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# Symmetric dimethylarginine and renal function analysis in horses with dehydration

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## Abstract

**Background:** Acute dehydration caused by a variety of diseases in horses can lead to acute kidney injury. However, current renal biomarkers usually indicate renal damage late in the course of the disease. A novel biomarker would be helpful to diagnose renal disease earlier.

**Objectives:** (1) To estimate the correlation of serum symmetric dimethylarginine (SDMA) concentrations with the degree of dehydration, traditional renal biomarkers and renal function analysis, and (2) to determine the value of SDMA as a prognostic and early biomarker of renal injury in horses.

**Study design:** Prospective cohort.

**Methods:** Serum SDMA, creatinine and urea concentrations and renal function analysis were measured in 41 horses with dehydration at 4 time points until 48 h after admission. Horses were grouped according to their dehydration level into mildly, moderately and severely dehydrated groups.

**Results:** Serum SDMA concentrations at admission correlated with creatinine concentrations ($r = .412$, $P < .001$). Differences in SDMA concentrations at admission were detected among dehydration levels but not between survivors and nonsurvivors. Significant correlations of SDMA concentrations with other markers of renal function analysis and short-term outcome were not observed.

**Main limitations:** Besides the small sample size and low statistical power, missing urine samples at specific time points were also 1 of the main limitations. Only 1 of the horses developed acute kidney injury, which made the evaluation of the predictive value of SDMA difficult.

**Conclusions:** SDMA concentrations correlated significantly with creatinine concentrations in dehydrated horses. Further research is needed to reveal the application of SDMA in horse.

KEYWORDS

horse, acute kidney injury, dehydration, glomerular filtration rate, SDMA

## 1 | INTRODUCTION

Symmetric dimethylarginine (SDMA) and its enantiomer asymmetric dimethylarginine are both amino acids derived from tissue endogenous proteins. While a major part of asymmetric dimethylarginine is metabolised by the enzyme dimethylarginine dimethylaminohydrolase, more than 90% of SDMA is excreted by the kidneys.[1–3] One meta-analysis paper including 18 studies in human medicine showed that plasma SDMA had a significant correlation with the glomerular filtration rate (GFR).[4] Studies in small animals found that SDMA has a better diagnostic value than creatinine for detecting a decrease in GFR since it can detect a decrease of <30%, while creatinine only increases after a 75% loss of nephron function.[5–7] The plasma concentration of SDMA increased both in dogs with acute kidney injury (AKI) and chronic kidney disease (CKD). Moreover, it was less effected by extrarenal factors, such as lean body mass, age and gender, than creatinine in some studies, which makes it more suitable for detecting CKD patients with weight loss.[8,9] Primary kidney disease is believed to be comparably rare in horses. The prevalence of AKI in hospitalised horses was 14.8% in 1 study and the severity was lower than in other animal species.[10] However, the risk of developing AKI could be higher in diseases leading to dehydration and hypovolaemia, such as colic or diarrhoea.[11] Early detection of renal injury and adequate therapy would be beneficial in these horses and drives researchers to search for a more sensitive biomarker. SDMA has not been widely studied in horses.

The aim of this study was to compare the concentration of SDMA with traditional renal biomarkers and establish its relationship with kidney function analysis in dehydrated horses. We hypothesised that SDMA concentrations would: (1) correlate significantly with dehydration and current renal markers, especially markers that are known to detect a decrease in GFR, and (2) provide a reliable value regarding short-term prognosis. The result should provide a prospective view as to whether SDMA is a potential renal marker to help diagnose early kidney injury in horses.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and study population

This study was a prospective investigation performed on clinically dehydrated horses. Serum SDMA, urea and creatinine concentrations, urine renal markers and short-term prognosis until discharge were included and analysed.

Horses that were presented to the equine clinic, Free University of Berlin, between August 2018 and December 2019, with at least 6% dehydration without primary or history of kidney disease were included in this cohort study. Horses that had at least 2 or more abnormal criteria on admission were included. The criteria considered were as follows: heart rate >60 beats/min, packed cell volume (PCV) >40%, total protein concentration (TP) >70 g/L, capillary refill time >2 s, lactate >0.9 mmol/L and clinical signs indicative of hypovolaemic shock including cold extremities, pale mucous membranes or decreased jugular fill. The assessment of the grade of dehydration was based on the clinical examination, PCV and TP at admission (Table S1).[12] Foals younger than 3 mo were excluded from the study in order to avoid spurious hypercreatininaemia in foals.[13]

The horses were divided into 3 groups: those with (1) mild dehydration (6%–8% dehydration), (2) moderate dehydration (8%–10% dehydration) and (3) severe dehydration (>10% or if horses were in hypovolaemic shock).

