Aus der Klinik für Pferde, Allgemeine Chirurgie und Radiologie,
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Diagnostic relevance of symmetric dimethylarginine and renal function analysis in horses

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin (Dr. med. vet.)
an der
Freien Universität Berlin

vorgelegt von
Hsiao-Chien Lo
Tierärztin aus Chiayi, Taiwan

Berlin 2021
Journal-Nr.: 4315

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des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Uwe Rösler
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Zweiter Gutachter:	PD Dr. Ann Kristin Barton
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Deskriptoren (nach CAB-Thesaurus):

Horses, kidney, renal function, arginine, acute kidney injury (MeSH), dehydration, glomerular, filtration rate, renal function test, diagnostic techniques

Tag der Promotion: 15.12.2021

Meine Familie und mein Freund Kuan-Chieh

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List of abbreviations

AKI	Acute kidney injury
GFR	Glomerular filtration rate
ICU	Intensive care unit
BUN	Blood urea nitrogen
SDMA	Symmetric dimethylarginine
RFA	Renal function analysis
ADMA	Asymmetric dimethylarginine
CKD	Chronic kidney disease
uSG	Urine specific gravity
FE_{Na+}	Fractional excretion of sodium
uTP	Urine total protein
GGT	Gamma-glutamyltransferase
ANS	Akute Nierenschädigung
NFA	Nierenfunktionsanalyse

I. Introduction

Primary renal diseases in horses are believed to be rare in comparison with small animals and humans. The information of the prevalence of primary renal diseases in horses nowadays is also still limited. Secondary acute kidney injury (AKI) in horses which induced by ischemic and hypovolemic hypoperfusion, is the most common renal emergency encountered. Any disorder, such as colic or diarrhea, which results in a decrease of the glomerular filtration rate (GFR), could lead to possibly prerenal azotemia (Muffert 2017; May et al. 2012). Once the hypovolemia and hypoperfusion persist, transient AKI could turn into intrinsic AKI (Toribio 2007). One study from a population of horses showed that 14.8% of hospitalized patients developed hospital-acquired AKI (Savage et al. 2019). The AKI in humans and dogs during hospitalization is associated with the outcome, cost and mortality of the patients (Thoen and Kerl 2011; Chertow et al. 2005). Similarly, one study indicated that the mortality rate was three times higher in horses with persistent renal insufficiency over 72 hours compared with the patients that had resolved azotemia within 3 days (Groover et al. 2006).

It is quite challenging for veterinarians to make a timely the diagnosis because the clinical signs of AKI in horses are non-specific and mild at onset. There are several diagnostic tools to help recognize AKI: the GFR is the best and most accurate indication to estimate the renal function and recognize even the tiny changes of renal disturbance (Lippi et al. 2019). However, measurement of the GFR is challenging, time-consuming, expensive, and requires specific equipment and trained technicians. Although there are several simple methods, such as iohexol clearance in horses, to help estimate the GFR, it is still not applicable for most equine practice (Meucci et al. 2015; Wilson et al. 2009). In addition to GFR, alteration of urine output has been showen to be a sensitive marker of AKI in ICU patients in humans. Consecutive oliguria has also demonstrated a predictive value of AKI (Leedahl et al. 2014; Macedo et al. 2011). “Kidney disease improving global outcome” suggested that a decrease of urine output by 0.5 mL/kg/h for 6 hours is one clinical criteria for diagnosis of the AKI (Kellum and Lameire 2013). However, continual measurement of urine output in horses is very difficult and imprecise in the clinic. Therefore, conventional renal biomarkers such as serum creatinine and blood urea nitrogen (BUN), remain the parameters most used to help recognize AKI.

Nevertheless, depending on creatinine and BUN to diagnose AKI still shows limitations: 1) both biomarkers have several extra renal factors which could lead to false

negative or positive diagnose. They are affected by the age, gender, muscle mass and even the nutrient intake of the patients (Muffert 2017; Hokamp and Nabity 2016; Hall et al. 2015) 2) In addition, BUN is unfortunately a poor marker of GFR since it can diffuse easily in the renal tubular (Toribio 2007) 3) Although creatinine correlates well with GFR, it increases only when > 75% functional nephrons are lost and the renal injury has then already reached a steady damaged state (Chen et al. 2017; Hokamp and Nabity 2016). The limitations above and lack of novel renal markers can cause a delayed diagnosis and treatment afterwards. Furthermore, it might be one of the reasons why AKI is not so often diagnosed in equine medicine. It has driven researchers to search for another better, earlier renal biomarker to represent the dynamic real-time kidney condition.

