Agreement between lacrimal fluid and serum for detecting urea nitrogen and creatinine in dogs

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OBJECTIVE
To determine whether urea nitrogen and creatinine levels differ in lacrimal fluid (LF) and serum (SER) in nonazotemic (control) and azotemic dogs and whether there is an agreement between LF and SER.

METHODS
A prospective observational study was performed at the Auburn University Small Animal Teaching Hospital between May 2023 and March 2024. Forty control and 38 azotemic dogs were enrolled. Twenty microliters of LF per eye was collected with microcapillary tubes, and 3 mL of blood was drawn. Bland-Altman plot and intraclass correlation coefficient (ICC) were used to evaluate the agreement between LF and SER.

RESULTS
There was good agreement between LF and SER levels of urea nitrogen in the control group (Bland-Altman plot mean bias of –0.8108 ± 2.407 mg/dL; ICC of 0.874 [95% CI, 0.773 to 0.934]) and the azotemic group (Bland-Altman plot mean bias of –9.681 ± 23.89 mg/dL; ICC of 0.82 [95% CI, 0.658 to 0.906]). There was poor agreement between LF and SER concentrations for creatinine in the control and azotemic groups, with only 26 dogs with creatinine detectable in LF.

CONCLUSIONS
Lacrimal fluid and SER concentrations of urea nitrogen showed good agreement in both the control and azotemic groups, whereas poor agreement was found for creatinine in both groups.

CLINICAL RELEVANCE
Measurement of urea nitrogen in LF may provide an alternative to blood for diagnosing uremia. However, additional research is necessary before substituting LF for SER.

Keywords: tear, azotemia, uremia, blood, canine

In veterinary medicine, the blood concentrations of urea nitrogen and creatinine are the gold-standard parameters for assessing prerenal, renal, and postrenal azotemia. Blood collection can be challenging to perform in small or severely hypovolemic patients. In those patients, collecting the minimal volume of blood needed for initial diagnosis can be challenging and, therefore, not always feasible. Venipuncture can also be arduous in patients bruised by multiple blood draws. Moreover, it can be stressful and lead to patient deterioration, particularly in those who are anemic or have a bleeding disorder. Thus, the ability to diagnose azotemia without the use of blood would be clinically useful.

Assessing azotemia with the use of a bodily fluid other than blood has been previously described in veterinary medicine. Urea nitrogen and creatinine have been successfully measured in canine saliva with concentrations similar to serum (SER) levels by use of a commercially available spectrophotometric assay. Urea nitrogen and creatinine can also be measured in vitreous and aqueous humor; SER concentrations are similar. Lacrimal fluid (LF) is another sample alternative that has the potential to detect systemic azotemia. The blood-tear barrier is composed of the corneal barrier and the tear barrier. Its mechanism remains unclear, but it is believed that increased permeability of the conjunctival vessels secondary to a systemic illness may result in leakage of systemic substances into the tear film.
Previous studies have examined the correlation between different species’ blood and LF concentrations of urea nitrogen and creatinine. Zapata et al. measured urea nitrogen and creatinine in healthy horses and only found a correlation between urea nitrogen concentration in plasma and LF concentrations. Steinmetz et al. performed a similar study in cats and found the same conclusion for urea nitrogen. Drawbacks reported from this study were the difficulty collecting an adequate amount of LF and the low detectability of creatinine in LF. Thus, it was hard to determine whether the lack of an adequate sample could have affected urea nitrogen and creatinine measurement. A study of humans by Kang et al. found a correlation between SER and LF for urea nitrogen and creatinine levels. To the investigators’ knowledge, no similar study has been published of dogs. Whether canine LF can be used to diagnose azotemia remains unknown.

This study had 4 aims: (1) to determine whether there is a significant difference between LF and SER urea nitrogen levels in the control group and the azotemic group, (2) to determine whether there is a significant difference between LF and SER creatinine levels in both groups, (3) to evaluate the agreement between LF and SER concentrations of urea nitrogen in both groups, and (4) to evaluate the agreement between LF and SER concentrations of creatinine in both groups.

The investigators hypothesized that there would be a significant difference between LF and SER urea nitrogen and creatinine concentrations in the control and azotemic groups, and that there would be an agreement between LF and SER concentrations of urea nitrogen and creatinine in the control and azotemic groups.

Methods

Case selection

Dogs presenting to the Auburn University Small Animal Teaching Hospital between May 2023 and March 2024 were eligible for inclusion. The Auburn University IACUC approved this study (2023-5176), and signed informed consent was obtained from all owners before inclusion.