Horses with 6%–8% dehydration were rehydrated either with infusion therapy (Ringer-lactate or Ringer's solution [B Braun Melsungen AG, Melsungen, Germany]) for at least 24 h, as indicated by PCV and TP, or water by nasogastric tube; horses with moderate or severe dehydration were treated with infusion therapy in all cases. Other treatments were chosen based on the horses' main complaint.

Survivors were defined as the horses which survived to discharge; nonsurvivors were those which died or were subjected to euthanasia during the hospitalisation. Horses which were subjected to euthanasia due to financial constraints were excluded from the analysis for the prognostic value of SDMA in order to reduce the statistical error.

AKI was defined as an increase in serum creatinine concentration≧26.5 μmol/L within 48 h, according to the veterinary AKI staging system.[10]

### 2.2 | Blood sample collection

A total of 10-ml full blood was taken from the external jugular vein at time point 0 ($T_0$) when the horses arrived at the clinic before infusion therapy. Further such samples were also taken at 12, 24 or 48 ± 2 h ($T_{12}$, $T_{24}$ and $T_{48}$, respectively) after admission. Each sample was filled into a serum tube with a clot activator (Sarstedt AG & Co, Nümbrecht, Germany) and centrifuged at 3800 g for 10 min. Two 1.2-ml samples were frozen at −80°C for each time point for later corrections or estimation if necessary; 1 sample was kept at 4°C until sent to an external laboratory (SYNLAB. vet GmbH) and analysed within 24 h. The concentrations of serum creatinine, urea nitrogen, glucose, TP, albumin and electrolytes were measured using an automated AU680 clinical chemistry analyser (Beckman Coulter GmbH). Serum SDMA concentrations were measured with a DLD SDMA ELISA Kit (DLD Diagnostika GmbH). The latter has been validated in healthy horses and horses with AKI.[14]

### 2.3 | Urine sample collection

Urine samples were taken from mares at $T_0$ with a urine catheter before or within the first 30 min of infusion therapy; stallions' and geldings' urine was collected during surgery, if the horse underwent surgery directly after admission or from the midstream of naturally voided urine in the stable within 30 min of admission. The urine samples at $T_{12}$, $T_{24}$ and $T_{48}$ were taken in the stable during spontaneous

urination after admission. All urine samples were analysed by a dipstick Combur9 Test (Roche Deutschland Holding GmbH) in the clinic. Urine (10 ml) collected in a sterile urine collection tube (Labor- und Medizintechnik Specht GmbH) was sent to an external laboratory (SYNLAB. vet GmbH) for renal function analysis together with serum samples from the same time point. The urine-specific gravity (SG), fractional excretion of electrolytes, urine TP (uTP) and the gamma-glutamyltransferase (GGT)/creatinine ratio were measured by a refractometer and the AU680 clinical chemistry analyser (Beckman Coulter GmbH) within 24 h. Sediment interpretation was performed by technicians with a microscope at an external laboratory (SYNLAB. vet GmbH).

### 2.4 | Data analysis

Analysis was performed using IBM SPSS software (IBM Deutschland GmbH) for Windows, version 25. Serum concentrations of SDMA, creatinine and urea, urine SG, fractional excretion of sodium ($FE_{Na+}$), uTP and GGT/creatinine ratio were analysed by Shapiro-Wilk tests to check the distribution of parameters. The Kendall's tau b coefficient test was used to test the correlations between concentrations of SDMA, creatinine and urea and parameters of renal function analysis, respectively, from $T_0$ to $T_{48}$. The correlation between changes in SDMA concentrations and other parameters from $T_0$ to $T_{12}$ was performed by Kendall's tau b test in order to evaluate the reaction of SDMA and renal markers to the initial rehydration therapy. The Kruskal-Wallis test was used to analyse the differences in SDMA concentrations among the 3 dehydration groups. The distribution of serum creatinine and urea concentrations among 3 dehydration groups at $T_0$ were also analysed by Kruskal-Wallis test. The distribution of serum SDMA concentrations at $T_0$ in survivors/nonsurvivors groups was analysed by Mann-Whitney U test. Linear mixed regression models with repeat measurement were applied to access the association between the concentrations of SDMA at 4 time points and the 3 dehydration groups independently. Mauchly's test for sphericity was applied and the Huynh-Feldt correction was used to determine differences between the time points and interactions between time point and group. Model diagnostics included the visual inspection of normality and homoscedasticity of the residuals per time point. The level of significance was set at 5% for all analyses.