Application of biomarkers aims to achieve the early detection of the disease, monitor and manage the disease staging, assess the prognosis and predict the risk of specific illness (Puntmann 2009). Consequently, a measurable biomarker should be found in urine, plasma or serum that are easy to obtain from the patients (Chen et al. 2017). There are several novel biomarkers which have been developed in human and small animal medicine in recent decades. One of the popular markers is symmetric dimethylarginine (SDMA).

This thesis aimed to reveal the diagnostic value of the novel marker SDMA in dehydrated horses under risk of AKI. Furthermore, the role that renal function analysis (RFA) could play in the prediction of AKI was also investigated.

The first project focused on “Symmetric dimethylarginine and renal function analysis in horses with dehydration.” Symmetric dimethylarginine (SDMA) and its enantiomer asymmetric dimethylarginine (ADMA) are both amino acids which were derived from tissue endogenous proteins and later methylated. They were first isolated from human urine in 1970 (Kakimoto and Akazawa 1970). While the major part of ADMA will be metabolized by the enzyme dimethylarginine dimethylaminohydrolase, more than 90% of SDMA is excreted by the kidney (Oliva-Damaso et al. 2019; Schwedhelm and Böger 2011; Pedersen 2006). Owing to this characteristic, SDMA is thought to be a potential novel renal biomarker and has been widely researched in the last few years. Studies in humans and small animals has shown that SDMA has an outstanding correlation with GFR and is supposed to detect an < 30% decrease of GFR in dogs and cats, which is much earlier than creatinine (Dahlem et al. 2017; El-Khoury et al. 2016; Hokamp and Nabity 2016; Nabity et al. 2015; Kielstein et al. 2006). In humans, SDMA increased significantly in 6 hours and peaked at 24 hours after unilateral nephrectomy (artificial 50% acute reduced GFR) (Kielstein et al. 2011) The International Renal Interest Society added SDMA as one of the standard parameters for diagnosing and

staging AKI and chronic kidney disease(CKD) in small animals in 2015. This application also showed that the prevalence of kidney disease which were predicted by creatinine alone was actually higher than was known to date (Relford et al. 2016). One study that included 165 healthy draft horses found that the normal range of SDMA was similar to small animals and it had no correlation to age or breed (Schott 2nd et al. 2021). A study that compared healthy horses and horses with AKI also found a significantly higher concentration of SDMA in AKI patients without any correlation to age, sex or body weight (Siwinska et al. 2020).

The aim of this study attempted 1) to estimate and compare SDMA with conventional renal biomarkers including creatinine, BUN and parameters of RFA in dehydrated horses, which underwent the risk of AKI, to see whether SDMA has a potential value to predict AKI earlier than the other biomarkers; and 2) to determine the short-term prognostic value of SDMA in horses within 48 hours after admission.

The second project is about “Renal function analysis and dehydrated status in horses.” Renal function analysis, the microscopic examination of urine sediments and reagent strip analysis are commonly used to differentiate and locate the precise site of injury in the urinary tract and kidney. These might allow an earlier detection of reduced renal function (Pressler 2015). Selected parameters, including urine specific gravity (uSG), fractional excretion of sodium (FE_{Na+}), urine total protein (uTP) and the GGT/Creatinine ratio, were compared with creatinine and BUN in dehydrated horses to see whether there is any potential correlation between these markers and the severity of dehydration. Although the ability of urine concentration is not a sensitive marker for renal damage, as the loss of nephrons could occur before the loss of concentrating ability, it could still help to estimate the dehydration status and distinguish prerenal and intrinsic AKI (Gratwick 2020; Schott 2nd et al. 2018). In addition to the ability to differentiate AKI, such as uSG, changes in the FE_{Na+} could also be an indicator of subclinical tubular damage (Vanmassenhove et al. 2013; Ulutas and Sahal 2005). Studies in human showed that proteinuria is an independent risk factor of AKI and associated with patients’ outcome and the need for intensive care (Han et al. 2014; Hu et al. 2012; Hsu and Hsu 2011). Horses with colic which underwent surgery and subsequently tended to develop acquired AKI had a GGT/Creatinine ratio above the reference range in one study. Furthermore, an increased GGT/Creatinine ratio could be observed in ponies that received gentamicin (Gratwick 2020; Arosalo et al. 2007). Both studies indicated that the GGT/Creatinine ratio might be a potential marker for early recognition of renal damage.

