PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Serum Ionized Calcium and the Risk of Acute Respiratory Failure in Hospitalized Patients: A Single-Center Cohort Study in the United States
AUTHORS	Thongprayoon, Charat; Cheungpasitporn, Wisit; Chewcharat, Api; Mao, Michael; Kianoush, K

VERSION 1 – REVIEW

REVIEWER	Adrian Martineau Queen Mary University of London, UK
REVIEW RETURNED	22-Nov-2019

GENERAL COMMENTS	This manuscript reports findings of a retrospective cohort study conducted in ~25,000 adults admitted to the Mayo Clinic from 2009 through 2013. The main positive finding is of a statistically significant inverse association between serum ionized calcium concentration at hospital admission and subsequent risk of acute respiratory failure requiring mechanical ventilation. Strengths include the large sample size, which affords power to assess a potential effect of the exposure of interest on a hard endpoint. There are significant limitations however: 1. What proportion of patients admitted to the Mayo Clinic during the study period had ionized ca measured? What was the indication? How did they differ from the other admissions? These details need to be reported to allow the reader to assess generalizability of study findings. 2. The introduction does not convey the rationale for the study. The mechanistic basis for hypothesizing that low ionised ca is a risk factor for acute respiratory failure needs to be spelt out more clearly. 3. There is potential for reverse causality to operate here. Patients with incipient type 1 respiratory failure may tend to be hyperventilating at admission, which will reduce ionized calcium. Was the observed association similar for type 1 vs type 2 resp failure? Dissecting apart these two outcomes could afford mechanistic insights. 4. Was the association specific for ionized ca? Was it seen for total ca (corrected for albumin?) 5. Could there be confounding by 25-hydroxyvitamin D level? One might expect patients with low 25OHD levels to have low ionized ca levels as well. There is a large and growing literature

	implicating inadequate vitamin D status as a risk factor for adverse outcomes in the context of illness presenting to critical care. 6. Why was baseline serum ionised ca not simply analysed as a continuous variable? 7. '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.' This seems to have been a post hoc decision – does the positive association reported here represent a self-fulfilling prophecy? 8. 'There was no missing data in this study'. Given the size and retrospective nature of this study, I find this statement hard to believe. Can every patient studies really have had complete data for every covariate investigated? 9. Conclusion: 'The impact of calcium replacement among patients with hypocalcemia on development of ARF needs to be investigated in prospective investigations.' This goes beyond the data in my opinion – confounding and/or reverse causality have by no means been excluded. In my opinion, this evidence is not in itself strong enough to justify an intervention study.

REVIEWER	Philip Yang Emory University, Atlanta, GA, USA
REVIEW RETURNED	09-Jan-2020

GENERAL COMMENTS	This paper by Dr. Thongprayoon and colleagues studied the association between serum ionized calcium level on hospital admission and ARF. The authors found that hypocalcemia was associated with increased odds of ARF with a linear relationship. It is an interesting paper that draws strength from a large sample size, and reports a novel finding that correlates an easily measurable lab value with an important clinical outcome. However, there are several issues that need to be addressed before the manuscript can be accepted. The following feedback is provided for the authors' consideration. Major points: 1. Neuromuscular irritability and muscle weakness are proposed as potential physiologic explanations for the association between hypocalcemia and ARF. There are several additional factors that may act as confounders: first, underlying neuromuscular diseases (e.g. ALS, myasthenia gravis, etc.), which are known to cause both acute and chronic respiratory complications; second, morbid obesity, which contributes to respiratory failure due to impaired chest wall mechanics; third, underlying chronic respiratory failure (e.g. presence of tracheostomy, chronic oxygen requirement, etc.), which predisposes patients to acute-on-chronic respiratory failure. These underlying conditions should either be adjusted for in the multivariate logistic regression analysis, or excluded from the study. 2. ABG at admission, even if available, is unlikely to provide useful information regarding ARF occurring later in the hospitalization. On the other hand, most patients who receive mechanical ventilation will likely have an ABG immediately before and/or after intubation, which does provide useful information. Therefore, for the patients needing mechanical ventilation, the authors should determine the

