BRAZILIAN JOURNAL OF BIOMETRICS

ISSN:2764-5290

ARTICLE

Longitudinal serum chloride as a marker for poor prognosis in severely ill COVID-19 patients: A joint model approach

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Abstract

Coronavirus Disease 2019 (COVID-19) has been responsible for an international health crisis that demanded incredible efforts from the scientific community to understand the disease, its risk factors, and consequences. In this work, we seek to investigate the role of serum chloride and main Strong Ion Difference (mSID) on the survival of severely ill COVID-19 patients treated in the Intensive Care Unit (ICU). Electrolyte measurements were taken daily from each patient from their admission to death or discharge. ICU survival time was measured in days from admission, and discharged patients were considered censored. The longitudinal trajectories of chloride and mSID were associated with patients' survival times using joint models for longitudinal and time-to-event data. A total of 58 COVID-19 patients were enrolled, and 21 died during hospitalization. Older patients had lower concentrations of chloride overall, and both chloride and mSID increased over time for patients on average. Age was a significant risk factor for ICU mortality, along with the slope of estimated longitudinal trajectory of chloride. Patients with decreasing chloride levels during ICU stay had increased hazard of death. This results of this study suggest that acquired hypochloremia may be an important marker of disease progression and risk of death in patients with severe COVID-19. As such, chloride should be further validated as longitudinal marker for monitoring prognosis during the course of ICU stay. Neither the current values or the slope of the trajectory of mSID were associated with mortality in this sample. The association between the longitudinal trajectory's slope and ICU mortality is important to understand the dynamics of the disease on a patient level, and could only be quantified by using the joint model framework.

Keywords: SARS-CoV-2, COVID-19, Longitudinal data, Survival analysis, Joint modeling

1.   Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS–CoV–2), the virus responsible for COVID–19, has been a major health concern worldwide since its emergence in November 2019. As a respiratory disease that can progress to severe stages, affecting multiple organs and systems, it is important to understand the dynamics of laboratory parameters that indicate disease progression and future prognosis (Tezcan *et al.,* 2020). Electrolytes maintain and regulate cellular functions in the human body (Shrimanker & Bhattarai, 2022). Imbalances in serum electrolytes can have detrimental consequences if not promptly dealt with, especially when associated with severe diseases such as COVID-19. Different imbalances in electrolytes associate with severe illness and poor outcomes in COVID-19 patients (Sultana *et al.,* 2020; Tezcan *et al.,* 2020), and therefore their monitoring may have important implications in the management and prognosis of critically ill patients.

Chloride is an important electrolyte, found predominantly in the extracellular fluid. Chloride levels are regulated by kidney function, and its imbalance can lead to excess water gain conditions such as congestive heart failure (Shrimanker & Bhattarai, 2022). Imbalances in chloride levels have been associated with poor outcomes in critically ill patients. The presence of low chloride levels, known as hypochloremia, has been associated with higher frequencies of intensive care unit (ICU) admission, use of mechanical ventilation, mortality (Tezcan *et al.,* 2020), and development of acute kidney injury (AKI) (Kimura *et al.,* 2020). ICU mortality and AKI have also been associated with hyperchloremia (Chowdhury *et al.,* 2012; Neyra *et al.,* 2015). Additionally, main Strong Ion Difference (mSID), the difference between sodium and chloride measurements, is also a possible prognostic tool for critically ill patients (Mallat *et al.,* 2013), and other authors have studied its relation to kidney impairment and mortality (Cusack *et al.,* 2002; Ho *et al.,* 2016; Kimura *et al.,* 2020). Although electrolyte imbalances reported in cross-sectional studies might be related to underlying patient characteristics, the pathological processes that occur as a consequence of COVID-19 itself can also lead to imbalances during the disease course. Particularly, SARS-CoV-2 acts directly on the renin-aldosterone-angiotensin system, which regulates electrolyte homeostasis(LIPPI; SOUTH; HENRY, 2020). It has been suggested that electrolyte levels may be successful indicators of disease progression (Atila *et al.,* 2021), and that the correction of unbalanced levels may improve patient outcomes (de La Flor *et al.,* 2021; Tan *et al.,* 2020).