Moreover, since no strips have been designed for horses yet, comparison between microscopic examination, RFA and dipsticks could help determine the reliability of a commercial reagent from human medicine in dehydrated horses.

A renal marker for AKI is expected to improve the recognition of early renal dysfunction. Whether totally novel biomarkers such as SDMA or known markers with advanced correlation with AKI, they might bring a new vision to equine renal pathology. This thesis attempted to investigate the diagnostic relevance of SDMA and its correlations with creatinine, BUN and parameters of RFA in dehydrated patients to see whether any of them is of value as a renal biomarker in horses. It was hypothesized that SDMA will not only correlate with traditional renal parameters, but also associate with the levels of dehydration and short-term prognosis. Furthermore, SDMA or one of the markers from RFA will help to detect AKI in horses under risk earlier than traditional biomarkers.

II. Research publications in journals

Publication I

2.1 Symmetric dimethylarginine and renal function analysis in horses with dehydration

Published: Equine veterinary journal – 00 (online ahead of print) (2021) 1–9

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DOI: 10.1111/evj.13484

The original publication is shown in the following pages.

Received: 14 December 2020 | Accepted: 28 May 2021

DOI: 10.1111/evj.13484

ORIGINAL ARTICLE

Symmetric dimethylarginine and renal function analysis in horses with dehydration

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Funding information
The measurement of symmetric dimethylarginine (SDMA, object Biomarker in this study) and renal functional analysis were provided and executed by SYNLAB.vet GmbH, Berlin

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 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.

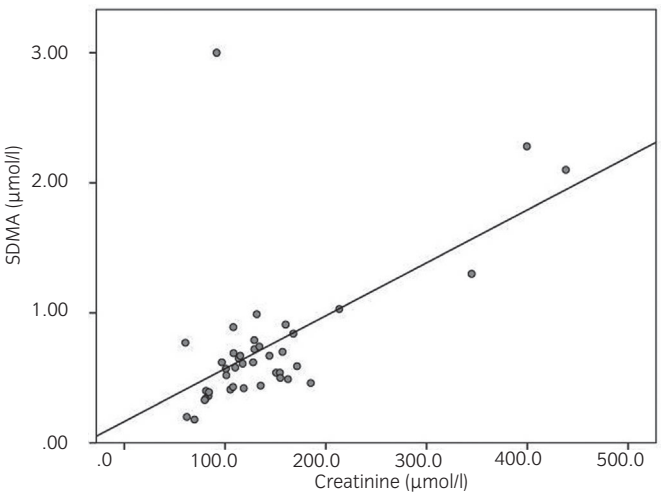

FIGURE 1 Correlation between serum symmetric dimethylarginine (SDMA) and creatinine concentrations at time point 0. Creatinine concentrations correlated moderately with SDMA ($r = .412$, $P < .001$)

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).

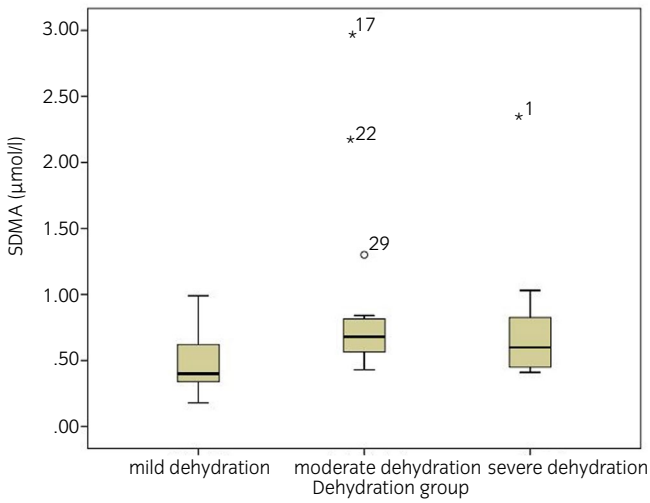

FIGURE 2 Distribution of symmetric dimethylarginine (SDMA) concentrations at time point 0 according to dehydration status. The SDMA concentrations varied significantly among 3 dehydration groups (P = .03)

