* 2019:e13461. doi: 10.1111/ijcp.13461 [published Online First: 2019/12/13]

21. Thongprayoon C, Cheungpasitporn W, Chewcharat A, et al. The Association of Low Admission Serum Creatinine with the Risk of Respiratory Failure Requiring Mechanical Ventilation: A Retrospective Cohort Study. *Sci Rep* 2019;9(1):18743. doi: 10.1038/s41598-019-55362-w [published Online First: 2019/12/12]

22. Yang P, Formanek P, Scaglione S, et al. Risk factors and outcomes of acute respiratory distress syndrome in critically ill patients with cirrhosis. *Hepatol Res* 2019;49(3):335-43. doi: 10.1111/hepr.13240 [published Online First: 2018/08/29]

23. Thongprayoon C, Cheungpasitporn W, Chewcharat A, et al. Risk of acute respiratory failure among hospitalized patients with various admission serum albumin levels: A cohort study. *Medicine (Baltimore)* 2020;99(9):e19352. doi: 10.1097/MD.0000000000019352 [published Online First: 2020/03/03]

24. Thongprayoon C, Cheungpasitporn W, Chewcharat A, et al. Risk of respiratory failure among hospitalized patients with various admission serum potassium levels. *Hosp Pract (1995)* 2020:1-5. doi: 10.1080/21548331.2020.1729621 [published Online First: 2020/02/18]

25. Thongprayoon C, Cheungpasitporn W, Srivali N, et al. Admission serum magnesium levels and the risk of acute respiratory failure. *Int J Clin Pract* 2015;69(11):1303-8. doi: 10.1111/ijcp.12696 [published Online First: 2015/07/25]

26. Chernow B, Zaloga G, McFADDEN E, et al. Hypocalcemia in critically ill patients. *Critical care medicine* 1982;10(12):848-51.

27. Zaloga GP. Hypocalcemia in critically ill patients. *Critical care medicine* 1992;20(2):251-62.

28. Zaloga GP, Chernow B. Hypocalcemia in critical illness. *JAMA* 1986;256(14):1924-29.

29. Aguilera I, Vaughan R. Calcium and the anaesthetist. *Anaesthesia* 2000;55(8):779-90.

30. Guo Y, He L, Liu Y, et al. A rare case report of multiple myeloma presenting with paralytic ileus and type II respiratory failure due to hypercalcemic crisis. *Medicine (Baltimore)* 2017;96(52):e9215-e15. doi: 10.1097/MD.0000000000009215

31. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150(9):604-12. doi: 10.7326/0003-4819-150-9-200905050-00006 [published Online First: 2009/05/06]

32. Charlson M, Szatrowski TP, Peterson J, et al. Validation of a combined comorbidity index. *J Clin Epidemiol* 1994;47(11):1245-51. [published Online First: 1994/11/01]

33. Armstrong C, Cota G. Calcium block of Na+ channels and its effect on closing rate. *Proceedings of the National Academy of Sciences* 1999;96(7):4154-57.

34. Thongprayoon C, Cheungpasitporn W, Mao MA, et al. Admission calcium levels and risk of acute kidney injury in hospitalised patients. *International journal of clinical practice* 2018;72(4):e13057.

35. Thongprayoon C, Cheungpasitporn W, Hansrivijit P, et al. Impact of Changes in Serum Calcium Levels on In-Hospital Mortality. *Medicina (Kaunas)* 2020;56(3) doi: 10.3390/medicina56030106 [published Online First: 2020/03/07]

36. Newman DB, Fidahussein SS, Kashiwagi DT, et al. Reversible cardiac dysfunction associated with hypocalcemia: a systematic review and meta-analysis of individual patient data. *Heart Fail Rev* 2014;19(2):199-205. doi: 10.1007/s10741-013-9371-1 [published Online First: 2013/01/29]

37. Moe SM. Disorders involving calcium, phosphorus, and magnesium. *Primary Care: Clinics in Office Practice* 2008;35(2):215-37.