Abnormal chloride measures at hospital admission have been associated with poor prognosis and overall mortality(DUAN et al., 2020; SULTANA et al., 2020; TEZCAN et al., 2020) through methods such as univariate testing and logistic regression analysis. However, these results only consider the status of chloride deregulation at the time of presentation, given the cross-sectional nature of the studies. By evaluating disease progression over time, longitudinal measures can be used to increase the performance of regression models and provide valuable insight into the dynamics of disease. When dealing with longitudinal data, it is important to consider the mechanism that leads to imbalance and missing values. In the case of ICU data, patients may provide a longer or shorter sequence of information given the length of their stay in the ICU. When the sequence is interrupted by patient's death, this might be a case of non-random dropout, in which traditional mixed effects models cannot be employed. Joint models for longitudinal and time-to-event data overcome this issue by modelling the longitudinal measures and the dropout mechanism (survival) simultaneously. In this study we aim to examine serum chloride alterations in COVID-19 patients being managed in the ICU of the Security Forces Hospital in Saudi Arabia, measured daily from admission to discharge or death, and explore the association between these longitudinal measures and patient survival via joint models.

2. Matherials and Methods

2.1 Study design, data collection and outcomes

This retrospective cohort study was approved by the Institutional Review Board of the Security Forces Hospital in accordance with the National Committee of Bio Ethics in Saudi Arabia and received a waiver of informed consent due to no greater than minimal risk to participants. This study was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local and national legislation.

The observational unit consists of adult patients presenting to the ICU with reverse transcriptase polymerase chain reaction (RT-PCR)-confirmed SARS-CoV-2 infection, during the period between the 8th of June 2020 and the 18th of August 2020.Chloride measurements were taken at ICU admission, as well as in the following days until death or discharge. The levels were quantified using an Ion–Selective Electrode (Roche Cobas 8000; Roche Diagnostics, Basel, Switzerland). Patients' baseline characteristics such as age, sex and comorbidities were also recorded.

This study has two main outcomes, which were modeled jointly in order to make inferences on the relationship between them. The first encompasses the longitudinal measures of serum chloride, and the second is the number of days of survival in the ICU for each patient. Patient survival was also analyzed jointly with mSID, a secondary outcome.

2.2 Statistical analysis

The joint model approach was utilized to accommodate longitudinal trajectories truncated by death, as well as estimate the association between the longitudinal markers and survival time. This methodology combines the use of Linear Mixed Effects (LME) models for longitudinal chloride and mSID measures and proportional hazards regression for the survival time.

Given a standard linear mixed model:

$$\begin{cases} \boldsymbol{y}_i = X_i\boldsymbol{\beta} + Z_i\boldsymbol{b}_i + \boldsymbol{\varepsilon}_i, \\ \boldsymbol{b}_i \sim \mathcal{N}(0, D), \\ \boldsymbol{\varepsilon}_i \sim \mathcal{N}(0, \sigma^2 \boldsymbol{I}_{n_i}), \end{cases} \tag{1}$$

where $\boldsymbol{y}_i$ is a vector of responses of dimension n_i that assumes values y_{ij} for the ith individual at the jth time point. X_i and Z_i are known design matrices, for the q_y fixed-effects regression coefficients $\boldsymbol{\beta}$ and the q_b random-effects regression coefficients $\boldsymbol{b}_i$. A multivariate normal distribution with mean zero and variance-covariance matrix D is assumed for the random effects, which are independent of the error terms $\boldsymbol{\varepsilon}_i$, also normally distributed with mean zero and variance matrix $\sigma^2 I_{n_i}$. Responses from the same subject at different time points are conditionally independent, given the covariates and random effects, and have conditional normal distributions. Assuming two random effects, the matrix $D = \begin{bmatrix} \sigma^2_{b_{i1}} & \rho_b \\ \rho_b & \sigma^2_{b_{i2}} \end{bmatrix}$.