	type of ARF based on ABG around the time of intubation. While a causal relationship cannot be established from this retrospective study, this can still provide insight as to which of the suggested reasons for the association between hypocalcemia and ARF (hypercapnic from neuromuscular/diaphragm weakness vs. hypoxic from cardiac dysfunction/pulmonary edema vs. metabolic derangements) may be more responsible. Minor points: 1. ESRD should be included as a comorbidity in Table 1. While baseline GFR and AKI are reported and included the multivariate logistic regression, it would be helpful to know the proportion of ESRD patients who have impaired calcium homeostasis. 2. Please specify whether the definition of "mechanical ventilation" only included invasive mechanical ventilation via endotracheal tube, or also included patients requiring non-invasive mechanical ventilation modalities such as CPAP or BPAP. 3. The predominance of Caucasian patients in the study (>90% of patients) should be addressed as a limitation, as it affects the generalizability of the results. 4. Page 7, line 45, typo/grammar. Patients or the public were "not" involved in the design, …. 5. Page 9, line 39. The sentence beginning with "While either admission serum ionized calcium…" is an incomplete sentence. 6. Page 10, line 10, typo/grammar. Low ionized serum calcium may also "cause" cardiac dysfunction…. 7. Page 10, line 51. It would be premature to call ionized calcium "the main" predictor of ARF; consider revising to state "a predictor" instead.

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer#1

Comment

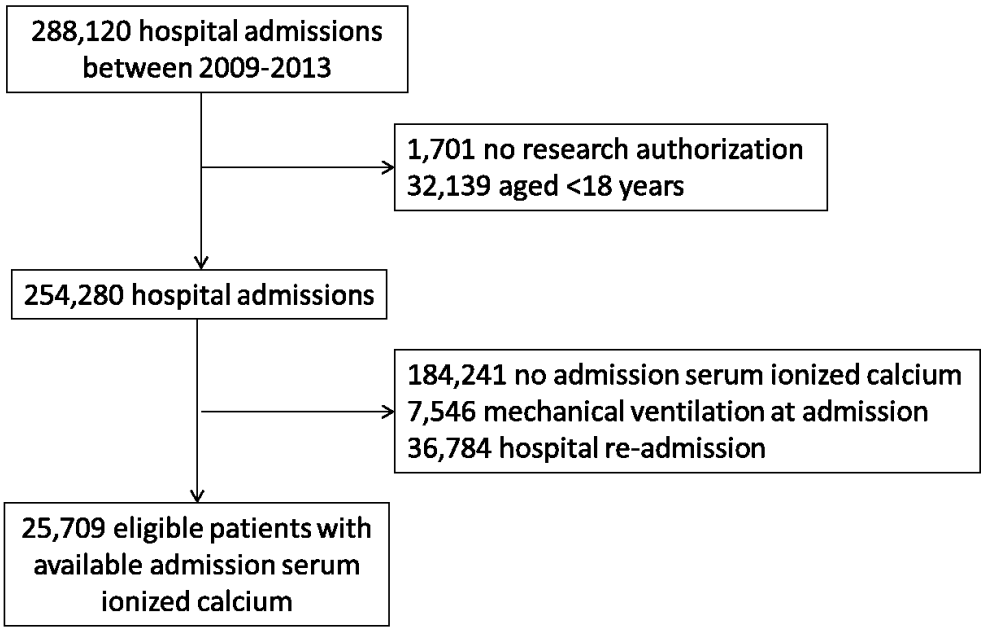
This manuscript reports findings of a retrospective cohort study conducted in ~25,000 adults admitted to the Mayo Clinic from 2009 through 2013. The main positive finding is of a statistically significant inverse association between serum ionized calcium concentration at hospital admission and subsequent risk of acute respiratory failure requiring mechanical ventilation. Strengths include the large sample size, which affords power to assess a potential effect of the exposure of interest on a hard endpoint. There are significant limitations however:

Response: We thank you for reviewing our manuscript and for your critical evaluation.

