

## ORIGINAL ARTICLE

## Mineral Bone Disorder

# Estimating serum-ionized magnesium concentration in hemodialysis patients

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## Abstract

**Introduction:** Cardiovascular mortality is significantly increased in kidney failure with replacement therapy (KFRT) patients, which is partly mediated by enhanced vascular calcification. Magnesium appears to have anticalcifying capabilities, and hypomagnesemia has been associated with increased mortality in KFRT patients. Ionized magnesium represents the biologically and physiologically active form. As serum ionized magnesium ( $Mg_{ion}$ ) is difficult to assess in clinical routine estimating equations derived from routinely assessed laboratory parameters could facilitate medical treatment.

**Methods:** We developed equations to estimate serum  $Mg_{ion}$  using linear regression analysis in 191 hemodialysis (HD) patients. Reference test was measured ionized magnesium ( $Mg_{ion}$ ). As index tests, we chose estimated  $Mg_{ion}$  using total magnesium ( $Mg_{tot}$ ) and other laboratory and demographic variable candidates. Equations were internally validated, using 749 subsequent  $Mg_{ion}$  measurements.

**Findings:** The median patient age was 65 years, 67.5% of the patients were male. Median (interquartile range [IQR]) measured  $Mg_{ion}$  was 0.64 [0.57, 0.72] mmol/L, 11 (6%) patients were hypo- (i.e., <0.45 mmol/L) and 127 (66%) were hypermagnesemic (>0.60 mmol/L). The final equation at the end of the development process included  $Mg_{tot}$ , serum ionized, and total calcium concentrations. In the validation dataset, bias (i.e., median difference between measured and estimated  $Mg_{ion}$ , -0.017 [-0.020, -0.014] mmol/L) and precision (i.e., IQR of bias 0.043 [0.039, 0.047] mmol/L) were small, 90% [88, 93] of estimated values were  $\pm 10\%$  of measured values. The equation detected normomagnesemia with overall good diagnostic accuracy (area under the receiver-operating curve 0.91 [0.89, 0.93]).

**Discussion:**  $Mg_{ion}$  can be estimated from equations containing routinely assessed laboratory variables with high accuracy and good overall performance. These equations might simplify the assessment of ionized magnesium

Christopher Holzmann-Littig and Claudius Kuechle contributed equally to this study.

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levels in the individual hemodialysis patients and help the treating physician to guide the overall treatment.

#### KEYWORDS

calcification, equation, hemodialysis, magnesium, mortality

## INTRODUCTION

Kidney failure with replacement therapy (KFRT)<sup>1</sup> patients are at increased risk for cardiovascular morbidity and mortality, which is in part thought to be due to amplified vascular calcification.<sup>2,3</sup> Magnesium appears to counteract vascular calcification,<sup>4,5</sup> which may explain the association between lower all-cause mortality in KFRT<sup>1</sup> patients and higher serum magnesium concentrations.<sup>6</sup> In turn, hypomagnesemia is associated with several complications, such as hypertension, metabolic syndrome, increased vascular calcification, and thereby associated with higher risk of mortality.<sup>7–10</sup> Therefore, current guidelines recommend to avoid hypomagnesemia in the setting of kidney impairment.<sup>7</sup>

Magnesium is the fourth most common cation in the body, with 99% of total magnesium stored in the skeletal system.<sup>11</sup> It is involved in approximately 80% of human metabolic processes.<sup>12</sup> Of the circulating magnesium, ionized magnesium represents the biologically and physiologically most active form.<sup>11,13,14</sup> Under physiological conditions, approximately 59%–72% of magnesium is present in ionized, 5%–11% in complexed (among others to bicarbonate, phosphate, citrate, etc.), and in 23%–31% protein bound (mainly albumin) form.<sup>15</sup> The amount of ionized magnesium ( $Mg_{ion}$ ) primarily depends on protein binding, but also on the pH.<sup>15</sup> Therefore, the  $Mg_{ion}$  fraction in the blood can vary substantially, especially in hemodialysis (HD) patients due to variable nutrition status and acid–base disturbances.<sup>16</sup> In a recent study, the fraction of  $Mg_{ion}$  was reduced to only 50% of total magnesium in HD patients.<sup>17</sup> Patients with normal  $Mg_{ion}$  values had increased total magnesium values.<sup>17</sup> This is consistent with the fact that higher total magnesium levels in HD patients are associated with lower total mortality.<sup>8</sup> Nevertheless, severe hypermagnesemia (in rare cases triggered by supplementation) is associated with disturbed consciousness, hypotension, bradycardia, and respiratory failure and should thus be avoided.<sup>18</sup> Therefore, the measurement of  $Mg_{ion}$  in this population could provide a more concise picture of the actual  $Mg_{ion}$  content in the blood in order to reach normomagnesemia. Furthermore, ionized hypermagnesemia has been shown to be associated stronger with higher mortality in critically ill patients compared to total serum hypermagnesemia.<sup>19</sup> Another study has shown that ionized hypomagnesemia is associated with