And given a proportional hazards model:

$$h_i(t|\boldsymbol{w}_i) = h_0(t) \exp(\boldsymbol{\gamma}'\boldsymbol{w}_i), \tag{2}$$

where $h_i(t)$ is the ith subject's hazard of death at time t, $\boldsymbol{w}'_i = (w_{i1}, ..., w_{ip})$ corresponds to the covariate vector and $\boldsymbol{\gamma}$ the vector of p respective regression coefficients. The $h_0(t)$ function is the baseline hazard function, the hazard function of a subject whose $\boldsymbol{\gamma}'\boldsymbol{w}_i = 0$, and is assumed to have a Weibull distribution.

The time-dependent slopes joint model takes the form:

$$h_i(t) = h_0(t) \exp\{\boldsymbol{\gamma}' \boldsymbol{w}_i + \alpha_1 m_i(t) + \alpha_2 m_i'(t)\}, \tag{3}$$

where $m_i(t)$ is a term that denotes the true value of the longitudinal variable at time t for the ith subject, and $m_i'(t)$ is the first derivative of the longitudinal trajectory of the longitudinal variable at time t, that represents its slope at that time point for the ith subject. α_1 and α_2 are association parameters that quantify the relationship between the hazard of death and the longitudinal marker value at time t, as well as the marker's rate of change at time t (Rizopoulos, 2012).

Estimation of the joint model is based on the joint distribution of the observed outcomes $\{T_i, \delta_i, \boldsymbol{\gamma}_i\}$, where the vector $\boldsymbol{\gamma}_i$ contains the observations of the ith individual at each available time point j, T_i the observed event times, δ_i is the indicator of censoring, and assuming that the random effects $\boldsymbol{b}_i$ account not only for the correlation between the repeated measurements in the longitudinal data but also the association between the longitudinal and the event outcomes.

Formally, we define $\boldsymbol{\theta} = (\boldsymbol{\theta}_t', \boldsymbol{\theta}_\gamma', \boldsymbol{\theta}_b')'$ the full parameter vector, where $\boldsymbol{\theta}_t$ corresponds to the $p + p_h$ parameters for the event time outcome, p the number of regression coefficients in γ and p_h the parameters of the baseline hazard distribution. $\boldsymbol{\theta}_\gamma$ corresponds to the parameters for the longitudinal outcome (a vector of $q_\gamma + 1$ elements, q_γ the size of the covariates and intercepts parameter vector $\boldsymbol{\beta}$, plus the estimated variance for the errors σ^2) and $\boldsymbol{\theta}_b$ corresponds to the vector of $q_b * (q_b + 1)/2$ parameters of the random-effects covariance matrix. Therefore, the event outcomes and the longitudinal outcome are conditionally independent, given the random effects and parameters, and longitudinal measurements of the same subject are also independent given the random effects and parameters,

$$p(T_i, \delta_i, \boldsymbol{\gamma}_i | \boldsymbol{b}_i; \boldsymbol{\theta}) = p(T_i, \delta_i | \boldsymbol{b}_i; \boldsymbol{\theta}) p(\boldsymbol{\gamma}_i | \boldsymbol{b}_i; \boldsymbol{\theta}), \text{ and} \tag{4}$$

$$p(\boldsymbol{\gamma}_i | \boldsymbol{b}_i; \boldsymbol{\theta}) = \prod_j p\{\gamma_i(t_{ij}) | \boldsymbol{b}_i; \boldsymbol{\theta}\}. \tag{5}$$

The log-likelihood contribution for the ith subject can be defined as

$$\log p(T_i, \delta_i, \boldsymbol{\gamma}_i; \boldsymbol{\theta}) = \log \int p(T_i, \delta_i, \boldsymbol{\gamma}_i, \boldsymbol{b}_i; \boldsymbol{\theta}) d\boldsymbol{b}_i$$
$$= \log \int p(T_i, \delta_i | \boldsymbol{b}_i; \boldsymbol{\theta}_t, \boldsymbol{\beta}) \Big[\prod_j p\{\gamma_i(t_{ij}) | \boldsymbol{b}_i; \boldsymbol{\theta}_\gamma\} \Big] p(\boldsymbol{b}_i; \boldsymbol{\theta}_b) d\boldsymbol{b}_i, \tag{6}$$