## 3 | RESULTS

### 3.1 | Study population

A total of 57 horses met the inclusion criteria. Sixteen were excluded due to lack of obvious laboratory changes which made grading of the accurate dehydration status impossible. The remaining 41 horses were included in the analyses. Equine data and final diagnosis can be seen in Table 1.

Most horses were admitted as emergency cases. Thirteen horses were assigned to the mild dehydration group with 6%–8% dehydration according to PCV/TP and clinical characterisations. Sixteen horses were in the moderately dehydrated group with 8%–10% dehydration. Twelve horses were in hypovolaemic shock on admission and belonged to the severely dehydrated group. A total of 46.3% (19/41) of horses in the current study underwent surgery because of the primary disease: 18 had colic surgery and 1 had orthopaedic surgery. Seventeen horses were treated with gentamicin during the sampling period and 33 horses received nonsteroidal anti-inflammatory drugs.

### 3.2 | Renal parameters and renal function analysis

A total of 26.8% (11/41) of the horses had increased serum concentrations of SDMA at $T_0$ using a cut-off at 0.75 µmol/L.[14] Of the horses with increased SDMA concentrations, the median concentration was 0.99 (IQR: 0.87-1.70) µmol/L. A total of 22% (9/41) of horses had serum creatinine concentrations above the reference range (71-159 µmol/L, reference range from external laboratory [SYNLAB. vet GmbH]) with a median value of 185.0 (IQR: 167.7-344.7) µmol/L; 6 of these 9 horses also had increased serum SDMA concentrations. A total of 26.8% (11/41) horses had increased urea concentrations (3.2-8.2 mmol/L, reference range from external laboratory [SYNLAB. vet GmbH]) with a median concentration of 9.21 (IQR:8.68-10.76) mmol/L; 5 of these horses had increased SDMA concentrations. A total of 45.5% (5/11) of horses with increased SDMA concentrations still have creatinine concentrations within the reference range, while 33.3% (3/9) of horses with increased creatinine concentrations have SDMA concentrations within the normal range. A total of 12.2% (5/41) horses have increased serum concentrations of SDMA, creatinine and urea simultaneously on admission. The results of serum SDMA concentration and other renal markers in the 3 dehydration groups are presented in Table 2.

Urine samples were collected from 28 horses at $T_0$; samples in the other 13 horses could not be obtained at this time point. A total of 17.9% (5/28) of horses were sampled before the beginning of infusion therapy at $T_0$. The urine SG and uTP were increased in 42.9% (12/28) and 17.9% (5/28) of horses respectively. One horse's urine was too concentrated to carry through the whole renal function analysis. Unfortunately, even after dilution of the sample, a homogeneous solution was not formed and could not be analysed by the laboratory equipment. Therefore, the $FE_{Na+}$ and GGT/creatinine ratio were measured in 27 horses and found to be increased in 18.5% (5/27) of cases.

Serum SDMA concentrations correlated moderately with creatinine concentrations ($r = .412$, $P < .001$; Figure 1) but not serum urea concentrations ($r = .142$, $P = .2$) at $T_0$. Creatinine concentrations have a positive correlation with serum urea concentrations ($r = .406$, $P < .001$). There were no correlations between SDMA and creatinine or urea concentrations from $T_{12}$ to $T_{48}$ (Table S2).