supraventricular and ventricular dysrhythmias, seizures, and hypotension in critically ill patients.<sup>20</sup> Also, the correction of  $Mg_{ion}$  has been associated with a lower incidence of postoperative ventricular tachycardia after cardiopulmonary bypass operation.<sup>21</sup> Furthermore, a negative correlation between  $Mg_{ion}$  and QT dispersion has been detected, potentially indicating a role in myocardial electrical stability in hemodialysis patients.<sup>22</sup> However, accurate measurement of  $Mg_{ion}$  is methodologically challenging and cost-intensive in clinical practice. Consequently, estimating equations (i.e., similar to those for glomerular filtration rate [eGFR]) would facilitate the physician to assess the  $Mg_{ion}$  content in the blood. However, only few studies developed equations to estimate  $Mg_{ion}$  in small cohorts.<sup>16,17,23</sup>

Hence, the aim of the current study was to develop an equation for the estimation of  $Mg_{ion}$  based on routinely assessed laboratory and demographic variables in a cohort of chronic HD patients.

## MATERIALS AND METHODS

### Study design

This is a retrospective, cross-sectional single-center cohort study to develop and validate equations to estimate  $Mg_{ion}$  from routinely assessed laboratory and demographic parameters. The study was approved by the local ethics committee (Technical University of Munich) with the number 66/20S-KH. As this is a retrospective data analysis of clinically collected laboratory values, a waiver of an informed consent statement was approved by the ethics committee.

### Participants

All patients were recruited from our outpatient HD center from January 1, 2014 to December 31, 2019. Laboratory and demographic variables were collected from chart review and electronic data sources. By collecting multiple time points per patient, we included a total of 940 data time points from 191 patients. For the external validation cohort, 69 different patients of our outpatient dialysis unit from 2011 to 2013 or 2020/2021 were included in the study.

## Test methods

As reference test, we utilized  $Mg_{ion}$  concentration, as index tests equations for estimated  $Mg_{ion}$  concentration developed from multivariable linear regression analyses evaluating 33 candidate laboratory variables and demographic parameters.

## Variable assessment

Serum samples were collected before the start of each HD session after a long interval (i.e., 2 days without HD treatment). Ionized calcium ( $Ca_{ion}$ ) and  $Mg_{ion}$  were measured by direct potentiometric determination using an ion sensitive electrode with a Nova CRT 8 Electrolyte Analyzer (nova<sup>®</sup> biomedical, USA). A report from 2006 states that the thiocyanate negative interference has been “improved but not eliminated.”<sup>24</sup> We cannot exclude other interfering inorganic and organic acids but have no reason to believe that these possible inferences invalidate our calculations. Calculated from internal quality controls, the coefficient of variation (CV) of  $Mg_{ion}$  is 1.5%, the CV of ionized calcium is 1.19%. Total calcium ( $Ca_{tot}$ ) was measured with the Cobas<sup>®</sup> 8000 Analyzer (Roche, Switzerland) using the c702 module. Total magnesium ( $Mg_{tot}$ ) measurements were performed with the cobas<sup>®</sup> modular analyzer using the c502 module (Roche systems), with the Xylidyl Blue colorimetric method. Quality control is performed in accordance with German RiliBAEK (Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations).<sup>25</sup> This includes regular internal quality controls and successful participation in external quality assessment schemes. Analytical performance specification (comparable to total allowable error) is 6% for total calcium and 7.5% for total magnesium. Measurement methods for other laboratory variables can be found in Table S1.

## Analysis

### Equation development (development dataset)

We selected the first time point of  $Mg_{ion}$  measurement in each patient to establish an equation development cohort, so every patient contributed to the development cohort with one measurement. We prespecified a process for equation development similar to methods published previously.<sup>26–28</sup> We used least squares linear regression and ANOVA to assess linearity between the predictor variables and  $Mg_{ion}$ . We selected candidate variables for the linear regression process in case of significant correlation with  $Mg_{ion}$  in Pearson product-moment correlation testing. We

then evaluated the association of  $Mg_{ion}$  with each candidate variable using univariable linear regression analysis and ranked the variables according to the model’s root-mean-square-error (RMSE, standard deviation of mean difference between measured and estimated  $Mg_{ion}$ ). A lower RMSE implies a better model fit. We performed multivariable linear regression analysis by adding each variable of the previous step separately to the model with the lowest RMSE. A variable was retained for the next step if it was significant in the model and improved (reduced) RMSE of the model by  $\geq 2\%$  compared to the model without the variable. We subsequently added variables until no further significant improvement of the model was achieved.