where the conditional density for the survival part $p(T_i, \delta_i | \boldsymbol{b}_i; \boldsymbol{\theta}_t, \boldsymbol{\beta})$ takes the form

$$p(T_i, \delta_i | \boldsymbol{b}_i; \boldsymbol{\theta}_t, \boldsymbol{\beta}) = h_i(T_i | \mathcal{M}_i(T_i); \boldsymbol{\theta}_t, \boldsymbol{\theta})^{\delta_i} S_i(T_i | \mathcal{M}_i(T_i); \boldsymbol{\theta}_t, \boldsymbol{\beta})$$
$$= \big[h_0(T_i) \exp\{\boldsymbol{\gamma}' \boldsymbol{w}_i + \alpha_1 m_i(T_i) + \alpha_2 m_i'(T_i)\} \big]^{\delta_i}$$
$$\exp\Big(- \int_0^{T_i} h_0(s) \exp\{\boldsymbol{\gamma}' \boldsymbol{w}_i + \alpha_1 m_i(s) + \alpha_2 m_i'(s)\} ds \Big), \tag{7}$$

and the joint density for the longitudinal responses together with the random effects is given by

$$p(\boldsymbol{\gamma}_i | \boldsymbol{b}_i; \boldsymbol{\theta}) p(\boldsymbol{b}_i; \boldsymbol{\theta}) = \prod_j p\{\gamma_i(t_{ij}) | \boldsymbol{b}_i; \boldsymbol{\theta}_\gamma\} p(\boldsymbol{b}_i; \boldsymbol{\theta}_b)$$
$$= (2\pi\sigma^2)^{-\frac{n_i}{2}} \exp\{-\|\boldsymbol{\gamma}_i - X_i\boldsymbol{\beta} - Z_i\boldsymbol{b}_i\|^2 / 2\sigma^2\}$$
$$(2\pi)^{\frac{q_b}{2}} \det(D)^{-\frac{1}{2}} \exp(-\boldsymbol{b}_i' D^{-1} \boldsymbol{b}_i / 2), \tag{8}$$

where $\|x\| = [\sum_i x_i^2]^{1/2}$ denotes the Euclidean vector norm.

Maximization of the log–likelihood function is done using the Expectation–Maximization algorithm (Rizopoulos, 2010).

Regression coefficients are presented, as well as their standard errors, Confidence Intervals (CI) and respective p–values. Statistical analysis was performed using R software (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria) and packages survival (Therneau, 2022), nlme (Pinheiro *et al.,* 2022) and JM (Rizopoulos, 2010). P–values below 5% were considered statistically significant.

3.   Results

3.1   Patient cohort and outcomes

Overall patient characteristics can be observed according to patient outcomes in Table 1. Categorical data were reported as absolute number (n) and relative frequency (%), whilst continuous variables were reported as median and interquartile range (IQR). In order to identify any baseline differences between characteristics of deceased and survivors, categorical variables were compared using the chi–squared test or Fisher's exact test, and continuous variables were compared using the Mann–Whitney U test.

A total of 58 patients with laboratory–confirmed COVID–19 were included in this study. Sixteen patients were female and 42 were male, ranging from 27 to 87 years of age. There was a significant difference in age between patients who died and those who survived, according to Mann–Whitney's U test. Deceased patients tended to be of older age. Twenty–nine patients presented with a diagnosis of hypertension and 3 with coronary artery disease, whilst 2 patients had been previously diagnosed with heart failure, 17 with hyperlipidemia and 33 with diabetes. Chronic obstructive pulmonary disease was present in one patient, chronic kidney disease in 7, and 4 had a history of stroke. These patients spent a minimum of two and a maximum of 58 days in the ICU, with a median ICU time of 12.5 days. A total of 21/58 (36.2%) patients died during the course of their ICU stay. We did not find any significant differences between deceased and survivors regarding the presence of comorbidities.