Inclusion criteria

Adult dogs older than 1 year of age presenting to the Auburn University Small Animal Teaching Hospital Emergency and Critical Care service with a previous or new diagnosis of azotemia were included in the azotemic group. Azotemia was defined as an SER creatinine level over 1.4 mg/dL as determined by the International Renal Interest Society staging of chronic kidney disease and a concentration of urea nitrogen > 29 mg/dL as determined by the upper reference interval established by our laboratory. Pre-renal, renal, and postrenal azotemia were not differentiated in this study. The control group consisted of staff- and student-owned dogs with creatinine ≤ 1.4 mg/dL and urea nitrogen ≤ 29 mg/dL that received preventative drugs as their only medication.

Exclusion criteria

Patients were excluded if signed consent for study participation was not obtained, if their temperament did not allow safe LF collection, or if they had at least one of the following exclusion criteria: the presence of an ocular disease affecting tear production (keratoconjunctivitis sicca, glaucoma, uveitis, corneal ulcers, blepharitis, or meibomianitis), administration of trimethoprim sulfadiazine antibiotics, diagnosis of diabetes mellitus, or administration of an injectable sedative (opioids, α-2 agonists, or benzodiazepines) within 12 hours before enrollment. If a patient only had an increase of urea nitrogen or creatinine, they were not included in this study.

Study design

This study was designed as a prospective non-randomized observational study. A single investigator collected 20 μL of LF from each eye using plain microcapillary glass tubes (Statspin 40-mm microhematocrit tubes; Iris Sample Processing) placed in the ventral conjunctiva. Immediately after LF collection, with an 18- to 22-gauge needle, 3 mL of blood from a peripheral vein was collected in a plain tube and let sit for about 10 minutes or until a clot was formed. The sample was centrifuged at 1,000 X g for at least 4 minutes for SER extraction. Both the SER and LF were transferred to a cryotube and stored at –80 °C until analysis. A chemistry analyzer (Heska Element DCX Veterinary Chemistry Analyzer; Antech) using the colorimetric method was utilized for sample measurement. This machine required a minimum of 10 μL per test. Ten microliters of LF and 20 μL of SER were used to measure creatinine and urea nitrogen each.

Following sample collection, all patients were allowed a minimum 30-minute wait time before an ophthalmic examination was performed to allow LF to replenish. During regular hours, patients included in this study received a complete ophthalmic examination performed by the same board-certified ophthalmologist (SB) that included a slit lamp and indirect ophthalmoscopy, a Schirmer tear test, fluorescein stain with tear breakup time, and intraocular pressure measurement. After regular hours, the ophthalmic examination was performed by the primary investigator (WT) and included a Schirmer tear test, fluorescein stain, Tyndall, and intraocular pressure. Data prospectively collected during the study included weight, age, sex, neuter status, and breed.

Statistical analysis

Sample size calculation was performed with the mean and SD from a previous study in which urea nitrogen and creatinine were measured in a fluid other than blood in dogs. With a power of 80% and an α of 0.05, a sample size of 30 dogs per group was needed. Descriptive statistics was used for demographic variables. For each variable, probability and residual plots were generated to verify that data followed a normal distribution. Moreover, the normality of the data was evaluated with the Kolmogorov-Smirnov test. Accordingly, urea nitrogen concentrations were compared with the paired t test, while creatinine
concentrations were compared with the Wilcoxon matched-pairs signed test. Agreement between measurements was assessed with Bland-Altman plots and analysis adjusted for repeated measures. The bias, SD of the bias, and 95% limits of agreement are reported. Pearson and Spearman correlation coefficients were calculated for urea nitrogen and creatinine, respectively. Furthermore, an intraclass correlation coefficient (ICC) calculation was performed. Intraclass correlation coefficient values below 0.5 were indicative of poor reliability; values between 0.5 and 0.75 indicated moderate reliability; values between 0.76 and 0.90 indicated good reliability; and values above 0.90 indicated excellent reliability. Data were analyzed with R for Mac (version 4.2.2; The R Project for Statistical Computing) and Prism (version 10.0; GraphPad Software), with an overall α set to \( P < .05 \).

**Results**

During the study period, 40 healthy control dogs were enrolled. Three dogs were excluded after the ophthalmic examination due to the following reasons: keratoconjunctivitis sicca, irregular corneal surface, and inflammatory mature cataract. For the azotemic group, 38 dogs were enrolled in the study. One dog had corneal hemorrhage and keratoconjunctivitis sicca and therefore was excluded from this study. Another dog was euthanized before an ophthalmic examination was performed, but since no gross abnormality was seen upon LF collection, this dog was included for analyses. Therefore, each group included 37 dogs eligible for analyses.

**Demographics**

**Control group**

The median age was 5 years (range, 1 to 15 years). Sex consisted of 13 (35.1%) spayed females, 2 (5.4%) intact females, 21 (56.8%) neutered males, and 1 (2.7%) intact male. The median body weight was 22.2 kg (range, 5.9 to 45 kg). Breeds represented were mixed breed (16/37 [43.2%]), Labrador Retriever (7/37 [18.9%]), German Shepherd Dog (3/37 [8.1%]), Pug (2/37 [5.4%]), Beagle (2/37 [5.4%]), and 1 each of the following breeds: German Shorthair Pointer, Basset Hound, Dachshund, Weimaraner, and pit bull-type dog.