### Equation validation (internal validation dataset)

The equations with the lowest RMSE containing one, two, three, and so forth variables were fitted in the development cohort and forwarded to the internal validation dataset to evaluate performance. All other remaining time points with  $Mg_{ion}$  measurements available were included in the validation dataset. One hundred and twenty-four patients were measured at more than one time point and could therefore be included in the internal validation. A median interquartile range (IQR) of 4 [2; 9.5] time points per patient was used for validation. We compared measured vs. estimated  $Mg_{ion}$  graphically by plotting the residuals of the regression model (difference between measured and estimated  $Mg_{ion}$ ) against estimated  $Mg_{ion}$ .<sup>28,29</sup> We defined bias as the median of the residuals and precision as the IQR of the residuals.<sup>27,28,30</sup> We defined accuracy as the percentage of estimated  $Mg_{ion}$  within  $\pm 10\%$  of measured  $Mg_{ion}$ . We calculated 95%-confidence intervals for bias, precision, and accuracy by bootstrapping with 2000 replicates.<sup>31</sup> We compared accuracy between equations using the McNemar Test for paired data. We assessed the area under the receiver operating characteristic curve (AUC) for predicting normomagnesemia (i.e., 0.45–0.60 mmol/L) using receiver-operating-characteristic (ROC) curve. Analyses were performed using R, version 3.4.1 (R Development Core Team). *p*-values  $< 0.05$  were considered to be significant. Results have not been adjusted for multiple testing.

### Equation validation (external validation dataset)

The equations which performed best in the internal validation dataset were then forwarded to an external

validation dataset to evaluate performance. Sixty-nine patients of our outpatient dialysis unit who were not part of the study with blood samples collected and analyzed either in the years before (2011–2013) or after (2020/2021) the study period were included to ensure random assignment. One measurement per patient was used for validation. Similar to the internal validation cohort, we compared measured vs. estimated  $Mg_{ion}$  graphically by plotting the residuals of the regression model against estimated  $Mg_{ion}$ . We assessed the same performance measures as in the internal validation cohort.

## Albumin regression analysis

Albumin values were not available in most cases. To ensure that the effect of albumin was not underestimated, we performed a separate calculation in a cohort of 48 patients in whom these values were available. We correlated ionized magnesium with total protein and albumin and performed an univariable linear regression analysis to evaluate the association of these parameters with  $Mg_{ion}$ .

## RESULTS

### Participants

The median patient age ( $n = 191$ ) was 65.43 [51.96, 75.33] years, 67.5% of the patients were male. The overall median  $Mg_{tot}$  was 1.01 [0.89, 1.12] mmol/L,  $Mg_{ion}$  was 0.64 [0.57, 0.72] mmol/L, total serum protein was 6.10 [5.60, 6.60] g/dl, and dialysis vintage was 1.51 [0.56, 3.93] years at baseline (Tables 1 and 2). All patients were dialyzed using the Fresenius 5008 system. The bicarbonate and sodium concentration of the dialysate baths was adapted online, that is, to the current blood gas analysis according our hemodialysis protocol. The potassium concentration of the dialysate bath was adjusted depending on the initial potassium level. When heparin was used for anticoagulation, a dialysate with a calcium concentration of 1.25 mmol/L was used. In the case of citrate dialysis, the calcium supply was controlled with a syringe pump according to an internal clinic protocol. The magnesium concentration in the dialysate was 0.75 mmol/L for all dialysis sessions included in our study. Patients received hemodiafiltration as standard of care in the majority of cases.

### Equation development

In the initial step, we selected candidate variables for the linear regression process in case of significant

**TABLE 1** Study population, overall characteristics—Laboratory parameters

<i>n</i>	191
Total magnesium (mmol/L)	1.01 [0.89, 1.12]
Ionized magnesium (mmol/L)	0.64 [0.57, 0.72]
Total protein (g/dl)	6.10 [5.60, 6.60]
Total calcium (mmol/L)	2.14 [2.02, 2.26]
Ionized calcium (mmol/L)	1.15 [1.08, 1.22]
Phosphate (mg/dl)	5.00 [4.00, 6.35]
Creatinine (mg/dl)	6.40 [4.85, 8.60]
eGFR (ml/min)	7.00 [6.00, 11.00]
Blood-urea-nitrogen (mg/dl)	49.00 [37.00, 66.00]
Bilirubin (mg/dl)	0.30 [0.20, 0.40]
ALAT (U/L)	14.00 [11.00, 21.00]
ASAT (U/L)	18.00 [14.00, 23.00]
Iron ( $\mu$ g/dl)	52.00 [39.00, 69.50]
CRP (mg/dl)	0.90 [0.30, 2.60]
INR	1.10 [1.00, 1.20]
Leucocyte count (G/L)	6.22 [5.12, 8.09]
Hemoglobin (g/dl)	10.00 [9.20, 10.80]
Thrombocyte count (G/L)	196.00 [154.00, 246.75]
TSH ( $\mu$ IU/ml)	1.75 [1.13, 2.73]
Ferritin (ng/ml)	687.00 [301.50, 955.00]
Transferrin (mg/dl)	161.00 [135.00, 184.00]
Transferrin saturation (%)	23.00 [17.00, 32.00]
Hba1c (%)	5.10 [4.80, 5.70]
Parathyroid hormone (pg/ml)	174.10 [85.30, 379.80]
pH	7.36 [7.33, 7.40]
CO <sub>2</sub> (mmHg)	39.80 [35.40, 43.80]
Actual bicarbonate (mmol/L)	21.90 [19.95, 24.30]
Standardized bicarbonate (mmol/L)	21.40 [20.30, 23.35]
Base excess (mmol/L)	−3.30 [−4.88, −0.72]
Base excess extracellular fluid (mmol/L)	−3.70 [−5.50, −0.80]
Sodium (mmol/L)	135.50 [133.00, 138.00]
Potassium (mmol/L)	5.00 [4.50, 5.50]
Chloride (mmol/L)	103.00 [100.00, 105.00]
Anion gap (mmol/L)	15.40 [13.05, 17.80]
Glucose (mg/dl)	110.00 [91.00, 134.50]