Table 1. Baseline characteristics of COVID-19 positive patients

Variable	Total Sample (n = 58)	Died (n = 21)	Survived (n = 37)	p-value
Age, years[a]	57.0 (48.2-67.0)	65.0 (56.0-72.0)	52.0 (43.0-62.0)	0.015
Sex[b] Male	42 (72.4%)	17 (80.9%)	25 (67.6%)	0.365
Female	16 (27.5%)	4 (19.0%)	12 (32.4%)	
Hypertension[c]	29 (50.0%)	12 (57.1%)	17 (45.9%)	0.585
Coronary Artery Disease[b]	3 (5.2%)	2 (9.5%)	1 (2.7%)	0.546
Heart Failure[b]	2 (3.5%)	2 (9.5%)	0 (0.0%)	0.127
Hyperlipidemia[c]	17 (29.3%)	6 (28.6%)	11 (29.7%)	1
Diabetes[c]	33 (56.9%)	12 (57.1%)	21 (56.8%)	1
Chronic Obstructive Pulmonary Disease[b]	1 (1.7%)	1 (4.8%)	0 (0,0%)	0.362
Chronic Kidney Disease[b]	7 (12.1%)	3 (14.3%)	4 (10.8%)	0.695
History of Stroke[b]	4 (6.9%)	1 (1.7%)	3 (8.1%)	1
Days in ICU[a]	12.5 (6.0-21.7)	14.0 (6.0-24.0)	12.0 (7.0-21.0)	0.852

[a]Mann-Whitney's U test, [b]Fisher's Exact test, [c]Chi-Square test

Given the normal range of chloride between 98 and 106 mmol/L for adults and elderly patients (RN *et al.,* 2018), 15/58 (25.9%) patients were hypochloremic when admitted to the ICU, while 6/58 (10.3%) presented with hyperchloremia. Thirty–two patients had a measured serum chloride

concentration below the lower limit of normal at some point in their ICU stay, while 34 were above the upper limit on at least one occasion. The profile charts allow us to visualize each patient's sodium trajectory over the course of ICU treatment, both for deceased and discharged patients (Figure 1A). Chloride measurements show reasonable variation at baseline and over time for each subject. A slight difference in overall trajectories is suggested by the profile chart and regression lines presented, given that patients whose outcome was death appear to have decreasing levels of chloride over time, while discharged patients display stable or increasing measurements throughout their ICU stay. These observations suggest that an LME model for these longitudinal trajectories might benefit from a random intercept term as well as a random slope term. Figure 1B shows the profile chart for mSID, where the normal range of 32 through 34 mmol/L (Kimura *et al.,* 2020) is shaded for reference. Although we can also observe different baseline values and different slopes for each patient's trajectory, and all patients presented values above the normal range at some point during ICU stay, systematic differences between deceased and survivors are less clear.
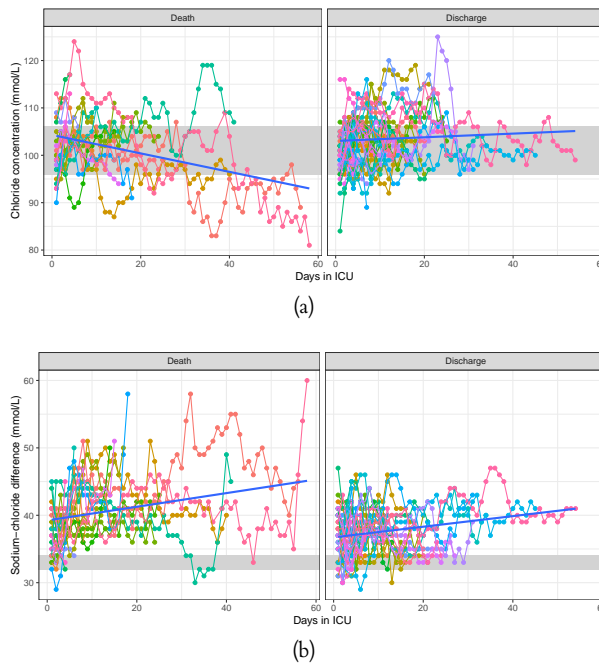

(a)

(b)