**Azotemic group**

The median age was 10 years (range, 1.5 to 16 years). Sex consisted of 21 (56.8%) spayed females, 1 (2.7%) intact female, 12 (32.4%) neutered males, and 3 (8.1%) intact males. The median body weight was 19.5 kg (range, 3.4 to 41.9 kg). Breeds represented included mixed breed (12/37 [32.4%]), Standard Poodle (2/37 [5.4%]), Toy Poodle (2/37 [5.4%]), Boxer (2/37 [5.4%]), Beagle (2/37 [5.4%]), German Shepherd Dog (2/37 [5.4%]), Yorkshire Terrier (2/37 [5.4%]), pit bull-type dog (2/37 [5.4%]), Golden-doodle (2/37 [5.4%]), and 1 each of the following breeds: Chihuahua, Pomeranian, Great Pyrenees, Staffordshire Terrier, French Bulldog, Doberman, Shih Tzu, Golden Retriever, and English Setter.

**Urea nitrogen concentration in LF and SER**

**Control group**

The mean urea nitrogen concentration was 16.38 ± 5.298 mg/dL in LF and 15.56 ± 4.623 mg/dL in SER (Figure 1). There was a significant difference between the 2 bodily fluids for urea nitrogen for the control group (\( P = .0478 \)).

**Azotemic group**

The mean urea nitrogen concentration was 83.3 ± 41.48 mg/dL in LF and 92.98 ± 42.37 mg/dL in SER (Figure 1). There was a significant difference between the 2 bodily fluids for urea nitrogen for the control group (\( P = .0478 \)).
between the 2 bodily fluids for urea nitrogen for the azotemic group ($P = .0186$).

**Agreement between LF and SER in the control group**

The Bland-Altman plot showed a mean bias of $-0.8108 \pm 2.407$ mg/dL with a limit of agreement from $-5.528$ to $3.906$ mg/dL between the LF and SER concentrations (Figure 2). The ICC was $0.874$ (95% CI, $0.773$ to $0.934$; $P < 0.0001$). On the basis of those results, the urea nitrogen concentration in LF and SER in the control group showed good agreement.

**Agreement between LF and SER in the azotemic group**

The Bland-Altman plot showed a mean bias of $-9.681 \pm 23.89$ mg/dL with a limit of agreement from $-56.51$ to $37.15$ mg/dL (Figure 3). The ICC was $0.82$ (95% CI, $0.658$ to $0.906$; $P < 0.0001$). On the basis of those results, the azotemic group’s urea nitrogen concentration in LF and SER showed good agreement.

**Creatinine concentration in LF and SER**

**Control group**

The LF creatinine concentration was below the detection limit (0.2 mg/dL) for 31 out of 37 (83.8%) dogs. The median creatinine levels were 0 mg/dL (range, 0 to 0.7 mg/dL) in LF and 0.8 mg/dL (range, 0.3 to 0.9 mg/dL) in SER (Figure 4). There was a significant difference between the 2 bodily fluids for creatinine in the control group ($P < .001$).

**Azotemic group**

The LF creatinine concentration was below the detection limit for 17 of the 37 (45.9%) dogs. The

**Figure 2**—Linear correlation (A) and Bland-Altman plot (B) comparing urea nitrogen (mg/dL) in lacrimal fluid and serum in the control group.

**Figure 3**—Linear correlation (A) and Bland-Altman plot (B) comparing urea nitrogen (mg/dL) in lacrimal fluid and serum in the azotemic group.
median creatinine levels were 0.2 mg/dL (range, 0 to 2.2 mg/dL) in LF and 2.6 mg/dL (range, 1.5 to 11.7 mg/dL) in SER (Figure 4). There was a significant difference between the 2 bodily fluids for creatinine in the azotemic group ($P < .001$).

**Agreement between LF and SER in the control group**

The Bland-Altman plot showed a mean bias of $-0.7270 \pm 0.3124$ mg/dL with a limit of agreement from $-1.339$ to $-0.1148$ mg/dL between the LF and SER concentrations. The ICC for creatinine was $-0.0264$ (95% CI, $-0.077$ to $0.077$; $P = .778$). On the basis of those results, creatinine concentration in LF and SER showed poor agreement in the control group. Only 6 (16.2%) dogs had a measurable creatinine level in LF, rendering Bland-Altman and linear correlation plots nonsensical and not illustrated.