38. Baird GS. Ionized calcium. *Clinica chimica acta* 2011;412(9-10):696-701.

39. Stefanidis C, Martineau AR, Nwokoro C, et al. Vitamin D for secondary prevention of acute wheeze attacks in preschool and school-age children. *Thorax* 2019;74(10):977-85. doi: 10.1136/thoraxjnl-2019-213278 [published Online First: 2019/07/05]

40. Martineau AR, Jolliffe DA, Greenberg L, et al. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. *Health Technol Assess* 2019;23(2):1-44. doi: 10.3310/hta23020

41. Jolliffe DA, Greenberg L, Hooper RL, et al. Vitamin D to prevent exacerbations of COPD: systematic review and meta-analysis of individual participant data from randomised controlled trials. *Thorax* 2019;74(4):337-45. doi: 10.1136/thoraxjnl-2018-212092 [published Online First: 2019/01/10]

Table

Table 1 Baseline clinical characteristics

Table 2 The association between admission serum ionized calcium levels and in-hospital acute respiratory failure requiring mechanical ventilation

Figure legend

Figure 1 The rate of mechanical ventilation use in hospital based on admission serum ionized calcium level

Table 1 baseline clinical characteristics

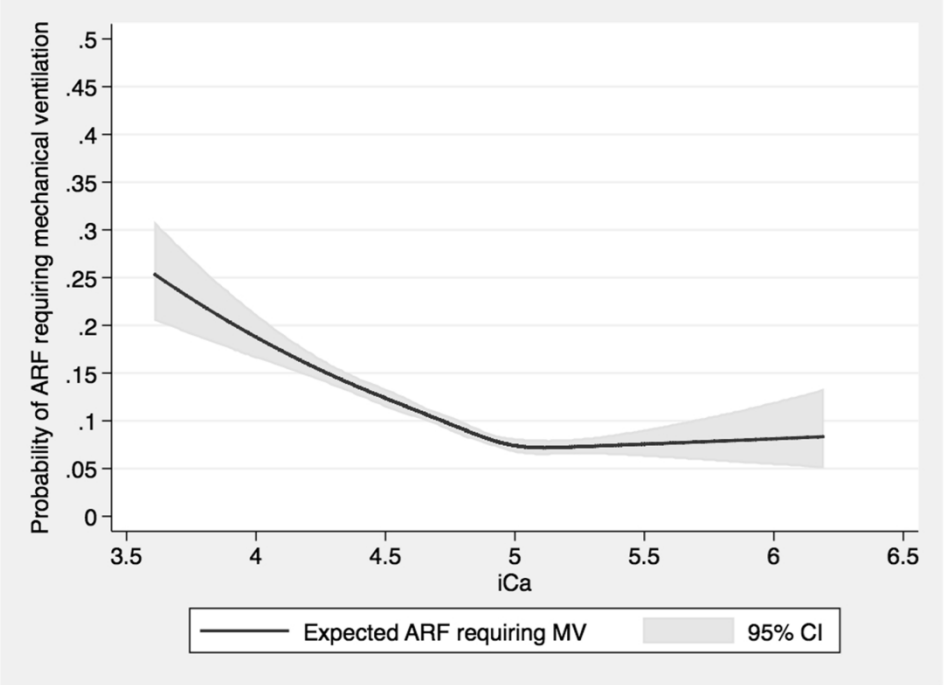
variables	All	Serum ionized calcium level at hospital admission (mg/dl)						
		≤4.39	4.40-4.59	4.60-4.79	4.80-4.99	5.00-5.19	≥5.20	p
N	25709	2336	3539	7108	7137	3589	2000	
Age (year)	63±17	60±17	63±17	64±17	64±17	63±18	65±17	<0.001
Male	13829 (54)	1207 (52)	1977 (56)	3974 (56)	3888 (54)	1853 (52)	930 (47)	<0.001
Caucasian	23712 (92)	2096 (90)	3269 (92)	6598 (93)	6596 (92)	3311 (92)	1842 (92)	<0.001
GFR (ml/min/1.73m2)	73±32	62±39	73±33	76±29	76±29	75±31	63±33	<0.001
Charlson score	2.3±2.6	2.4±2.7	2.4±2.7	2.2±2.6	2.1±2.5	2.1±2.5	2.6±2.8	<0.001
Comorbidities								
- Coronary artery disease	5516 (21)	410 (18)	678 (19)	1498 (21)	1614 (23)	839 (23)	477 (24)	<0.001
- Hypertension	14439 (56)	1266 (54)	1906 (54)	3906 (55)	4039 (57)	2069 (58)	1253 (63)	<0.001
- Diabetes mellitus	5884 (23)	545 (23)	787 (22)	1492 (21)	1649 (23)	894 (25)	517 (26)	<0.001
- Congestive heart failure	2051 (8)	170 (7)	319 (9)	547 (8)	538 (8)	289 (8)	188 (9)	0.01
- COPD	2663 (10)	209 (9)	353 (10)	728 (10)	764 (11)	396 (11)	213 (11)	0.13
- Asthma	2047 (8)	174 (7)	275 (8)	558 (8)	592 (8)	312 (9)	136 (7)	0.13
- Dementia	491 (2)	20 (0.8)	43 (1)	131 (2)	154 (2)	84 (2)	59 (3)	<0.001
- Stroke	2326 (9)	165 (7)	264 (7)	579 (8)	679 (10)	403 (11)	236 (12)	<0.001
- Cirrhosis	759 (3)	121 (5)	145 (4)	208 (3)	156 (2)	73 (2)	56 (3)	<0.001
- End-stage renal disease	1261 (5)	345 (15)	227 (6)	260 (4)	209 (3)	116 (3)	104 (5)	<0.001
- Obesity	8962 (35)	786 (34)	1289 (36)	2531 (36)	2574 (36)	1138 (32)	644 (32)	<0.001
Principal diagnosis								<0.001
- Cardiovascular	5554 (22)	295 (13)	566 (16)	1549 (22)	1832 (26)	908 (25)	404 (20)	
- Hematology/Oncology	5526 (21)	415 (18)	932 (26)	1804 (25)	1402 (20)	599 (17)	374 (19)	
- Infectious disease	1044 (4)	236 (10)	204 (6)	248 (3)	180 (3)	97 (3)	79 (4)	
- Endocrine/metabolic	1078 (4)	136 (6)	149 (4)	252 (4)	253 (4)	128 (4)	160 (8)	
- Respiratory	1104 (4)	94 (4)	144 (4)	294 (4)	346 (5)	140 (4)	86 (4)	
- Gastrointestinal	2774 (11)	336 (14)	436 (12)	739 (10)	720 (10)	381 (11)	162 (8)	
- Genitourinary	1104 (4)	223 (10)	165 (5)	228 (3)	210 (3)	117 (3)	161 (8)	
- Injury and poisoning	3419 (13)	330 (14)	490 (14)	957 (13)	937 (13)	487 (14)	218 (11)	
- Other	4106 (16)	271 (12)	453 (13)	1037 (15)	1257 (18)	732 (20)	356 (18)	
Acute kidney injury at admission	5671 (22)	869 (37)	850 (24)	1287 (18)	1255 (18)	707 (20)	703 (35)	<0.001
Vasopressor use at admission	1306 (5)	183 (8)	211 (6)	392 (6)	345 (5)	109 (3)	66 (3)	<0.001