Figure 1. Profile charts for (a) chloride and (b) mSID over time according to patient outcome. Shaded area represents normal range. Solid blue line is a simple linear regression.
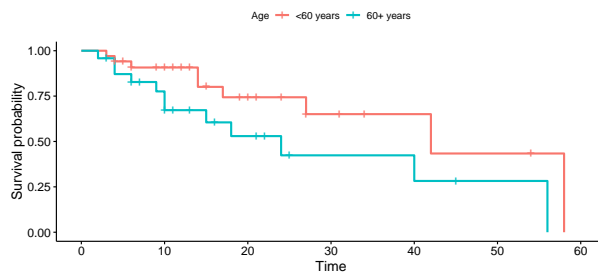

Figure 2. Kaplan-Meier survival curves according to age group.

As for the survival outcome, given that age was significantly different between survivors and deceased, the Kaplan-Meier estimator was also used to plot different survival curves according to patient's age groups. A large gap can be observed between the curves in Figure 2, which suggests that age is a determining factor when estimating risk of death in the ICU. Median survival time was estimated at 42 days for those under 60 years of age, and 24 days for those aged 60 years or older.

3.2   Joint modeling

Taking into account the behaviour seen in the descriptive analysis, we specified the longitudinal sub models for Chloride and mSID measurements as LME models with random intercept and random slopes. As age is known to be an important prognostic factor in COVID-19 patients, and we observed it to have an important role in this cohort's survival, patients' scaled age was used as an independent variable in the models, such that one unit of age corresponds to one standard deviation of 14.4 years from the mean of 56.7 years. Time in the ICU was also reparameterized so that one unit of time corresponds to 3 days in the ICU. The survival sub model is a proportional hazards regression model, where patients who did not die during their ICU stay are censored. Individuals' age was also included in the model as a covariable, scaled in the same way as mentioned for the LME model. For the joint model specification, to avoid underestimation of standard errors (HSIEH; TSENG; WANG, 2006), the baseline hazard function of the survival part was assumed to follow a Weibull distribution. Model estimates for chloride are presented in Table 2, and Table 3 for mSID. Standard errors for model coefficients are the standard asymptotic maximum likelihood estimators: $\mathrm{v\hat{a}r}(\hat{\boldsymbol{\theta}}) = \left\{ -\sum_{i=1}^{n} \frac{\partial^2 \log p(\boldsymbol{\gamma}_i, T_i, \delta_i; \boldsymbol{\theta})}{\partial \boldsymbol{\theta}^\top \partial \boldsymbol{\theta}} \Big|_{\boldsymbol{\theta}=\hat{\boldsymbol{\theta}}} \right\}^{-1}$, and p-values result from z-tests, given the parameters' asymptotic normal distributions.

Table 2. Joint Model Coefficients for Chloride and Survival

	Coefficient	Std. Error	p-value
Longitudinal Process			
Intercept	100,58	0,535	<0.001
Age	-0,733	0,304	0,016
Time	0,388	0,076	<0.001
Event Process			
Intercept	-7,338	2,054	<0.001
Age	0,429	0,251	0,087
α_1	0,018	0,015	0,216
α_2	-1,061	0,531	0,045
$\sigma_{b_{i1}} = 5.179, \sigma_{b_{i2}} = 1.076, \rho_b = -0.623$			

Therefore, the model expression for chloride and survival takes the form:

$$h_i(t) = h_0(t) \exp\{-7.338 + 0.429 * Age +$$
$$+0.018 * [(100.58 + b_{i1}) - 0.733 * Age + (0.388 + b_{i2}) * Time] - \tag{9}$$
$$-1.061 * [0.388 + b_{i2}]\},$$

and the expression for mSID and survival takes the form:

$$h_i(t) = h_0(t) \exp\{-5.311 + 0.344 * Age +$$
$$+0.021 * [(37.944 + b_{i1}) - 0.349 * Age + (0.277 + b_{i2}) * Time] - \tag{10}$$
$$-0.277 * [0.277 + b_{i2}]\}.$$

