Feline renal biomarkers: from reference intervals to clinical application

Rebekah Mack DVM, DACVIM-SAIM, IDEXX Medical Affairs Specialist
Objectives

- Understanding the development and utilization of a reference interval
- Understanding the difference between use of a reference interval for evaluating change in renal function and applying the IRIS CKD staging guidelines
- Exploring additional renal biomarkers to improve diagnosis of kidney disease
- Discussing feline kidney disease and concurrent conditions
- Exploring a case example
Understanding the development and utilization of a reference interval
Timeline of the development of reference intervals

1969 First introduced in human medicine in

1977 Human guidelines established

1978 First comprehensive veterinary publication

1996 established American Society for Veterinary Clinical Pathology

2001 established

Healthy Human Populations

+ Disease-State Human Populations

Healthy Human Populations

Healthy Veterinary Populations


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Definition of a reference interval

**Definition:** “is an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others.”

**Mechanics of reference interval (RI):**

- No RI is completely “right” or “wrong.”
- The majority of RIs in use today refer to the central 95% of the reference population of subjects.
- By definition, 5% of all results from “healthy” individuals will fall outside of the reported RI and, as such, will be flagged as being “abnormal.”

3) Ekelund, Suzanne. Reference Intervals and percentiles – implications for the healthy patient. Acutecaretesting.org
How do you “develop” a reference interval?

Population
- 120 clinically healthy individuals
- Equal sex representation
- Geographic representation
- Appropriate age (>1 year)

Calculate using **nonparametric** methods
In case you forgot…

Nonparametric data:
- Does not have a normal distribution (not a perfect bell curve)
- Better for large data sets
- Considers outliers without having to remove them

1) Ekelund, Suzanne. Reference Intervals and percentiles – implications for the healthy patient. Acutecaretesting.org
How do you “develop” a reference interval?

Most universities, and commercial labs develop independent reference intervals unique to them.

Analyzers

Reagent & Methodology

Population & Geography
Veterinary reference interval development can differ

Limitations:
- Unique species
- Sample quantities
- Individual samples

These limitations can lead to the use of different statistical methods and less “robust” reference intervals
Benefits of Reference Intervals

**Medical Decision Point** – provide guidance for when to institute further testing or care

Can allow for a single moment in time (testing) to help determine the health of an individual

Leads to differentials and medical action
Downside of reference intervals

95% of the population represented – 5% will fall outside the confidence intervals

Population based vs Individual based - describe fluctuations in healthy populations or individuals which make establishing health status critical for interpretation

Difficult, time-consuming, and expensive to establish

Intra- and Interindividual biological variation

Preanalytical Aspects

Analytical Aspects

Calculations

Intra- and Interindividual biological variation

Biological variation within and between individuals

- Differences in analyte can make it better for population based RI (which we use) or patient based
- This can make a population based RI less accurate for determining abnormality in a single patient

1) http://eclinpath.com/test-basics/reference-intervals/
Preanalytical

- Samples must be from clinical practice

<table>
<thead>
<tr>
<th>Subject Preparation</th>
<th>Methodological Factors: Specimen Collection</th>
<th>Methodological Factors: Specimen Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fasting vs non-fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Drug regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Physical activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Time of day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• With or without tourniquet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Body posture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Anticoagulant, type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Sampling equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Interferents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Transportation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Time before centrifugation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Storage before measurement</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analytical
Calculations

Proper statistical methods used for population and population distribution
Downside of individual reference intervals

- Often imperfect for evaluation of all patients
- Variability on how well an entire population can be represented within a RI
- Complex to properly establish
- Must be maintained as methodologies, analyzers, or patient populations change
Individual patient vs population reference interval

### Individual patient details:
- Slow muscle and weight loss
- Intermittent hyporexia

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Creatinine (mg/dL)</th>
<th>Creatinine (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/9/17</td>
<td>2:52 PM</td>
<td>1.8</td>
<td>159</td>
</tr>
<tr>
<td>1/19/17</td>
<td>12:14 AM</td>
<td>1.7</td>
<td>150</td>
</tr>
<tr>
<td>12/9/16</td>
<td>12:25 AM</td>
<td>1.6</td>
<td>141</td>
</tr>
<tr>
<td>10/28/16</td>
<td>3:09 PM</td>
<td>1.7</td>
<td>150</td>
</tr>
<tr>
<td>9/23/16</td>
<td>3:20 PM</td>
<td>2.0</td>
<td>176</td>
</tr>
<tr>
<td>8/23/16</td>
<td>2:45 PM</td>
<td>2.1</td>
<td>185</td>
</tr>
<tr>
<td>5/20/16</td>
<td>12:45 AM</td>
<td>2.3</td>
<td>203</td>
</tr>
</tbody>
</table>

**Trending Up**: High Normal

**Trending Down**: Slow muscle and weight loss

**High Normal**: Intermittent hyporexia
Application of Reference Interval vs Treatment Guideline: Creatinine as a clinical example
Diagnosis of kidney disease does not improve with lowering the creatinine reference interval—let us show you why
Reference intervals are determined for each laboratory test by universal standards protocols.