Comment #1

What proportion of patients admitted to the Mayo Clinic during the study period had ionized ca measured? What was the indication? How did they differ from the other admissions? These details need to be reported to allow the reader to assess generalizability of study findings.

Response: We appreciate the reviewer's important comment. We agree, and we have additionally included Figure S1 to demonstrate the selection process of patients included in this study, as the reviewer suggested.



The following statements in **bold** have been added to the result section to describe the selection procession of patients included in this study

288,120 hospital admissions during the study period were screened. Patients without research authorization (n=1,701), age <18 years (n=32,139), without admission serum ionized calcium measurement (n=184,241), those on mechanical ventilation at hospital admission (n=7,546), and individuals with re-admission (n=36,784) were excluded. A total of 25,709 eligible patients with available admission serum ionized calcium were analyzed (Figure S1).

We also added Table S2 to compare clinical characteristics between included patients and patients without admission serum ionized calcium measurement as the reviewer's suggestion.

	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 - Cardiovascular - Hematology/Oncology - Infectious disease - Endocrine/metabolic - Respiratory - Gastrointestinal - Genitourinary - Injury and poisoning - Other	 21% 15% 3% 3% 4% 9% 4% 15% 25%	 22% 21% 4% 4% 4% 11% 4% 13% 16%	<0.001

As shown in Table S2, there were differences in clinical characteristics between included patients and patients without admission serum ionized calcium measurement. The caution of selection bias should be recognized when interpreting the result of the study. The following statements in **bold** have been added to the limitation section.

"Serum ionized calcium is not routinely measured in hospitalized patients at the time of hospital admission. A potential selection should be cautioned when interpreting the result of the study. The included patients with available admission serum ionized calcium were older, lower eGFR, higher comorbidity burden, were more primarily admitted for hematology/oncology or gastrointestinal disease than patients without admission serum ionized calcium who were not included in the analysis (Table S2)."

Comment #2

The introduction does not convey the rationale for the study. The mechanistic basis for hypothesizing that low ionised ca is a risk factor for acute respiratory failure needs to be spelt out more clearly.

Response: The reviewer raises an important comment. We agree and we have added the rationale for the study and the mechanistic basis for this study in the introduction of our revised manuscript. The following statements in **bold** have been added to the introduction section.

"**Acute respiratory failure (ARF) is a common and life-threatening condition among hospitalized patients,[11 12] associated with high morbidity and mortality worldwide.[13-18] Alterations of serum calcium have been linked to the development of ARF in several reports.[19-23] 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.[19-23] 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 unknown.**

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."

Comment #3

There is potential for reverse causality to operate here. Patients with incipient type 1 respiratory failure may tend to be hyperventilating at admission, which will reduce ionized calcium. Was the observed association similar for type 1 vs type 2 resp failure? Dissecting apart these two outcomes could afford mechanistic insights.

Response: The reviewer raises an important point. As the reviewer alluded to, the assessment of oxygenation depends on both pO2 and FiO2. Unfortunately, due to the lack of accurate documentation of FiO2 in our arterial blood gas results, the severity of hypoxic respiratory failure could not be reliably assessed based on the review of blood gas alone. However, we were able to assess if patients had hypercapnic respiratory failure by assessing pCO2 alone. We defined hypercapnic respiratory failure as arterial pCO2 before mechanical ventilation ≥50 mmHg.

We additionally performed a sensitivity analysis to assess the association between admission serum ionized calcium levels and the risk of hypercapnic ARF requiring mechanical ventilation, as shown in Table S1.

Serum ionized calcium level at hospital admission (mg/dl)	Hypercapnic 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

The following statements in **bold** have been added to the result section.

In a sensitivity analysis, admission serum ionized calcium level of ≤4.79 mg/dL was significantly associated with the increased risk of hypercapnic ARF requiring mechanical ventilation (Table S1).

The following statements in **bold** have been added to the discussion

"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 hypercapnic ARF. We demonstrated that low admission serum ionized calcium of ≤4.79 mg/dL was significantly associated with the increased risk of hypercapnic ARF requiring mechanical ventilation. 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 increased risk of ARF requiring mechanical ventilation."