Note: All values defined as (median [IQR]).

Abbreviations: alat, alanine aminotransferase; asat, aspartate aminotransferase; CO<sub>2</sub>, carbon dioxide; crp, c-reactive protein; eGFR, estimated glomerular filtration rate; inr, international normalized ratio; tsh, thyrotropin.

correlation with  $Mg_{ion}$  in Pearson product-moment correlation testing.  $Mg_{tot}$ , potassium, creatinine, estimated glomerular filtration rate (eGFR),  $Ca_{tot}$ ,  $Ca_{ion}$ ,

**TABLE 2** Study population, overall characteristics—Age, gender, dialysis regimen, and underlying disease

<i>n</i>	191
Age (years)	65.43 [51.96, 75.33]
Sex (male, %)	129 (67.5)
Dialysis vintage (years)	1.51 [0.56, 3.93]
Dialysis regimen <i>N</i> (%)	
<3×/week	31 (16.4)
3×/week	154 (81.5)
>3×/week	4 (2.1)
Dialysis session duration (hs)	4.00 [4.00, 4.00]
Dialysis access <i>N</i> (%)	
Fistula	74 (38.9)
Catheter	113 (60.5)
Unknown	4 (0.5)
Underlying disease <i>N</i> (%)	
Hypertensive nephropathy	11 (5.8)
Diabetic nephropathy	17 (8.9)
Diabetic + hypertensive nephropathy	11 (5.8)
Glomerulonephritis	31 (16.2)
fsgs	11 (5.8)
Other	63 (33.0)
Unknown	47 (24.6)
RKF (ml)	500 [100, 1500]

Note: All values defined as (median [IQR]); fsgs, focal segmental glomerulosclerosis; rkf, residual kidney function.

transferrin, blood urea nitrogen (BUN), phosphate, total serum protein, c-reactive protein (crp), iron, and parathyroid hormone correlated significantly to  $Mg_{ion}$  (Table S2).

In univariable linear regression analysis,  $Mg_{tot}$  showed the nominally strongest association with  $Mg_{ion}$ , followed by potassium, creatinine,  $Ca_{tot}$ , eGFR,  $Ca_{ion}$ , transferrin, BUN, phosphate, crp, total serum protein, iron, and parathyroid hormone (Table S3).

In the next step, we added these variables to  $Mg_{tot}$  in separate multivariable linear regression models. In these models, only  $Ca_{ion}$ ,  $Ca_{tot}$ , and phosphate significantly reduced the RMSE by more than 2%. Adding  $Ca_{ion}$  to  $Mg_{tot}$  led to the nominally greatest reduction RMSE (12.3%, Table S4). In the next step,  $Ca_{tot}$  and phosphate were added to  $Mg_{tot}$  and  $Ca_{ion}$  in separate models. Only  $Ca_{tot}$  reduced the RMSE of the model beyond the threshold of 2% (4.6%, Table S4), while phosphate did not (1.3%, Table S4). Therefore,  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  were included in the final model.

Equations including one, two, and three variables derived from the development cohort were then forwarded to the internal validation cohort (Table 3):

**TABLE 3** Equations for estimation of serum-ionized magnesium concentrations

Variable	Equation
Total mg	$0.036 + (0.609 \times Mg_{tot})$
Total mg + ionized ca	$-0.165 + (0.598 \times Mg_{tot}) + (0.184 \times Ca_{ion})$
Total mg + ionized ca + total ca	$-0.129 + (0.620 \times Mg_{tot}) + (0.337 \times Ca_{ion}) + (-0.110 \times Ca_{tot})$

Abbreviations: Mg, magnesium; Ca, calcium;  $Mg_{tot}$ , total magnesium;  $Ca_{ion}$ , ionized calcium;  $Ca_{tot}$ , total calcium.

Containing total Mg:  $0.036 + (0.609 \times Mg_{tot})$   
 Containing total Mg + ionized Ca:  $-0.165 + (0.598 \times Mg_{tot}) + (0.184 \times Ca_{ion})$   
 Containing Total Mg + ionized Ca + total Ca:  $-0.129 + (0.620 \times Mg_{tot}) + (0.337 \times Ca_{ion}) + (-0.110 \times Ca_{tot})$

### Internal validation

Median bias was small and ranged from  $-0.017$  to  $-0.021$  mmol/L for all equations (Table 4). The equation including  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  showed the nominally lowest bias with  $-0.017$  [ $-0.020$ ,  $-0.014$ ] mmol/L. Median precision was between 0.043 and 0.054 mmol/L for all equations. The equation including  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  was most precise. Accuracy was nominally the highest for the equation including  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  with 90.4% (Figure 1).