**Agreement between LF and SER in the azotemic group**

The Bland-Altman plot showed a mean bias of $-3.232 \pm 2.302$ mg/dL with a limit of agreement from $-7.744$ to $1.280$ mg/dL. The ICC for creatinine was $0.0168$ (95% CI; $-0.08$ to $0.162$; $P = .388$). On the basis of those results, creatinine concentration in LF and SER showed poor agreement in the azotemic group. Only 20 (54.0%) dogs had a measurable creatinine level in LF, rendering Bland-Altman and linear correlation plots nonsensical and not illustrated.

**Discussion**

This study evaluated LF concentrations of urea nitrogen and creatinine with corresponding SER concentrations in control and azotemic dogs. No obvious signs of pain, irritation, or patient intolerance were observed during LF sampling with microcapillary glass tubes.

This study found a clinically significant difference in urea nitrogen in the 2 bodily fluids used in both groups, similar to previous studies of horses, cats, and humans. Although LF and SER concentrations of urea nitrogen showed good agreement, the limits of agreement were wide. This finding suggested that LF urea nitrogen cannot be used interchangeably with the SER concentration. Therefore, reference intervals should be established for urea nitrogen in LF in dogs.

For creatinine, a significant difference was also found in the 2 bodily fluids used in both groups. This difference was suspected to be caused by the low number of patients with detectable creatinine levels in LF. In the control group, creatinine was only detectable in 6 out of 37 (16.2%) dogs, whereas in the azotemic group only 20 (54.0%) dogs had measurable levels. Steinmetz et al also had similar findings in which creatinine was detectable in only 25% of the study cats. In the latter study, cats with a plasma concentration $\geq 254$ μmol/L ($\geq 2.87$ mg/dL) had a detectable creatinine in LF. In our study, similar findings were not found. The poor agreement between LF and SER concentrations of creatinine was reflected in the Bland-Altman plot and low correlation coefficient. Similar to the previous studies of cats and horses, creatinine measured in LF and plasma did not correlate. This finding contrasted with that of Kang et al, who found that creatinine measured in LF correlated in the plasma of human patients. Although previous studies in other species used plasma concentration instead of SER, it has been shown that creatinine levels are 0.06 to 0.1 mg/dL higher when measured in SER than in plasma. This difference was likely insignificant for clinical purposes. Several hypotheses can explain why creatinine was hardly detectable in LF. First, the

![Figure 4](image-url) — Lacrimal fluid and serum concentrations of creatinine (mg/dL) in the control group (A) and azotemic group (B).
molecular weight of creatinine is 113 Da, almost twice the molecular weight of urea nitrogen (60 Da). This difference can affect its ability to readily cross the blood-tear barrier. Second, the volume of LF used for measurement was the minimum volume required by the machine due to difficulties in collecting LF in some dogs. This limitation can influence the detection ability of creatinine in LF.

This study had several limitations. First, the volume used to measure LF was the minimum volume required by the machine utilized. Since the normal LF volume in dogs is approximately 65 µL, there was a limited volume of LF that could be collected each time. A problem during LF sampling was the difficulty of LF collection in severely dehydrated patients. In humans, systemic dehydration has been associated with increased tear osmolality, but changes in LF volume or composition remain to be elucidated. Therefore, it is unknown how LF collection will be impacted, as dehydration is often associated with azotemia. Second, this study used microcapillary glass tubes to collect the LF samples similar to a previous study of horses. Van Haeringen et al. found that when microcapillary tubes were used instead of filter paper strips to collect LF, lactate dehydrogenase and glucose measured lower in LF. This is suspected to be secondary to mechanical irritation of the corneal epithelium caused by the paper strips, which in turn leads to increased diffusion of substances through the LF. Since this study used microcapillary tubes to collect LF, creatinine levels may have been measured lower in LF compared to SER.

With its average basal turnover rate of 12.2%/min in physiologic conditions, LF may provide a simpler, noninvasive, nonpainful alternative to blood to evaluate systemic diseases. Moreover, due to its small turnover rate, LF may provide an unlimited resource with only mild systemic impacts. The blood-tear barrier is not a well-defined concept. Unlike the blood-aqueous barrier and the blood-retinal barrier, the blood-tear barrier is located outside of the eye on the corneal surface. It is believed that increased permeability of the conjunctival vessels secondary to a systemic illness may result in leakage of systemic substances into the tear film. It is unclear how permeable this barrier is; thus, further studies will be required to better understand the function of this barrier.

In conclusion, our research indicated that urea nitrogen detection in LF of dogs showed good agreement with SER concentration, offering potential clinical utility in cases involving coagulopathy, anemia, or challenging venous access. However, the detection of creatinine in LF among dogs was inconsistent, rendering it clinically unhelpful. Further investigations are necessary to ascertain the interchangeability between LF and SER concentrations of urea nitrogen.

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Disclosures
Dr. Gerken is a member of the JAVMA Scientific Review Board, but was not involved in the editorial evaluation of or decision to accept this article for publication.

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