Finally, we acknowledge this potential reverse causality in the limitation section.

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 requiring mechanical ventilation. However, regardless of which mechanistic pathway, we were able to highlight the importance of serum ionized calcium as a predictor of ARF requiring mechanical ventilation.

Comment #4

Was the association specific for ionized ca? Was it seen for total ca (corrected for albumin?)

Response: As the reviewer's suggestion, we additionally performed the sensitivity analysis in patients with available albumin-corrected total serum calcium, which showed a similar inverse association between total serum calcium and risk of ARF requiring mechanical ventilation. That is, hypocalcemia (serum calcium ≤8.5 mg/dL) was associated with the increased risk of ARF requiring mechanical ventilation, compared with normocalcemia (serum calcium 8.6-10.0 mg/dL). In contrast, hypercalcemia (serum calcium ≥10.1 mg/dL) was not significantly associated with the increased risk of ARF requiring mechanical ventilation. The following statements in **bold** have been added to the result section.

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 increased risk of ARF requiring mechanical ventilation (OR 1.45; 95% CI 1.28-1.63), compared with the admission serum calcium levels of 8.6-10.0 mg/dL. In contrast, admission serum calcium of ≥10.1 mg/dL was not significantly associated with the increased risk of ARF requiring mechanical ventilation (OR 1.08; 95% CI 0.85-1.37). A decrease in admission serum calcium by 1 mg/dL was significantly associated with the increased risk of ARF requiring mechanical ventilation with an adjusted OR of 1.10 (95% CI 1.04-1.17).

Comment #5

Could there be confounding by 25-hydroxyvitamin D level? One might expect patients with low 25OHD levels to have low ionized ca levels as well. There is a large and growing literature implicating inadequate vitamin D status as a risk factor for adverse outcomes in the context of illness presenting to critical care.

Response: The reviewer raises an excellent point that there is a large and growing literature implicating inadequate vitamin D status as a risk factor for adverse outcomes including acute wheeze attacks, acute respiratory infections, and exacerbations of COPD (Thorax, 74 (10), 977-985, Health Technol Assess, 23 (2), 1-44, and Thorax, 74 (4), 337-345). The information about 25(OH)D levels was limited in our database of hospitalized patients. We agree with the reviewer and have additionally included this important point in our limitation of the study.

"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.[34-36] 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 requiring mechanical ventilation."

Comment #6

Why was baseline serum ionised ca not simply analysed as a continuous variable?

Response: The reviewer raises an important point. We agree. Additionally, categorized serum ionized calcium to explore the possibility of non-linear association with the risk of respiratory failure requiring mechanical ventilation. The following statements in **bold** have been added to the result section to show the association of admission serum ionized calcium as the continuous variable with the risk of respiratory failure.

A decrease in admission serum ionized calcium by 1 mg/dL was associated with an increased risk of ARF requiring mechanical ventilation with an adjusted OR of 1.85 (95% CI 1.66-2.09).

Comment #7

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.' This seems to have been a post hoc decision – does the positive association reported here represent a self-fulfilling prophecy?

Response: Thank you for the excellent comment. In our institution, the normal reference range of serum ionized calcium in adult patients is 4.57-5.43 mg/dL. Therefore, serum ionized calcium of 4.80-4.99 and 5.00-5.19 were considered the center of the normal reference range. The analysis using serum ionized calcium of 4.80-4.99 mg/dL as the reference group showed a similar inverse association between admission serum ionized calcium and the risk of respiratory failure requiring mechanical ventilation, as shown in the table below. That is, lower serum ionized calcium of less than 4.80 was progressively associated with the increased risk of respiratory failure requiring mechanical ventilation.