Table 3. Joint Model Coefficients for mSID and Survival

	Coefficient	Std. Error	p-value
Longitudinal Process			
Intercept	37,944	0,341	0,000
Age	-0,349	0,239	0,143
Time	0,277	0,031	0,000
Event Process			
Intercept	-5,311	0,925	0,000
Age	0,344	0,231	0,137
α_1	0,021	0,023	0,364
α_2	-0,277	0,618	0,654
$\sigma_{b_{i1}} = 2.777$, $\sigma_{b_{i2}} = 0.765$, $\rho_b = -0.480$			

Residual plots were used to evaluate each model's adequacy. Figure 3 presents the standardized conditional residuals for the longitudinal process of each model and the Cox-Snell residuals for the survival process. It is expected that conditional residuals are randomly distributed around zero and have constant variability. These assumptions are reasonably met by both models, but better by the chloride and survival model, whereas the mSID model shows some lack of fit when estimating higher values of mSID. We also expect that the Cox-Snell residuals survival function follows a unit exponential distribution, and the plots show relative closeness between the two functions, indicating a good fit of the survival part of the model.
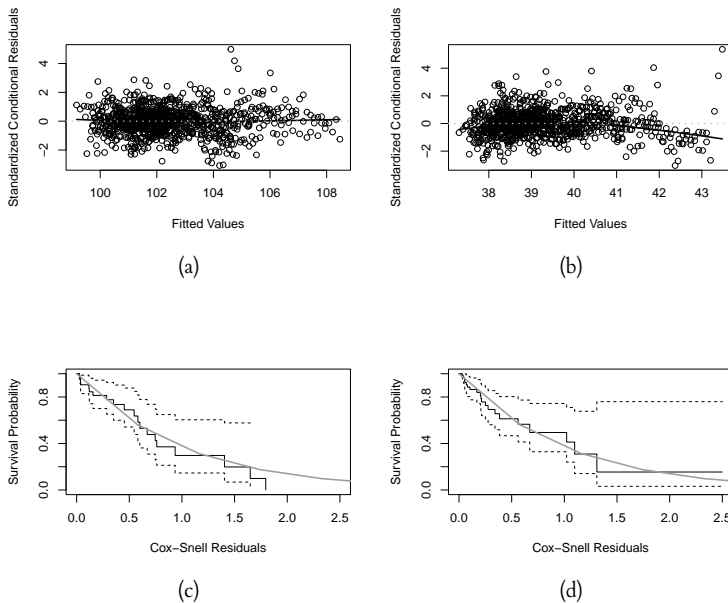

Figure 3. Standardized conditional residuals for the chloride and survival model (a) and the mSID and survival model (b), and estimated survival functions for the Cox-Snell residuals of (c) the chloride and survival model and (d) the mSID and survival model, where dashed lines represent the function's 95% confidence interval, and solid grey lines represent the unit exponential.

Patients' age was found to be significantly associated with chloride measurements, every 14.4 years corresponding to a mean decrease of 0.73 mmol/L in chloride (95% CI: –1.33 to –0.14, p=0.016). The overall mean effect of time was also significant, such that chloride measurements increased by a mean of 0.388 mmol/L every three days in the ICU (95% CI: 0.239 to 0.537, p <0.001). The current value association parameter α_1 was not significant, meaning there was no association between a patient's chloride concentration at a specific time point and their hazard of death at that same time. In contrast, the slope association parameter alpha 2 was estimated at –1.061, corresponding to a hazard ratio of 0.35. The negative coefficient indicates that longitudinal chloride trajectories with positive slopes were associated with decreased hazard of death, while negative slopes (i.e., trajectories that decrease over time) were associated with increased hazard of death.

The joint model for mSID showed a significant average increase in sodium–chloride differences over time, a mean increase of 0.277 units every 3 days. Longitudinal values of mSID did not, however, associate with ICU mortality.