**TABLE 1** Patient data, final diagnosis, main treatment and prognosis

| Variable | 6%–8% Dehydration N (%) | 8%–10% Dehydration N (%) | >10% Dehydration and shock N (%) |
|---|---|---|---|
| Sex | N = 13 | N = 16 | N = 12 |
| Mare | 7 (53.8) | 8 (50) | 7 (58.4) |
| Gelding | 6 (46.2) | 8 (50) | 4 (33.3) |
| Stallion | | | 1 (8.3) |
| Age (years) (median and range) | 16 (5-28) | 14 (7-26) | 15.5 (8-27)[a] |
| BCS (median and range) | 5 (2-6) | 6 (4-8) | 5 (4-7) |
| Breed | | | |
| Thoroughbred | 1 (7.7) | 2 (12.5) | 3 (25) |
| Warmblood | 6 (46.1) | 5 (31.3) | 2 (16.7) |
| Pony | 1 (7.7) | 5 (31.3) | 5 (41.7) |
| Draught horse | 1 (7.7) | 1 (6.2) | 1 (8.3) |
| Other | 4 (30.8) | 3 (18.7) | 1 (8.3) |
| Primary diagnosis | | | |
| Intestinal tract disease | 9 (69.2) | 12 (75) | 10 (83.4) |
| Tumour | 1 (7.7) | | |
| Orthopaedic problem | | 1 (6.2) | |
| Intoxication | | 2 (12.6) | |
| Respiratory disease | | | 1 (8.3) |
| Other | 3 (23.1) | 1 (6.2) | 1 (8.3) |
| Main treatment | | | |
| Surgery | 7 (53.8) | 7 (43.8) | 5 (41.7) |
| Conservative treatment | 6 (46.2) | 9 (56.2) | 7 (58.3) |
| NSAID | 11 (84.6) | 13 (81.3) | 9 (75) |
| Aminoglycoside | 8 (61.5) | 3 (18.8) | 6 (50) |
| Short-term outcome[b] | | | |
| Survivors | 8 (72.7) | 5 (31.2) | 5 (41.7) |
| Nonsurvivors | 3 (27.3) | 11 (68.8) | 7 (58.3) |

Abbreviations: BCS, body condition score; N, number of subset.

[a]Included one 4-month-old stallion that was excluded from the calculation of median age.

[b]Survivors were defined as horses which survived to discharge; nonsurvivors died or were subjected to euthanasia during hospitalisation. Two horses in the mild dehydration group that were subjected to euthanasia due to financial constraints were excluded.

No significant correlations at $T_0$ were identified between SDMA concentrations and the parameters of renal function analysis: urine SG, $FE_{Na+}$, uTP and GGT/creatinine ratio. The urine TP had a moderate correlation with SDMA concentrations at $T_{12}$ ($r = .394$, $P = .04$) and $T_{48}$ ($r = .565$, $P = .01$). The GGT/creatinine ratio at $T_{24}$ correlated significantly with SDMA concentrations ($r = .547$, $P = .02$). Neither urine SG nor $FE_{Na+}$ correlated with SDMA concentrations from $T_0$ to $T_{48}$ (Table S2).

In order to compare the response of each marker with the rehydrated therapy, changes in serum SDMA concentrations and other renal markers from $T_0$ to $T_{12}$ were analysed. The SDMA and creatinine concentrations of most cases decreased after infusion therapy at $T_{12}$. Changes in SDMA concentrations within 12 h were positively correlated with changes in the concentrations of creatinine ($r = .441$, $P = .001$) and the GGT/creatinine ratio ($r = .691$, $P = .02$). Similar correlations were not examined after $T_{12}$ because the different therapy and progress of the primary disease of each horse could result in more study errors.

One horse had creatinine concentrations above the reference range persistently until $T_{48}$, which fit the criteria of AKI. The SDMA concentrations above the cut-off value over 48 h were observed in 2 horses, 1 of them was the horse with AKI, while another 1 had increased creatinine concentrations only until $T_{12}$. Two horses that did not have increased SDMA concentrations at $T_0$ developed increased SDMA concentrations above the cut-off value at $T_{24}$ and $T_{48,}$ respectively, meanwhile, they both had normal creatinine concentrations persistently throughout the study period.