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 ACCESSIBILITY STATEMENT

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

ACKNOWLEDGEMENTS

We would like to thank Racem B. Romdhane from Institute for Veterinary Epidemiology and Biostatistics, Free University of Berlin, for the helpful consultation of data analysis. Many thanks to Tanja Ahrens and the veterinarians, veterinary assistants from Equine Clinic in Free University Berlin who helped collect the samples, as well as the owners, horses and grooms who made this study possible.

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.

How to cite this article: Lo H-C, Winter JC, Merle R, Gehlen H. Symmetric dimethylarginine and renal function analysis in horses with dehydration. Equine Vet J. 2021;00:1–9. https://doi.org/10.1111/evj.13484

Publication II

2.2 Nierenfunktionsanalyse und Dehydratationszustand beim Pferd

Published: Pferdeheilkunde – Equine Medicine 37 (2021) 2 (März/April) 156–164

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DOI: 10.21836/PEM20210207

The original publication is only available online.

III. Discussion

This dissertation aimed to assess the diagnostic relevance of a novel renal SDMA in dehydrated horses. The correlations between SDMA and RFA, the conventional markers creatinine and BUN were analyzed. The RFA was also analyzed to reveal its association with the dehydration status of the patients.

As SDMA increased both in dogs with AKI and CKD and has been proven to be a reliable risk marker in all-cause mortality in humans who underwent coronary angiography (Dahlem et al. 2017; Schlesinger et al. 2016), not only the predictive relevance of AKI but also a short-term prognostic value of SDMA was also estimated in this study. In the first study, SDMA was increased in more patients at admission than creatinine. The SDMA correlated moderately with creatinine, but not BUN or the other markers including uSG, uTP, FE_{Na+} and the GGT/creatinine ratio before rehydration therapy. The association between the SDMA concentration at admission and the dehydration levels was significant, but no association was found between the SDMA and survivor/non-survivors. After the addition of the time series for repeated measurements, there was no significant difference of the SDMA between dehydration groups over 48 hours. Only one horse in this study was diagnosed with hospitalized acquired AKI through persistent elevated SDMA and creatinine until 48 hours after admission. The results indicated that the diagnostic value of SDMA might be similar to creatinine in horses. Its association with the dehydration status could be interpreted as evidence that SDMA might ultimately correlate with hypovolemia, hypoperfusion and the reduced GFR of kidney. However, similar to creatinine, it could also mean that SDMA would be affected by the hydration status of the patients. Similar conclusions were also drawn by one study that SDMA had significant differences before and after the correction of dehydration in dogs with azotemia (Choi et al. 2017). A total of 83.3% of horses with initially increased SDMA had a SDMA concentration which decreased towards the reference range after 12 h of rehydration therapy. Similar to one study in dogs, the SDMA showed no predictive value of a poor outcome in 48 hours in our group of horses (Köster et al. 2018). Furthermore, a larger variance between horses than within individual horses was found in this study. It means that one absolute SDMA concentration might not be suitable for the early recognition of decreased renal function. A serial monitoring of SDMA is, therefore, recommended to detect kidney injury in the individual.

The main limitation of the first study was the collection of urine samples: 1) at admission before any infusion, there was only limited urine or nothing left in the bladder in dehydrated horses; and 2) After the treatment, it was still a challenge to collect urine at specific time points every 12 hours until 48 hours. The lack of urine samples could have influenced the correlations between parameters of the RFA and SDMA. Moreover, the grouping of the dehydration severity was based on the clinical examination, packed cell volume and total protein at admission. It is unfortunately not precise as a measurement of plasma osmolality, which might lead to false grouping and affect the association between the SDMA and dehydration severity. Medications against a primary complication with suspected renal toxicity could not be avoided in this study, such as gentamicin and non-steroidal anti-inflammatory drugs, which might also play a role as influencing factors.