The AUC to detect normomagnesemia was highest for the equation including  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  with 0.910 (0.887, 0.933) (Table 5).

### External validation

Median bias ranged from  $-0.000$  to  $-0.010$  mmol/L for all equations (Table 4). Median precision was between 0.052 and 0.076 mmol/L for all equations. The equation including  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  was most precise. Accuracy was nominally the highest for the equation including  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  with 84.1% (Table 4).

The AUC to detect normomagnesemia for the equation including  $Mg_{tot}$ ,  $Ca_{ion}$ , and  $Ca_{tot}$  was 0.781 (0.660, 0.901) (Table 4).

### Albumin regression analysis

Numerically total protein correlated stronger with ionized magnesium ( $r = 0.42$ ,  $p < 0.01$ ) compared to

**TABLE 4** Performance of serum-ionized magnesium estimating equations

Variables	Bias (95% CI) <sup>a</sup> (mmol/L)	Precision (95% CI) <sup>b</sup> (mmol/L)	Accuracy (95% CI) <sup>c</sup> (%)
<i>Internal validation cohort</i>			
Total Mg	−0.021 (−0.024, −0.017)	0.054 (0.048, 0.060)	81.5 (78.7, 84.1)
Total Mg + ionized Ca	−0.020 (−0.023, −0.017)	0.049 (0.045, 0.053)	86.5 (84.0, 89.1)*
Total Mg + ionized Ca + total Ca	−0.017 (−0.020, −0.014)	0.043 (0.039, 0.047)	90.4 (88.3, 92.5)*
<i>External validation cohort</i>			
Total Mg	−0.000 (−0.019, 0.010)	0.076 (0.055, 0.090)	75.4 (65.2, 85.5)
Total Mg + ionized Ca	−0.010 (−0.019, 0.004)	0.066 (0.045, 0.086)	81.2 (71.0, 89.9)
Total Mg + ionized Ca + total Ca	−0.004 (−0.006, 0.020)	0.052 (0.039, 0.067)	84.1 (75.4, 92.8)

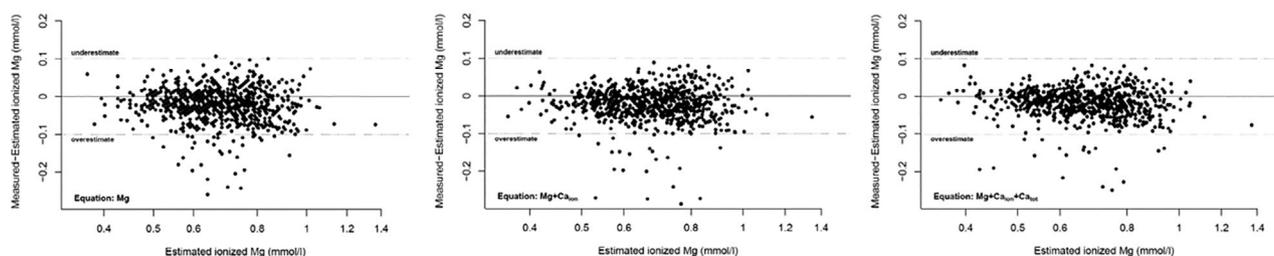
Abbreviations: CI, confidence interval; Mg, magnesium; Ca, calcium.

\*Significance level of  $p < 0.001$  for difference between the accuracy of the corresponding equation and the Mg equation.

<sup>a</sup>Bias defined as the standard deviation of mean difference between measured and estimated value.

<sup>b</sup>Precision defined as interquartile range of the differences between measured and estimated value.

<sup>c</sup>Accuracy defined as the percentage of estimates within 10% of measured value.



**FIGURE 1** Associations between estimated ionized serum magnesium concentrations and difference between measured and estimated ionized serum magnesium concentrations in the internal validation cohort. The differences between measured and estimated concentrations are presented on the y-axis, the estimated concentration on the x-axis. The specific variables used in the equations are indicated within the graphs. Positive differences indicate underestimation of measured concentrations by the estimated concentrations, negative differences overestimation. Abbreviations: Mg, serum magnesium;  $Ca_{ion}$ , serum ionized calcium concentrations;  $Ca_{tot}$ , serum total calcium concentrations