**TABLE 2** Concentrations of symmetric dimethylarginine and renal parameters at time point 0 in 3 dehydration groups

| Parameter/Unit<br>Reference range[a] | Dehydration groups | N | Minimum | Maximum | Median | IQR | *P* value[b] |
|---|---|---|---|---|---|---|---|
| Serum SDMA | Mild | 13 | 0.18 | 0.99 | 0.40 | 0.34-0.62 | .03 |
| (μmol/L) | Moderate | 16 | 0.43 | 3.00 | 0.68 | 0.58-0.80 | |
| | Severe | 12 | 0.41 | 2.28 | 0.60 | 0.46-0.78 | |
| | Survivor | 18 | 0.18 | 1.30 | 0.58 | 0.40-0.69 | .1 |
| 0.1-0.75 | Nonsurvivor | 21 | 0.41 | 3.00 | 0.67 | 0.54-0.84 | |
| Serum creatinine | Mild | 13 | 60.6 | 131.4 | 83.5 | 79.8-101.2 | <.001 |
| (μmol/L) | Moderate | 16 | 91.6 | 438.2 | 149.2 | 116.5-163.7 | |
| 71-159 | Severe | 12 | 96.8 | 399.5 | 134.7 | 113.8-166.3 | |
| Serum urea nitrogen | Mild | 13 | 3.28 | 8.90 | 5.00 | 4.26-6.12 | .04 |
| (mmol/L) | Moderate | 16 | 3.63 | 13.87 | 6.32 | 5.17-8.07 | |
| 3.2-8.2 | Severe | 12 | 4.96 | 10.78 | 7.62 | 5.93-9.48 | |
| uSG | Mild | 12 | 1.022 | 1.087 | 1.040 | 1.034-1.050 | .4 |
| (g/mL) | Moderate | 11 | 1.008 | 1.050 | 1.035 | 1.030-1.044 | |
| 1.020-1.040 | Severe | 5 | 1.017 | 1.054 | 1.032 | 1.030-1.050 | |
| Urine total protein | Mild | 12 | 182.0 | 11 164.0 | 621.0 | 395.8-988.8 | .4 |
| (mg/L) | Moderate | 11 | 201.0 | 5604.0 | 298.0 | 256.5-647.0 | |
| <1000 | Severe | 5 | 429.0 | 839.0 | 571.0 | 523.0-749.0 | |
| $FE_{Na+}$ | Mild | 11 | 0.02 | 2.04 | 0.21 | 0.10-0.94 | .5 |
| (%) | Moderate | 11 | 0.06 | 11.55 | 0.18 | 0.11-0.36 | |
| <1 | Severe | 5 | 0.11 | 1.83 | 0.46 | 028-0.69 | |
| GGT/creatinine ratio | Mild | 11 | 1.0 | 21.0 | 7.0 | 3.5-9.0 | .1 |
| | Moderate | 11 | 4.0 | 133.0 | 11.0 | 5.0-28.0 | |
| <25 | Severe | 5 | 5.0 | 53.0 | 22.0 | 7.0-46.0 | |

Abbreviations: $FE_{Na+}$, fractional excretion of sodium; GGT, gamma-glutamyl transpeptidase; IQR, interquartile range; N, number of subset; SDMA, symmetric dimethylarginine;uSG, urine-specific gravity.

[a]Reference range from external laboratory that conducted the examinations.

[b]*P* values indicate the differences between groups.

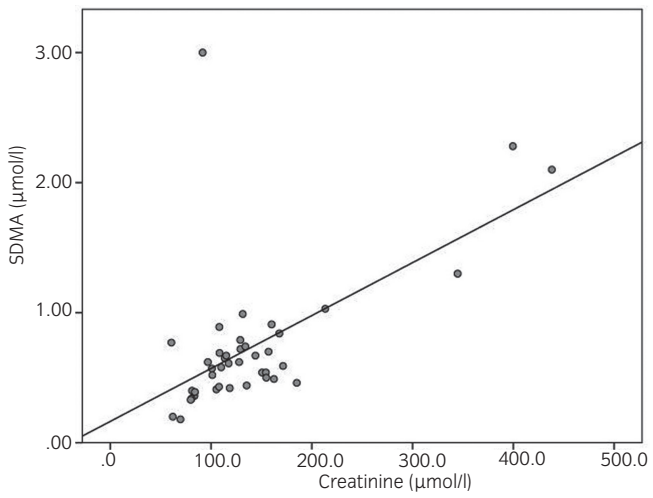


**FIGURE 1** Correlation between serum symmetric dimethylarginine (SDMA) and creatinine concentrations at time point 0. Creatinine concentrations correlated moderately with SDMA ($r = .412$, $P < .001$)

A total of 25% (7/28) of horses have no urine casts according to the sediment examination. The rest of the horses (19/28) have calcium carbonate, -oxalate and struvite within the physiological amount. Since the sediment examinations were all carried out in an external laboratory, although examined within 24 h, the rapid degeneration of the cast in alkaline urine could not be totally avoided in this study. In addition, the estimation of sediment amounts and types with numerical SDMA data is difficult and imprecise. Regardless of the type of the urine cast, there were no significant correlations between the SDMA concentrations and amounts of cast from $T_0$ to $T_{48}$. Findings of the other indicators including erythrocytes and leucocytes made the analysis between these indicators and SDMA concentrations impossible: pathologically increased erythrocytes and leucocytes were only found in 1 and 2 horses, respectively, throughout 48 h, meanwhile, the rest of the cases had no or acceptable normal amounts of erythrocytes and leucocytes in their urine. Without convincing statistical estimation, the results of the sediment examinations are not discussed further.