The RFA helps to localize the damage of the particular nephron segment. It is common used in animals with clinical signs of renal disease instead of early recognition of kidney injury. Therefore, in the second study, parameters of RFA were measured in dehydrated horses, which were under a high risk of AKI within 48 hours after admission. Creatinine and BUN were analyzed together with selected markers: uSG, FE_{Na+}, uTP and the GGT/Creatinine ratio. The marker elevated at admission in most patients was uSG, which is reasonable since most uSG is sensitive to acute hypertonic dehydration (Oppliger et al. 2005). However, the uSG had no significant association with the dehydration levels. This might be due to the delayed reaction and the dilution of urine present in the bladder during the acute dehydration (Steiner et al. 2007). Creatinine was the only parameter associated with the severity of dehydration in this study. The only marker associated with the short-term outcome was FE_{Na+}. The uSG had a moderate correlation with FE_{Na+} and the GGT/Creatinine ratio, which showed that the urine concentrating process, which was regulated by sodium absorption, might influence the GGT/Creatinine ratio. No parameter showed any significant differences between dehydration groups within 48 hours. Furthermore, the urine strip did not correspond to all of the exact findings from the microscopic examination and RFA including uTP, leukocytes and erythrocytes. The result reveals that the parameters of RFA selected were not suitable to detect early changes in renal function induced by dehydration.

There are several limitations in the second study: 1) the challenging collection of urine within 48 hours. 2) There were several factors that could have influenced the results of FE_{Na+} and uSG such as infusion and medication against dehydration and primary disorders. 3) There was no horse diagnosed with AKI within 48 hours in this group. Therefore, it is unknown, whether any of these parameters had a predictive value for AKI. 4) The low sensitivity of the urine dipstick makes it difficult to detect minor

changes in the urinary tract. It also makes the comparison between the urine strip and the RFA microscopic examination difficult and unreliable.

The results from both studies confirm the correlation between the SDMA and creatinine. The association of SDMA and the severity of dehydration at admission also makes it a potential marker for renal perfusion in horses. We did not have enough patients that developed AKI to compare the further correlations between selected parameters from RFA and SDMA and the dehydration status. There is no single biomarker that could provide perfect information to diagnose and evaluate the renal dysfunction and AKI. It is still worth seeing how SDMA and other markers play their role in dehydrated horses. Further studies to estimate the SDMA and other biomarkers in the process from dehydration to secondary AKI will be helpful to find a novel better renal biomarker for our equine patients.

IV. Declaration of my own portion of work in the research publications

Symmetric dimethylarginine and renal function analysis in horses with dehydration

Authors: Hsiao-Chien Lo, Judith C. Winter, Roswitha Merle and Heidrun Gehlen

Year: 2021

Journal: Equine veterinary journal, 00 (online ahead of print) (2021), 1-9

	Hsiao-Chien Lo	Judith C. Winter	Heidrun Gehlen	Merle Roswitha
Study design	35%	35%	30%	-
Data collection	75%	15%	10%	-
Study execution	60%	30%	10%	-
Data analysis and interpretation	55%	15%	10%	20%
Preparation of the manuscript	60%	25%	10%	5%

Nierenfunktionsanalyse und Dehydratationszustand beim Pferd

Authors: Hsiao-Chien Lo, Judith C. Winter and Heidrun Gehlen

Year: 2021

Journal: Pferdeheilkunde-Equine Medicine, 37(2021) 2, 156-164

	Hsiao-Chien Lo	Judith C. Winter	Heidrun Gehlen	Merle Roswitha
Study design	35%	35%	30%	-
Data collection	75%	15%	10%	-
Study execution	60%	30%	10%	-
Data analysis and interpretation	55%	15%	10%	20%
Preparation of the manuscript	60%	25%	10%	5%

V. Summary

Diagnostic relevance of symmetric dimethylarginine and renal function analysis in horses

Diagnosis of AKI in the early stages is challenging not only in human but also in veterinary medicine. The novel renal biomarker SDMA has been proven to be a sensitive indicator of changes of renal function with fewer extra-renal influence factors than creatinine in human and small animals. The diagnostic value of SDMA in horses is still unknown. Other than SDMA, RFA, urine reagent strips and microscopic examination of urine might also have value in predicting AKI.