**TABLE 5** Diagnostic accuracy of estimating equations to identify ionized normomagnesemia (0.45–0.60 mmol/L)

Variables	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
<i>Internal validation cohort</i>			
Total Mg	0.905 (0.882, 0.929)	0.900 (0.847, 0.934)	0.855 (0.809, 0.898)
Total Mg + ionized Ca	0.906 (0.883, 0.930)	0.925 (0.871, 0.963)	0.840 (0.790, 0.884)
Total Mg + ionized Ca + total Ca	0.910 (0.887, 0.933)	0.930 (0.880, 0.963)	0.860 (0.821, 0.902)
<i>External validation cohort</i>			
Total Mg	0.769 (0.650, 0.886)	0.893 (0.75, 1.00)	0.756 (0.585, 0.878)
Total Mg + ionized Ca	0.781 (0.664, 0.899)	0.964 (0.821, 1.00)	0.732 (0.561, 0.854)
Total Mg + ionized Ca + total Ca	0.781 (0.660, 0.901)	0.964 (0.857, 1.00)	0.756 (0.610, 0.878)

Abbreviations: AUC, area under the curve; Ca, calcium; CI, confidence interval; Mg, magnesium.

albumin ( $r = 0.36$ ,  $p = 0.02$ ). Likewise, the regression coefficient for total protein was numerically higher ( $r = 0.092$ ,  $p < 0.01$ ) compared to serum albumin ( $r = 0.087$ ,  $p = 0.03$ ).

## DISCUSSION

In this study, we developed and internally validated an equation to estimate  $Mg_{ion}$  from routinely assessed serum

variables and demographic variables. An equation containing three variables ( $Mg_{tot}$ ,  $Ca_{ion}$ ,  $Ca_{tot}$ ) performed well both in terms of accuracy to estimate the ionized value and to predict normomagnesemia.

To our knowledge, this is the equation developed in the largest HD population so far, including an internal and external validation process. Equations to estimate  $Mg_{ion}$  developed in previous studies included variables different from our study. Del Giorno et al. included potassium, bicarbonate, and albumin.<sup>16</sup> For equation development, they applied a different variable selection process in form of stepwise multiple regression analysis with backward elimination. Interestingly, the authors built two subsets of normo-albuminemic patients and hypo-albuminemic patients in order to take into account Mg protein binding. However, this led to further reduction in sample size of the subgroups, limiting the accuracy of model development. No validation process was performed.<sup>16</sup>

Basten et al. published a formula consisting of  $Mg_{tot}$  and serum albumin only.<sup>23</sup> The authors correlated different variables with  $Mg_{ion}$  showing the strongest correlation for  $Mg_{tot}$ . The authors then developed the formula using linear regression analysis, and taking into account the  $R^2$  value. However, the results from this study might be limited due to the fact of a relatively small cohort for equation development and the lack of a validation process.<sup>23</sup> Another study only included  $Mg_{tot}$  and the anion gap.<sup>17</sup> Also in this work, a linear regression approach was performed to develop the equation, but again no validation process was conducted.

In all studies including ours, it was found that  $Mg_{tot}$  has the nominally strongest association with  $Mg_{ion}$ . In contrast to the other studies, however, our study concludes that, in addition to total magnesium, only ionized calcium and total calcium improves the overall performance of the estimating equation. It is known that the ratio between bound and  $Mg_{ion}$  is influenced by the proteins to which it is bound, the temperature, pH, and other ions competing for the binding sites and other factors.<sup>15,32</sup> As calcium and magnesium are divalent positive ions that bind proteins, it seems logical that calcium concentrations influence the protein binding of magnesium. Other published equations captured these influencing factors by incorporating albumin or bicarbonate or anion gap. In HD patients, protein removal through the extracorporeal circulation might alter the protein binding of magnesium. Indeed, not only calcium but also magnesium binds to clotting proteins, such as F VIIa, FIXa, and FXa.<sup>33</sup> However, only approximately 8% of magnesium is bound to all globulins.<sup>32</sup> Constituting a fraction of globulins, clotting proteins account for even less bound magnesium. A theoretical loss of magnesium during clotting in

serum tubes has no relevance in clinical practice. In fact, many authors regard magnesium measured in serum or plasma as interchangeable.<sup>34</sup> Furthermore, as our equation includes total calcium and ionized calcium in addition to total magnesium, we assume that all these effects are considered in their entirety in our formula by the ratio of total calcium to ionized calcium. We therefore consider the formula calculated here to be more robust. In addition, ionized calcium is often included in standard blood gas analyses and therefore does not require additional resources/measurements.