Continuous data are presented as mean ± SD; categorical data are presented as count (percentage)

Table 2 The association between admission serum ionized calcium levels and in-hospital acute respiratory failure requiring mechanical ventilation

Serum ionized calcium level at hospital admission (mg/dl)	Mechanical ventilator in hospital	Univariate analysis		Multivariate analysis	
		OR (95% CI)	p	Adjusted OR (95 % CI)	P
≤4.39	388 (16.6)	2.53 (2.14-2.99)	<0.001	2.52 (2.12-3.00)	<0.001
4.40-4.59	429 (12.1)	1.75 (1.49-2.06)	<0.001	1.76 (1.49-2.07)	<0.001
4.60-4.79	742 (10.4)	1.48 (1.28-1.71)	<0.001	1.48 (1.27-1.72)	<0.001
4.80-4.99	590 (8.3)	1.14 (0.98-1.33)	0.08	1.13 (0.97-1.32)	0.11
5.00-5.19	262 (7.3)	1 (ref)	-	1 (ref)	-
≥5.20	152 (7.6)	1.04 (0.85-1.29)	0.68	1.14 (0.93-1.41)	0.22

Adjusted for age, sex, race, eGFR, Charlson Comorbidity Score, history of coronary artery disease, hypertension, diabetes mellitus, congestive heart failure, chronic obstructive pulmonary disease, asthma, dementia, stroke, cirrhosis, end-stage renal failure, obesity, principal diagnosis, acute kidney injury and vasopressor use at hospital admission



The rate of mechanical ventilation use in hospital based on admission serum ionized calcium level

254x190mm (300 x 300 DPI)

Table S1 The association between admission serum ionized calcium levels and hypercapnic respiratory failure requiring mechanical ventilation