In the first study, the SDMA in horses with different dehydration levels was analyzed with RFA, creatinine and BUN. The SDMA had a moderate correlation with creatinine and association with the dehydrated status before infusion. There was no other significant correlation with the other parameters and the outcome of patients. Its reaction to rehydration therapy correlated with creatinine and the GGT/creatinine ratio, showed that it might eventually indicate the perfusion status of equine kidney. Although SDMA did not show that it had a predictive value of AKI and prognosis in this study, it is still worth investigating it further in horses under risk of AKI and finding out the role it plays in the renal pathology in horses.

In the second study, parameters from RFA were estimated between different dehydration levels. The comparison between urine reagent strips and microscopic examination showed that the dipstick was not sensitive enough to detect any tiny early changes of renal function. No parameter besides creatinine had any significant association with the dehydration status. This showed that selected renal markers from RFA had no predictive value of AKI.

Although primary renal disease is believed to be rare in horses, secondary AKI induced by hypovolemic dehydration, renal toxic medication and endotoxemia could affect the survival rate, cost and the need for intensive care of the hospitalized patients. A reliable diagnostic tool, either biomarkers or another test, such as urine output estimation, will be helpful to recognize changes in renal function and injury, treat the horses earlier and achieve a better prognosis. The correlation between SDMA and creatinine/ dehydration indicated the potentiality of SDMA to be a possible new biomarker for equine renal injury. In order to discover the proper usage of SDMA and other renal markers, further studies will be needed in horses with AKI and other renal dysfunctions.

VI. Zusammenfassung

Diagnostische Relevanz vom symmetric dimethyarginine und Nierenfunktionsanalyse beim Pferd

Nicht nur beim Menschen, sondern auch beim Tier ist es eine Herausforderung, eine akute Nierenschädigung (ANS) frühzeitig zu diagnostizieren. Der Parameter symmetric dimethylarginine (SDMA) zieht dewegen die Aufmerksamkeit von den Forschern mehr und mehr auf sich. SDMA wurde als ein sensitiver Biomarker zur Änderung der Nierenfunktion nachgewiesen, der von weniger extrarenalen Faktoren im Vergleich zu Kreatinin beeinflusst ist. Allerdings ist die Verwendbarkeit von SDMA beim Pferd noch unbekannt. Auch eine Nierenfunktionsanalyse (NFA), Harnsticks und mikroskopische Untersuchungen des Urins können zur Diagnostik von ANS beitragen.

In der ersten Studie, wurde SDMA bei dehydrierten Pferden gemessen und analysiert mit NFA, Kreatinin und Harnstoff. SDMA hatte eine moderate Korrelation mit Kreatinin und unterschied sich signifikant in den drei Gruppen mit verschiedenem Dehydratationsgrad. Es gab keine Korrelationen zwischen SDMA und anderen ausgewählten Parametern von NFA. SDMA hat sich nicht mit kurzzeitiger Prognose assoziiert. Es wurden signifikante Korrelationen von der Reaktion zu rehydrierter Therapie innerhalb 12 Stunden zwischen Kreatinin, GGT/Kreatinin Ratio und SDMA beobachtet. Es hat dadurch angezeigt, dass SDMA eventuell den Perfusions-Status der Niere repräsentieren könnte. Aufgrund weniger Probanden und bestätigten ANS Patienten (nur bei einem Pferd in der Studie konnte schließlich ANS diagnostiziert werden) kann man keinen prognostischen Wert von SDMA beim Pferd berücksichtigen.

Im zweiten Versuch, wurden Parameter aus der NFA gemessen und in drei Dehydratationsgruppen evaluiert. Vergleiche zwischen den Ergebnissen vom Harnsticks und der mikroskopischen Untersuchung zeigten, dass die Harnsticks nicht sensitiv genug waren, um kleine Veränderung der Nierenfunktion nachzuweisen. Kein Marker außer Kreatinin hatte eine signifikante Assoziation mit dem Dehydratationsgrad. NFA scheint weswegen bei akut dehydrierten Patienten aus klinischer Sicht wenig diagnostischen Wert zu haben.