Our study has a couple of strengths. First, to our knowledge, we included the largest number of patients to develop an equation to estimate  $Mg_{ion}$ . Also, this is the first study including an internal and external validation process. In addition, we evaluated a large set of 33 potential co-variables, hereby increasing accuracy of the equation. We used a well validated process for equation development and validation. Moreover, we only included HD patients, which improves the accuracy and therefore applicability in this target population. However, our study has limitations. Blood samples had been taken after the long dialysis interval. Given this background, it should be noted that the laboratory phenotype after the long interval might deviate to some extent from what might be expected after a midweek interval. However, based on all measurements performed in the study, total protein was within the normal range (median 6.1 g/dl [5.6, 6.6]), so was the pH (median 7.36 [7.33, 7.40]). Bicarbonate was near normal with a median of 21.9 mmol/L [20.0, 24.3]. Only hyperphosphatemia of 5.0 mg/dl [4.0, 6.4] was detected (which may be expected in dialysis patients). A previous review described pre-dialytic total magnesium values ranging from  $0.94 \pm 0.18$  mmol/L<sup>35</sup> to  $1.19$  (1.05–1.13) mmol/L<sup>36</sup> and  $Mg_{ion}$  values from  $0.55 \pm 0.02$  mmol/L<sup>37</sup> to  $0.71$  (0.67–0.78) mmol/L.<sup>36,38</sup> Our study showed pre-dialytic median values of  $1.01$  [0.89, 1.12] mmol/L (total magnesium) and  $0.64$  [0.57, 0.72] mmol/L ( $Mg_{ion}$ ) and are therefore within the published predialytic range. Another recent study described predialysis calcium values of  $2.3 \pm 0.2$  mmol/L (total calcium) and  $1.14 \pm 0.12$  mmol/L (ionized calcium).<sup>39</sup> We measured median levels of  $2.14$  [2.02, 2.26] (total calcium) and  $1.15$  [1.08, 1.22] mmol/L (ionized calcium), again very well comparable to the published literature. We therefore suggest the general applicability of our equation independent of the length of the preceding intradialytic interval. Generalizability of our equation remains to be examined due to the small external validation cohort. Moreover, we included multiple measurements of one patient in the validation dataset. Therefore, each patient might not have contributed to the same extent. Finally, we did not evaluate serum albumin, the

protein magnesium primarily binds to, as a potential covariable in the equation development but total protein instead due to data availability. However, in a small subgroup analysis, total protein correlated stronger with ionized magnesium compared to albumin and the regression coefficient for total protein was numerically higher compared to serum albumin. Furthermore, albumin-magnesium binding strongly relies on the blood pH, which did not identify as a significant covariable to be integrated into the final equation. Finally, although several studies have shown that the ionized magnesium may vary substantially in hemodialysis patients even with normal total magnesium values<sup>16</sup> and ionized magnesium has been shown to be of prognostic value in critically ill patients,<sup>19,20</sup> further studies on the outcomes, related to the use of ionized magnesium in hemodialysis patients, need to be evaluated.

In conclusion, we developed and validated an equation to estimate  $Mg_{ion}$  in a HD population, which can be readily applied in clinical practice due to its reliance on routinely measured covariables. This equation enables the treating physician to estimate  $Mg_{ion}$  in order to guide medical treatment and improve patient care.

## CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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## REFERENCES

- Levey AS, Eckardt KU, Dorman NM, et al. Nomenclature for kidney function and disease: report of a kidney disease: Improving global outcomes (KDIGO) consensus conference. *Kidney Int.* 2020;97:1117–29.
- Goodman WG, Goldin J, Kuizon BD, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med.* 2000;342:1478–83.
- Disthabanchong S. Vascular calcification in chronic kidney disease: Pathogenesis and clinical implication. *World J Nephrol.* 2012;1:43–53.
- Henaut L, Massy ZA. Magnesium as a calcification inhibitor. *Adv Chronic Kidney Dis.* 2018;25:281–90.
- Schlieper G, Schurgers L, Brandenburg V, Reutelingsperger C, Floege J. Vascular calcification in chronic kidney disease: An update. *Nephrol Dial Transplant.* 2016;31:31–9.
- Schmaderer C, Braunisch MC, Suttman Y, et al. Reduced mortality in maintenance haemodialysis patients on high versus low dialysate magnesium: A pilot study. *Nutrients.* 2017; 9:926.
- Heaf JG. Chronic kidney disease-mineral bone disorder in the elderly peritoneal dialysis patient. *Perit Dial Int.* 2015;35: 640–4.
- Sakaguchi Y, Fujii N, Shoji T, Hayashi T, Rakugi H, Isaka Y. Hypomagnesemia is a significant predictor of cardiovascular and non-cardiovascular mortality in patients undergoing hemodialysis. *Kidney Int.* 2014;85:174–81.
- Reffelmann T, Ittermann T, Dorr M, et al. Low serum magnesium concentrations predict cardiovascular and all-cause mortality. *Atherosclerosis.* 2011;219:280–4.
- Ye H, Cao P, Zhang X, et al. Serum magnesium and cardiovascular mortality in peritoneal dialysis patients: A 5-year prospective cohort study. *Br J Nutr.* 2018;120:415–23.
- Jahnen-Dechent W, Ketteler M. Magnesium basics. *Clin Kidney J.* 2012;5(Suppl 1):i3–i14.
- Workinger JL, Doyle RP, Bortz J. Challenges in the diagnosis of magnesium status. *Nutrients.* 2018;10:1202.
- Sakaguchi Y, Hamano T, Isaka Y. Magnesium in hemodialysis patients: A new understanding of the old problem. *Contrib Nephrol.* 2018;196:58–63.
- Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. *Clin J Am Soc Nephrol.* 2015;10:1257–72.
- Ben Rayana MC, Burnett RW, Covington AK, et al. IFCC guideline for sampling, measuring and reporting ionized magnesium in plasma. *Clin Chem Lab Med.* 2008;46:21–6.
- Del Giorno R, Riva H, Donato G, Gabutti L. Ionized and total serum magnesium in hemodialysis: predictors and variability. A longitudinal cross-sectional study. *Clin Exp Nephrol.* 2018; 22:620–8.
- Sakaguchi Y, Hamano T, Kubota K, et al. Anion gap as a determinant of ionized fraction of divalent cations in hemodialysis patients. *Clin J Am Soc Nephrol.* 2018;13:274–81.
- Nishikawa M, Shimada N, Kanzaki M, et al. The characteristics of patients with hypermagnesemia who underwent emergency hemodialysis. *Acute Med Surg.* 2018;5:222–9.
- Escuela MP, Guerra M, Añón JM, et al. Total and ionized serum magnesium in critically ill patients. *Intensive Care Med.* 2005;31:151–6.
- Salem M, Stacey J, Chemow B. Ionized magnesium values in critically ill patients—A novel ion selective electrode for determining free extracellular magnesium concentrations. *Crit Care Med.* 1993;21:S256.
- Wilkes NJ, Mallett SV, Peachey T, Di Salvo C, Walesby R. Correction of ionized plasma magnesium during cardiopulmonary bypass reduces the risk of postoperative cardiac arrhythmia. *Anesth Analg.* 2002;95:828–34.
- Yokoyama A, Kikuchi K, Kawamura Y. Heart rate variability, arrhythmia and magnesium in hemodialysis patients. *Clin Calcium.* 2005;15:226–32.
- Basten MI. *Dissertation: Beobachtungsstudie zur Bedeutung des ionisierten Magnesiums bei chronischer Niereninsuffizienz* [Dissertation / PhD Thesis]. Aachen: Rheinisch-Westfälische Technische Hochschule Aachen 2018.
- Abudu N, Miller JJ, Elin RJ. The effect of thiocyanate on the Nova ion-selective electrode for magnesium. *Clin Chem.* 2006; 52(S6):A105.
- German Medical Association. Guideline of the German Medical Association on Quality Assurance of Medical Laboratory Examinations (RiliBÄK 2014). *Dtsch Arztebl.* 2014;111: A1583–618.