Serum ionized calcium level at hospital admission (mg/dl)	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	Adjusted OR (95 % CI)	P
≤4.39	2.21 (1.93-2.54)	<0.001	2.23 (1.93-2.58)	<0.001
4.40-4.59	1.53 (1.34-1.75)	<0.001	1.55 (1.36-1.78)	<0.001
4.60-4.79	1.29 (1.15-1.45)	<0.001	1.31 (1.16-1.47)	<0.001
4.80-4.99	1 (ref)	-	1 (ref)	-
5.00-5.19	0.87 (0.75-1.02)	0.08	0.88 (0.75-1.03)	0.11
≥5.20	0.91 (0.76-1.10)	0.33	1.01 (0.84-1.22)	0.92

In addition, the analysis using admission serum ionized calcium as a continuous variable confirmed this association (please see comment#6). However, we are happy to update the manuscript using serum ionized calcium of 4.80-4.99 mg/dL as the reference group if the reviewer thinks it is more reasonable.

Comment #8

There was no missing data in this study'. Given the size and retrospective nature of this study, I find this statement hard to believe. Can every patient studies really have had complete data for every covariate investigated?

complete. These data were abstracted from an institutional electronic database using previously validated algorithm and these variables were selected to include in the analysis because they did not have missing data. However, we later added the analysis of albumin-corrected total serum calcium as suggested by the reviewer. Albumin-corrected total serum calcium at the time of hospital admission was available in only 8,534 patients. Missing data were not imputed. Therefore, the previous statements regarding missing data were deleted.

Comment #7

Conclusion: 'The impact of calcium replacement among patients with hypocalcemia on development of ARF needs to be investigated in prospective investigations.' This goes beyond the data in my opinion – confounding and/or reverse causality have by no means been excluded. In my opinion, this evidence is not in itself strong enough to justify an intervention study.

Response: We agree with the reviewer, and this statement has been deleted as the reviewer suggested.

Response to Reviewer#2

Comment

This paper by Dr. Thongprayoon and colleagues studied the association between serum ionized calcium level on hospital admission and ARF. The authors found that hypocalcemia was associated with increased odds of ARF with a linear relationship. It is an interesting paper that draws strength from a large sample size, and reports a novel finding that correlates an easily measurable lab value with an important clinical outcome. However, there are several issues that need to be addressed before the manuscript can be accepted. The following feedback is provided for the authors' consideration.

Response: We thank you for reviewing our manuscript and for your critical evaluation.

Comment #1

Neuromuscular irritability and muscle weakness are proposed as potential physiologic explanations for the association between hypocalcemia and ARF. There are several additional factors that may act as confounders: first, underlying neuromuscular diseases (e.g. ALS, myasthenia gravis, etc.), which are known to cause both acute and chronic respiratory complications; second, morbid obesity, which contributes to respiratory failure due to impaired chest wall mechanics; third, underlying chronic respiratory failure (e.g. presence of tracheostomy, chronic oxygen requirement, etc.), which predisposes patients to acute-on-chronic respiratory failure. These underlying conditions should either be adjusted for in the multivariate logistic regression analysis, or excluded from the study.

Response: The reviewer raises an important point. We agree and we additionally adjusted for obesity, defined as body mass index >30 kg/m2, when we assessed the association between admission serum ionized calcium and the risk of ARF. However, our database did not contain information regarding underlying neuromuscular disease or chronic respiratory failure. The following statements in **bold** have been added to the limitation section.

Although we extensively adjusted for potential confounders, the association between admission serum ionized calcium and the risk of 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 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.**

Comment #2

ABG at admission, even if available, is unlikely to provide useful information regarding ARF occurring later in the hospitalization. On the other hand, most patients who receive mechanical ventilation will likely have an ABG immediately before and/or after intubation, which does provide useful information. Therefore, for the patients needing mechanical ventilation, the authors should determine the type of ARF based on ABG around the time of intubation. While a causal relationship cannot be established from this retrospective study, this can still provide insight as to which of the suggested reasons for the association between hypocalcemia and ARF (hypercapnic from neuromuscular/diaphragm weakness vs. hypoxic from cardiac dysfunction/pulmonary edema vs. metabolic derangements) may be more responsible.