Obwohl primäre Nierenerkrankungen relative selten beim Pferd vorkommen, spielt die sekundäre ANS, die von Dehydratation, toxischen Medikamenten und Endotoxämie ausgelöst werden kann, eine wichtige Rolle bei der Mortalität der Patienten, im Vergleich zu den Kosten und den Bedürfnissen von intensivemedizinischer Behandlung. Eine rechtzeitige Diagnosemethode, entweder durch bessere Biomarker für die Niere

oder ein spezieller Test, wird auf jeden Fall hilfreich für Tierärzte und Tierbesitzer. Die Korrelation zwischen SDMA und Kreatinin/ Dehydratation hat gezeigt, dass SDMA eventuell das Potenzial hat, ein neuer Biomarker für die Niere des Pferdes werden zu können. Weitere Forschungen zu SDMA Korrelation bei bestätigten ANS erkrankter Pferde sind erforderlich, damit man die Rolle von SDMA bei der Pathologie der Nierenerkrankung des Pferdes besser verstehen kann. Die genaue Überwachung von ANS erkrankten Pferden mittels NFA ist auch notwendig, um die Veränderung in der NFA zu evaluieren.

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VIII. List of publications

I. Lo, H. C., Winter, J. C., Gehlen, H. and Roswitha, M. 2021. Symmetric dimethylarginine and renal function analysis in horses with dehydration. Equine Vet J, 00, 1-9.

II. Lo, H. C., Winter, J. C. and Gehlen, H. 2021. Nierenfunktionsanalyse und Dehydratationszustand beim Pferd. Pferdeheilkunde-Equine Medicine, 37(2), 156-164.

IX. Acknowledgements

This dissertation could not have been completed without the support and contributions from the following people:

First of all, I would like to express my deep gratitude to Professor Heidrun Gehlen, my supervisor, for the opportunity given, freedom to think and design of the study, efficient discussions and her patience.

Regarding the great part of data collection, I am really grateful and appreciate all the help from our incredible team of internal medicine, residents and interns at the clinic for horses, Freie Universität Berlin. In addition, I would like to give a special great thanks to our laboratory technician, Tanja Ahrens. Without her, the measurement of laboratory parameters could not have been done so smoothly. Many thanks also to the grooms and rotation students who helped in handling horses and assisted in the sample collection. Finally, I also owe a great thank you to the owners and the horses that participated in this study.

I would like to thank Judith Winter, my co-supervisor and co-author, who arranged the integral design and collection method of the study, and also provided professional advice and encouragement, with so much patience, to correct the text and pushed me to finish the study at a better, more complete level. A big thank you also goes to Professor Roswitha Merle, for her helpful advice regarding data analysis and constructive interpretation of the results.

Deep appreciation goes to Pitiporn Leelamankong and Suphannika Phutthachalee for their support and suggestions regarding the “Doktorarbeit” career, and above all, their warm friendship to help me move so far.

I am constantly thankful and feel so lucky to have the support from the DAAD (Deutscher Akademischer Austauschdienst). The trusting and always caring team from DAAD accompanied me on this wonderful promotion journey in Germany.

Deep thanks also go to Kuan-Chieh Tseng, my boyfriend, and his family, who were always there to embrace my complaints and depression, taking care of my family and pet rabbit Dodo.

Last but not least, I would like to thank my family for supporting me unconditionally all the time with great love and understanding, which encouraged me to pursue my dreams and live my life bravely.

X. Source of funding and competing interests

Die Arbeiten wurden finanziell unterstützt durch SYNLAB.vet GmbH, Berlin, in denen sie die Kit für die Messung von SDMA und Nierenfunktionsanalyse angeboten haben.

Es besteht ein Interessenskonflikt durch finanzielle Unterstützung der Arbeiten mit Kooperation durch Ko-Autorin Judith C. Winter. Die Autorin (Hsiao-Chien Lo) hat vollständige Rechte und Zugang zu den Studiendaten. Sie ist auch verantwortlich für die Integrität der Daten und die Genauigkeit der Analyse. Es bestehen bei anderen Autoren keine Interessenskonflikte.

XI. Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegeben Quellen und Hilfe in Anspruch genommen habe.

Berlin, den 15.12.2021
Hsiao-Chien Lo

Druck: Mensch und Buch Verlag Berlin