26. Inker LA, Tighiouart H, Coresh J, et al. GFR estimation using beta-trace protein and beta2-microglobulin in CKD. *Am J Kidney Dis.* 2016;67:40–8.
27. Shafi T, Michels WM, Levey AS, et al. Estimating residual kidney function in dialysis patients without urine collection. *Kidney Int.* 2016;89:1099–110.
28. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* 2012;367:20–9.
29. Steubl D, Fan L, Michels WM, et al. Development and validation of residual kidney function estimating equations in dialysis patients. *Kidney Funct.* 2019;1:104–14.
30. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150:604–12.
31. Kulesa A, Krzywinski M, Blainey P, Altman N. Sampling distributions and the bootstrap. *Nat Methods.* 2015;12:477–8.
32. Kroll MH, Elin RJ. Relationships between magnesium and protein concentrations in serum. *Clin Chem.* 1985;31:244–6.
33. Messer AS, Velandar WH, Bajaj SP. Contribution of magnesium in binding of factor IXa to the phospholipid surface: Implications for vitamin K-dependent coagulation proteins. *J Thromb Haemost.* 2009;7:2151–3.
34. Thomas L. *Labor und Diagnose: Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik 2020.* Frankfurt, Germany: 2020; <https://www.labor-und-diagnose-2020.de/>, <https://www.clinical-laboratory-diagnostics-2020.com/>.
35. Saha H, Harmoinen A, Pietilä K, Mörsky P, Pasternack A. Measurement of serum ionized versus total levels of magnesium and calcium in hemodialysis patients. *Clin Nephrol.* 1996;46:326–31.
36. Dewitte K, Dhondt A, Lameire N, Stöckl D, Thienpont L. The ionized fraction of serum total magnesium in hemodialysis patients: Is it really lower than in healthy subjects? *Clin Nephrol.* 2002;58:205–10.
37. Saha HH, Harmoinen AP, Pasternack AI. Measurement of serum ionized magnesium in CAPD patients. *Perit Dial Int.* 1997;17:347–52.
38. Cunningham J, Rodríguez M, Messa P. Magnesium in chronic kidney disease stages 3 and 4 and in dialysis patients. *Clin Kidney J.* 2012;5(Suppl\_1):i39–51.
39. Jain A, Bhayana S, Vlasschaert M, House A. A formula to predict corrected calcium in haemodialysis patients. *Nephrol Dial Transplant.* 2008;23:2884–8.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Holzmann-Littig C, Kuechle C, Bietenbeck A, et al. Estimating serum-ionized magnesium concentration in hemodialysis patients. *Hemodialysis International.* 2021;1–9. <https://doi.org/10.1111/hdi.12944>