### 3.3 | Relationship between SDMA and dehydration groups within 48 h

The Kruskal-Wallis test revealed that there were significant differences in SDMA concentrations at $T_0$ among dehydration groups ($P$ =.03; Figure 2; Table 2). Moderately dehydrated animals had the highest SDMA concentrations and differed significantly from mildly dehydrated horses (Bonferroni-corrected post hoc test, $P$ = .03). No significant differences were observed between mild and severe dehydration groups ($P$ = .3), or between moderate and severe dehydration groups ($P$ > .9). Moderately and severely dehydrated horses had a median concentration of SDMA of 0.68 (IQR: 0.58-0.80) and 0.60 (IQR: 0.46-0.78) μmol/L at $T_0$, respectively, higher than mildly dehydrated horses with a median SDMA concentration of 0.4 (IQR: 0.34-0.62) μmol/L. After adding the animal as a random factor and running a linear mixed regression model, there was no statistically significant difference in the SDMA concentrations between the different dehydration groups ($P$ = .3). Neither the time point ($P$ = .2) nor the interaction between the groups and time point ($P$ = .3) showed any statistically significant effects. In conclusion, the differences in the SDMA concentrations in the 3 dehydration groups were only significant at $T_0$ but not at any of the other time points. Although higher mean concentrations of SDMA could be observed in the moderately and severely dehydrated group from $T_0$ to $T_{48}$, the distribution of SDMA concentrations in the 3 groups overlapped easily with each other. The intraclass correlation coefficient was calculated as 81.2%. This means that 81.2% of the variance was due to the variance between horses, whereas values did not differ much between the individual horses.

Besides SDMA, there were also significant differences in the serum creatinine and urea concentrations among 3 dehydration groups at $T_0$ ($P$ < .001 and $P$ = .04, respectively, Table 2).

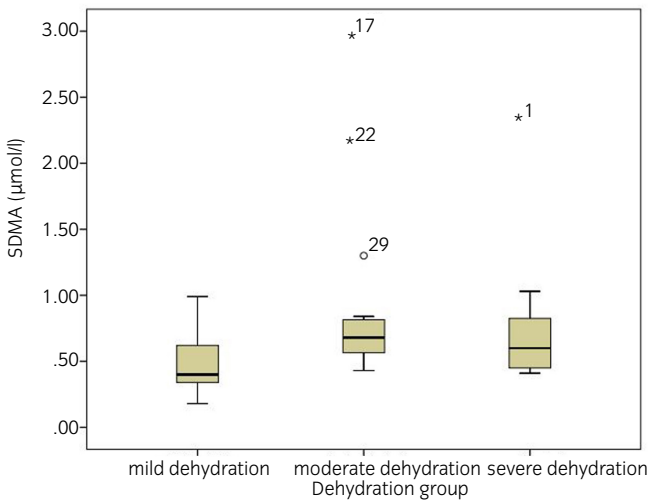


**FIGURE 2** Distribution of symmetric dimethylarginine (SDMA) concentrations at time point 0 according to dehydration status. The SDMA concentrations varied significantly among 3 dehydration groups ($P$ = .03)

### 3.4 | Prognostic value of SDMA

Twenty-eight of the 41 horses included in the analyses were alive until $T_{12}$, 25 horses at $T_{24}$ and 21 horses survived to $T_{48}$. Two horses that were subjected to euthanasia due to financial constraints were excluded from the statistical estimation. A total of 53.8% (21/39) horses were subjected to euthanasia in accordance with animal welfare and poor prognosis or deceased during hospitalisation, and 46.2% (18/39) of horses were discharged.

With the Mann-Whitney U test, there was no statistical significance in the association between SDMA concentrations at $T_0$ and survival ($P$ = .1). The median concentration of SDMA at $T_0$ in the survivor group was 0.58 (IQR: 0.40-0.69) μmol/L, while the median in the nonsurvivor group was 0.67 (IQR: 0.54-0.84) μmol/L. A total of 63.6% (7/11) of horses with increased SDMA concentrations at $T_0$ were subjected to euthanasia or died, while 50% (14/28) of horses did not survive to discharge despite normal SDMA concentrations.

## 4 | DISCUSSION

### 4.1 | Relationship between SDMA and current renal biomarkers

This study aimed to examine the association of SDMA concentrations with other markers of renal function in dehydrated horses to test its value as a potential marker of early kidney injury.