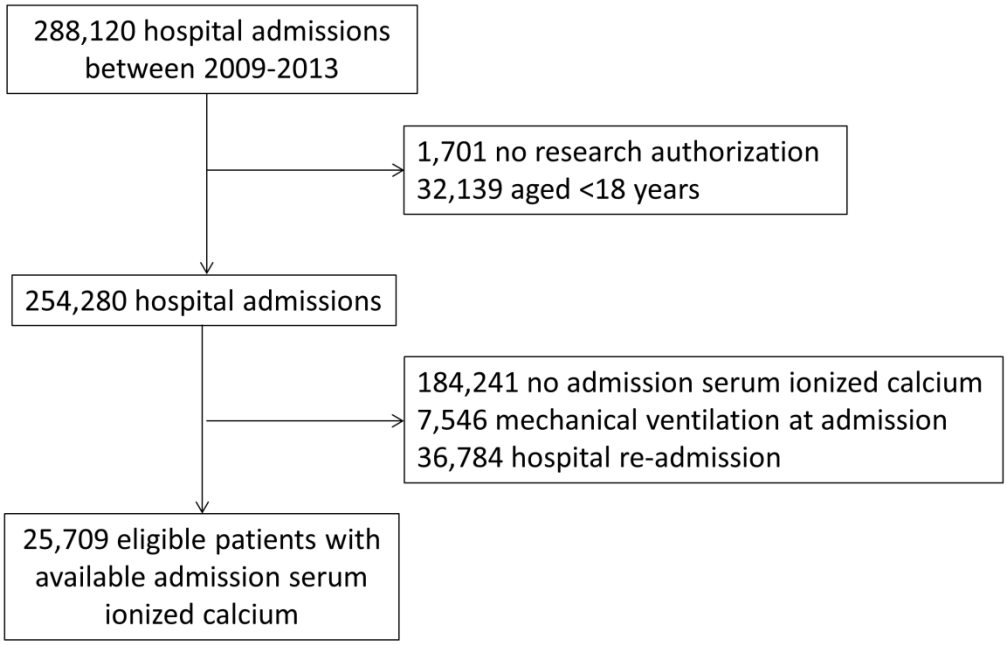
Serum ionized calcium level at hospital admission (mg/dl)	Hypercarbic respiratory failure	Univariate analysis		Multivariate analysis	
		OR (95% CI)	p	Adjusted OR (95 % CI)	P
≤4.39	81 (3.5)	4.12 (2.72-6.26)	<0.001	3.06 (1.99-4.71)	<0.001
4.40-4.59	70 (2.0)	2.31 (1.51-3.54)	<0.001	2.02 (1.32-3.12)	0.001
4.60-4.79	103 (1.5)	1.69 (1.13-2.53)	0.01	1.62 (1.08-2.43)	0.02
4.80-4.99	94 (1.3)	1.53 (1.02-2.30)	0.03	1.51 (0.98-2.27)	0.06
5.00-5.19	31 (0.9)	1 (ref)	-	1 (ref)	-
≥5.20	24 (1.2)	1.39 (0.82-2.38)	0.23	1.38 (0.80-2.37)	0.25

Adjusted for age, sex, race, eGFR, Charlson Comorbidity Score, history of coronary artery disease, hypertension, diabetes mellitus, congestive heart failure, chronic obstructive pulmonary disease, asthma, dementia, stroke, cirrhosis, end-stage renal disease, obesity, principal diagnosis, acute kidney injury and vasopressor use at hospital admission

Table S2 Clinical characteristics between patients with and without admission ionized calcium measurement

	No admission ionized calcium measurement	Included patients	p-value
Age (year)	61±18	63±17	<0.001
Male	53%	54%	<0.001
Caucasian	93%	92%	<0.001
GFR (ml/min/1.73m2)	78±28	73±32	<0.001
Charlson score	1.8±2.3	2.3±2.6	<0.001
Principal diagnosis			<0.001
- Cardiovascular	21%	22%	
- Hematology/Oncology	15%	21%	
- Infectious disease	3%	4%	
- Endocrine/metabolic	3%	4%	
- Respiratory	4%	4%	
- Gastrointestinal	9%	11%	
- Genitourinary	4%	4%	
- Injury and poisoning	15%	13%	
- Other	25%	16%	

Figure S1 study flow chart



STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	8,9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8,9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8,9
		(*b*) Describe any methods used to examine subgroups and interactions	8,9
		(*c*) Explain how missing data were addressed	8,9
		(*d*) If applicable, explain how loss to follow-up was addressed	8,9
		(*e*) Describe any sensitivity analyses	8,9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	Figure S1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	9,10 Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted	9,10, Table

		estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	2
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12,13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12,13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.