4.   Discussion

The results of this study reveal that, as an essential electrolyte, chloride has an important role in describing the progression of COVID–19 disease in severely ill patients treated in the ICU. Chloride imbalances have previously been associated with mortality in non–COVID–19 critically ill patients(Ji & Li, 2021; Marttinen *et al.,* 2016), and in those with severe acute conditions (Grodin *et al.,* 2015; Ter Maaten *et al.,* 2016). A study of hospitalized patients suffering from acute heart failure found that newly developed or persistent hypochloremia was associated with increased mortality, while baseline hypochloremia that resolved within 14 days was not (Ter Maaten *et al.,* 2016). These findings are in accordance with the results of the present study, where the rate of decrease in chloride concentration was significantly associated with lower survival time, while increase in chloride concentration was associated with enhanced survival. In our study, the longitudinal chloride trajectory slopes are subject–specific given the random effects declared in the LME model, and their standard deviation was estimated at 1.076. A patient's random slope adds to the overall effect of time, so that a patient with a decrease of one standard deviation from the mean slope would have an estimated slope of –0.688 (from 0.388 to –1.076), associated with a hazard ratio of 2.075, meaning a patient whose chloride measurements decrease 0.688 mmol/L every three days is, on average, twice as likely to die when compared to a patient with stable chloride throughout their hospitalization. These results also highlight the importance of longitudinal studies that consider the dynamics of these biomarkers over time in hospitalized patients.

Patients' older age was also associated with lower chloride values over time, which may be due to older patients' reduced homeostatic capacity (Rolls & Phillips, 1990). Hypochloremia in ICU patients may be related to gastrointestinal or renal losses of chloride ions, which can occur in the presence of renal disorders, gastrointestinal symptoms such as vomiting, and congestive heart failure (Bandak & Kashani, 2017). Acute renal involvement is expected in COVID–19 patients and is correlated with poor outcomes and higher mortality in these patients (Pourfridoni *et al.,* 2021). Gastrointestinal symptoms such as abdominal pain and vomiting have also been widely reported in relation to this disease (Henry *et al.,* 2020). Other authors report that COVID–19 may increase the odds of acute heart failure in both previously healthy patients (Bader *et al.,* 2021) and in those with previous history of heart failure (Rey *et al.,* 2020). Combined with the present study, previous investigations suggest that the effects of SARS-CoV-2 infection on kidney and heart function may translate into lowering chloride levels, making it an important marker of disease progression and poor prognosis. Moreover, hypochloremia may cause metabolic alkalosis, leading to further clinical decompensation (Tani *et al.,* 2012). Overall, it is likely that hypochloremia in COVID–19 patients is multi-factorial and clinically represents a declining capacity of the body to maintain homeostasis.

This study is limited by its small sample and observational nature. Although we were not able to

find a significant association between the longitudinal trajectories of mSID and death, this may be a result of an underpowered sample, considering that there was great variability between patients, resulting in large standard errors for model estimates. Some studies of critically ill patients without COVID–19 have had similar results, where chloride performed better as a prognostic tool than markers based on anion differences (Cusack *et al.,* 2002; Ho *et al.,* 2016), while larger samples have found that mSID correlates with the development of acute kidney injury (Kimura *et al.,* 2020). A strength in the methodology used in this study is the increase in information that can be found when collecting data from patients at many time points, instead of limiting data collection to the moment of hospital admission. Traditional regression models would not be able to evaluate the significance of the longitudinal trajectory's slope on the survival time, unlike the joint model parameterization presented in this work.

5.   Conclusions

Decreasing chloride during ICU stay was associated with increased mortality in COVID–19 patients, as was older age, independent of the current value of chloride on any given time, indicating that acquired hypochloremia may be an important marker of disease progression in severely ill patients. Older patients had overall lower values of chloride over time. The etiology of hypochloremia in COVID–19 patients is likely multi–factorial and clinically represents a declining capacity of the body to maintain homeostasis, thus correlating to poor outcomes and higher mortality. As such, chloride should be further validated as a longitudinal marker for monitoring prognosis during the course of ICU stay. We highlight the importance of longitudinal monitoring of ICU patients, as the analysis of markers' trajectories over time may be more informative and allow for medical care to be tailored to each patient in real-time. This relationship between a longitudinal slope and a survival outcome can only be inferred on by simultaneously estimating the two regression models using the joint model framework.

Acknowledgments

Conflicts of Interest

The authors declare no conflict of interest.

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