Response: The reviewer raises an important point. As the reviewer alluded to, the assessment of oxygenation depends on both pO2 and FiO2. Unfortunately, due to the lack of accurate documentation of FiO2 in our arterial blood gas results, the severity of hypoxic respiratory failure could not be reliably assessed based on the review of blood gas alone. However, we were able to assess if patients had hypercapnic respiratory failure by assessing pCO2 alone. We defined hypercapnic respiratory failure as arterial pCO2 before mechanical ventilation ≥50 mmHg.

We additionally performed a sensitivity analysis to assess the association between admission serum ionized calcium levels and the risk of hypercapnic ARF requiring mechanical ventilation, as shown in Table S1.

Serum ionized calcium level at hospital admission (mg/dl)	Hypercapnic 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

The following statements in **bold** have been added to the result section.

In a sensitivity analysis, admission serum ionized calcium level of ≤4.79 mg/dL was significantly associated with the increased risk of hypercapnic ARF requiring mechanical ventilation (Table S1).

The following statements in **bold** have been added to the discussion

"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 hypercapnic ARF. We demonstrated that low admission serum ionized calcium of ≤4.79 mg/dL was significantly associated with the increased risk of hypercapnic ARF requiring mechanical ventilation. 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 increased risk of ARF requiring mechanical ventilation."

Comment #3

ESRD should be included as a comorbidity in Table 1. While baseline GFR and AKI are reported and included the multivariate logistic regression, it would be helpful to know the proportion of ESRD patients who have impaired calcium homeostasis.

Response: We agree with the reviewer. The information about the end-stage renal disease was added in Table 1. In addition, we have additionally included end-stage renal disease in multivariable logistic regression.

Comment #4

Please specify whether the definition of “mechanical ventilation” only included invasive mechanical ventilation via an endotracheal tube, or also included patients requiring non-invasive mechanical ventilation modalities such as CPAP or BPAP.

Response: The reviewer raises an excellent point. In this study, due to the availability of information within our database, we only included invasive mechanical ventilation via the endotracheal tube as the outcome of interest but did not include non-invasive ventilation modalities. The following statements have been added to the method to describe the ascertainment of mechanical ventilation clearly.

The outcome of interest was ARF requiring i**nvasive mechanical ventilation** during hospitalization. **The requirement of invasive mechanical ventilation was obtained from our intensive care unit (ICU) DataMart that recorded 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 the use of non-invasive ventilation support** were not included as the outcome.

Comment #5

The predominance of Caucasian patients in the study (>90% of patients) should be addressed as a limitation, as it affects the generalizability of the results.

Response: We agree with the reviewer. The following statements in **bold** have been added to the limitation section

Finally, our study was a single-center study, and most of the included individuals were from the white race. This might limit the generalizability of the study.

Comment #6

Page 7, line 45, typo/grammar. Patients or the public were “not” involved in the design,

Response: We apologized for this error. The error has been corrected, as suggested.

Comment #7

Page 9, line 39. The sentence beginning with “While either admission serum ionized calcium…” is an incomplete sentence.

Response: We apologized for this error. We have reviewed and revised our discussion, as suggested. This statement has been deleted and revised.

Comment #8

Page 10, line 10, typo/grammar. Low ionized serum calcium may also “cause” cardiac dysfunction….

Response: We apologized for this error. The error has been corrected, as suggested.

Comment #9

Page 10, line 51. It would be premature to call ionized calcium “the main” predictor of ARF; consider revising to state “a predictor” instead.

Response: We agree with the reviewer. This change has been made, as suggested.

We greatly appreciated the editors’ and reviewers’ time and comments to improve our manuscript.

VERSION 2 – REVIEW

REVIEWER	Adrian Martineau Queen Mary University of London
REVIEW RETURNED	10-Feb-2020

GENERAL COMMENTS	Reviewers' comments have been satisfactorily addressed in the rebuttal and in the revision.

REVIEWER	Philip Yang Emory University, Atlanta, Georgia, USA
REVIEW RETURNED	13-Feb-2020

GENERAL COMMENTS	The authors have adequately addressed the reviewers' queries and have made appropriate revisions. The manuscript is significantly improved. It can be accepted for publication.