We found a moderate correlation between SDMA and creatinine concentrations at $T_0$, while there was no significant correlation between SDMA and serum urea concentrations from $T_0$ to $T_{48}$. The moderate correlation between SDMA and creatinine concentrations was similar to a study in dogs with AKI.[8] Furthermore, the changes in the SDMA and creatinine concentrations after rehydration therapy measured at 12 h were positively correlated, indicating that both SDMA and creatinine might have the similar ability to detect the decrease in GFR. There were inconsistencies among SDMA, creatinine and urea concentrations in some cases, results which may relate to extrarenal factors: 3 of 9 horses with increased creatinine concentrations at $T_0$ have SDMA concentrations within the normal range. Serum creatinine concentrations can increase due to not only the kidney injury but also the dehydrated status of the horses. Although the authors in 1 study of dehydrated dogs concluded that SDMA might be influenced by the prerenal volume status in dogs with azotaemia,[15] we postulate that hydration status may not impact SDMA as much as creatinine. We observed clearer differentiation in creatinine concentrations than SDMA concentrations among the 3 dehydration groups, suggesting that creatinine might be affected by dehydration more easily than SDMA. However, since 2 of these 3 horses died shortly after $T_0$, it remains unknown whether the hydrated status led to any effect on either biomarker within 12 h in the current study. These 3 horses were of middle age and with normal to obese body condition score. On the other hand, 5 of 11 horses with increased SDMA concentrations

have creatinine concentrations within the normal reference range. Four of these horses were older than 20 years and 1 was estimated to have a body condition score of 2 of 9. Decreased liver function, older age and less muscle mass might contribute towards the differences in our observations between creatinine and SDMA and may explain why which might cause the concentration of creatinine to remain within the normal range with a potential kidney injury or decrease in GFR.[9,16] Serum urea nitrogen is not a sensitive marker of GFR and is also affected by different extrarenal variations.[17] Serum urea and creatinine are impacted by similar extrarenal factors and were found to have moderate correlation with each other.

Two horses developed increased SDMA concentrations above the cut-off value at $T_{24}$ and $T_{48}$ with persisting normal creatinine concentrations. Although neither horse was defined as having AKI according to traditional creatinine criteria, it could still indicate that SDMA might detect an early kidney injury prior to creatinine.

Most studies in small animals and humans with AKI and CKD focused on the relationship between SDMA and creatinine or GFR. In the current study, we also compared SDMA concentrations with parameters of renal function measured in urine. No significant correlations of SDMA concentrations with urine SG, $FE_{Na+}$, uTP and the GGT/creatinine ratio were found in the present study from $T_0$ to $T_{48}$, except for the correlation with uTP at $T_{12}$ and $T_{48}$ and the GGT/creatinine ratio at $T_{24}$. A lack of complete urine sampling throughout the whole study period might have been a factor affecting these results. In addition, different external factors, such as infusion therapy with and without electrolytes, or medications have an impact on the renal parameters.

Urine SG has been used in the estimation of dehydration for a long time. In our study, urine SG was the parameter which showed the highest proportional increase at $T_0$ in 42.9% (12/28) of dehydrated horses. Although urine SG is sensitive to acute hypertonic dehydration, it could still lead to misclassified results. The urine in the bladder in horses with acute dehydration could still be physiologically diluted and, then, mixed with urine produced in the dehydrated state.[18,19] Furthermore, the SG is also affected by the infusion therapy, medications such as alpha-2 agonists from $T_{12}$ to $T_{48}$; thus, interpretation of our data was only possible at $T_0$. The $FE_{Na+}$ indicates the function and damage of the proximal tubule. However, it can be affected by breed, age, exercise, medication or crystalloid fluid therapy in horses.[20] Only 5 horses were sampled before the beginning of sodium-containing infusions at $T_0$. Two horses have increased GGT/creatinine ratios and $FE_{Na+}$ until $T_{24}$ and $T_{48}$, respectively, indicating advanced tubular damage. These 2 horses also have increased SDMA and creatinine concentrations at $T_0$ and a relatively high concentration of SDMA within the reference range until $T_{48}$, while their creatinine concentrations decreased continuously into the normal range after $T_{12}$. We postulate that SDMA might also reflect the tubular damage, while creatinine does not. A total of 18.5% (5/27) of horses had an increased GGT/creatinine ratio at $T_0$. One study showed that the GGT/creatinine ratio was increased in all colic horses that underwent surgery.[21] By contrast, only 1 of the 18 horses that underwent colic surgery had an increased GGT/creatinine ratio in the current study. The 5 horses with an increased GGT/creatinine ratio all have different primary complaints, ranging from colic to orthopaedic problems. The reason for the significant correlation between the GGT/creatinine ratio and SDMA concentrations only at $T_{24}$ is unclear. The positive correlation between changes in SDMA concentrations and the GGT/creatinine ratio within the first 12 h could be related to the acute temporary disturbance of the renal tubule caused by renal ischaemia during dehydration, but administration of potential nephrotoxic aminoglycosides, such as gentamicin, may also had influenced our results.[22] Proteinuria occurs in glomerular disease, bacteriuria or pyuria, and it may increase in equine urine after exercise.[12] Only 1 of the 5 horses that showed increased uTP at $T_0$ had an increased uTP at $T_{12}$ as well. Other than that, increased urine protein seemed to be a coincidental and transient result in each case at different time points and the moderate correlation between uTP and SDMA concentrations at $T_{12}$ and $T_{48}$ might be an accidental result.

Several studies showed that SDMA might be eliminated by the liver and other nonrenal enzymatic degradation in humans.[23–25] Furthermore, SDMA showed neither an advantage in predicting CKD in dogs with leishmaniosis nor the ability to detect CKD in cats with diabetes mellitus in several studies.[26,27] These results indicate that, in addition to being a potential marker of renal function, SDMA might also be involved in other physiological or pathological processes in human subjects and small animals. Similarly, the elimination process of SDMA might not only be limited to the kidneys in horses, which might have influenced the SDMA's correlation with renal function in the current study.

Although SDMA concentrations varied significantly among 3 dehydration groups at $T_0$, most differences were observed between the mild and moderate dehydration groups. Since the subgrouping of the horses depended only on their PCV/TP and clinical characteristics but not plasma osmolality, the wrong assignment could not be totally avoided which might have contributed to these statistical observations.

## 4.2 | Prognostic value

No significant association was identified in this study between the SDMA concentrations and outcome, whereas SDMA has been shown to be an independent prognostic indicator for long-term mortality in critical human patients and was associated with adverse clinical outcome 30 days after an ischaemic stroke.[28,29] In critically ill dogs, no significant difference in serum SDMA concentrations between survivors and nonsurvivors was found.[30] We did not show difference in SDMA concentrations in survivors compared with nonsurvivors. However, relying only on the absolute concentration of the marker at 1 time point might not be enough to identify the prognostic value. Serial monitoring has been recommended for early detection of renal injury earlier in dogs and may be worthy of further research in horses.[31,32]

### 4.3 | Limitations

The collection of the urine samples at $T_0$ was challenging despite our use of extended time zone (±2 h). The lack of urine samples in some cases, especially at $T_0$, could have influenced the correlations between the renal parameter and SDMA concentrations. In addition to the lack of complete urine and serum samples from all horses until $T_{48}$, the small sample size provides limited statistical power. Only 1 horse in the moderately dehydrated group had persistent azotaemia and increased creatinine concentrations accompanied by increased SDMA concentrations until $T_{48}$ indicating AKI. Due to the low statistical power, the results of this study should be interpreted cautiously.

Urine samples analysed here were collected by catheterisation or spontaneous voiding. Minor contamination of the samples could have had an impact on parameters of the renal function analysis. Our cases have a range of primary complaints and medications such as gentamicin, nonsteroidal anti-inflammatory drugs and anaesthetic agents that have unknown influence on the results.

## 5 | CONCLUSION

We observed moderate correlation between SDMA and serum creatinine concentrations but no persistently significant associations between renal function parameters and SDMA concentrations in dehydrated horses. SDMA concentrations were different between groups with different hydration status but SDMA was not different between survivors and nonsurvivors. Extrarenal factors are likely to have influenced our results and further studies of SDMA including serial monitoring will help clarify the role of this biomarker in equine renal disease.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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### CONFLICT OF INTERESTS

Author Hsiao-Chien, Lo received support for this work from SYNLAB.vet GmbH, Berlin, including part of study design, measurement of symmetric dimethylarginine (SDMA; object Biomarker) and renal functional analysis with the coordination of co-author Judith C. Winter. The author has full access to the study data and takes complete responsibility for the integrity of the data and accuracy of data analysis. Other co-authors have declared no competing interests.

### AUTHOR CONTRIBUTIONS

Hsiao-Chien, Lo was the principal author and contributed to study design, data collection and data analysis and manuscript preparation. JC Winter contributed to study design, project coordination and revising the content. M. Roswitha contributed to data analysis and interpretation. H. Gehlen was the senior author and contributed to overall study design, project coordination, data analysis and revising the manuscript. All authors gave their final approval of the manuscript.

### ETHICAL ANIMAL RESEARCH

This study was approved by the Ethics Committee of Free University Berlin.

### INFORMED CONSENT

Owners consented for their horses to take part in this study.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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