IDEXX follows regulatory standards to establish reference intervals, specifically the Clinical and Laboratory Standards Institute.¹

Determinations study clinically healthy populations.

Reference intervals are not universal and may differ based on laboratory, methodology, and population.

Sources:
The IRIS guidelines do not propose reference intervals.

International Renal Interest Society (IRIS) guidelines are not reference intervals.

The IRIS guidelines provide disease-staging criteria to inform treatment after diagnosis of chronic kidney disease (CKD).

Sources:
IDEXX creatinine reference interval study for cats: A reference interval is determined, not created

175 feline patients
Adults of all breeds

7 clinics + 3 reference laboratories
Small-animal private practices from diverse geographic locations

Sample distribution
Determined from sample data, reference interval based on central 95%

Source:
1. Data on file at IDEXX Reference Laboratories, Inc. Westbrook, Maine USA.

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What happens if we change our upper reference limit to 1.6 mg/dL (140μmol/L)?

Distribution of creatinine in apparently healthy cats

<table>
<thead>
<tr>
<th>Creatinine (mg/dL)</th>
<th>Percent of cats with creatinine value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 (44 µmol/L)</td>
<td>2.5%</td>
</tr>
<tr>
<td>1.6</td>
<td>95%</td>
</tr>
<tr>
<td>2.3 (203 µmol/L)</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

IRIS recommends staging CKD at Stage 1 with a stable creatinine of less than 1.6 mg/dL.²

Sources:
The math: The effect on our cat population

Distribution of creatinine in apparently healthy cats

Percent of cats with creatinine value

- Lower reference interval
- Upper reference interval

Creatinine (mg/dL)

- 0.5 (44 µmol/L)
- 1.6
- 2.3 (203 µmol/L)

25.5%

of clinically healthy cats now identified as abnormal (sum of shaded areas).

Source:
1. Data on file at IDEXX Reference Laboratories, Inc. Westbrook, Maine USA.
Using the IRIS Stage 1 threshold for the upper limit of the reference interval would misdiagnose one in four clinically healthy cats.

Sources:
The consequence of misdiagnosis of disease

- Unnecessary diagnostics for healthy cat
- Unnecessary costs for the owner
- Unwarranted treatment for a healthy cat
- Inappropriate diagnosis of an irreversible and progressive condition for healthy cats
Exploring additional renal biomarkers to improve diagnosis of kidney disease
The importance of understanding kidney function and detecting early disease

The range between IRIS Stage 1 threshold and the IDEXX creatinine reference interval

Source:
The importance of understanding kidney function and detecting early disease

Impaired GFR
Identify these cats correctly with probable impairment of GFR (aligns with elevated SDMA).

n = 352,767 (26.2%)

Source:
The importance of understanding kidney function and detecting early disease

Number of cats at each creatinine value

SDMA 0–14 µg/dL
SDMA >14 µg/dL

Functional GFR
You could misidentify these clinically healthy cats (creatinine >1.6 mg/dL, normal SDMA).
\( n = 622,104 \) (46.3%)

Impaired GFR
Identify these cats correctly with probable impairment of GFR (aligns with elevated SDMA).
\( n = 352,767 \) (26.2%)

Source:
The importance of understanding kidney function and detecting early disease

Functional GFR
You could misidentify these clinically healthy cats (creatinine >1.6 mg/dL, normal SDMA).

Impaired GFR
You could miss these cats using creatinine alone.

Impaired GFR
Identify these cats correctly with probable impairment of GFR (aligns with elevated SDMA).

Source:
Using a different test is better than arbitrarily changing a RI

Impaired GFR
You could miss these cats using creatinine alone.

n = 209,795 (6.4%)
Commercially available renal biomarkers

1. SDMA, Creatinine, BUN
2. Phosphorus, Hematocrit, Potassium, Magnesium
3. Urinalysis: USG, Proteinuria, Urine protein: creatinine ratio
SDMA and Feline Concurrent Disease
What are their kidneys telling you?
Listen closer with IDEXX SDMA®

**Detects**

diseases of the kidney sooner¹–³

Chronic kidney disease
Acute kidney injury
Pyelonephritis
Upper urinary obstruction
Kidney stones
Glomerulonephritis
Congenital disease

**Reflects**

other disease processes affecting the kidneys⁴

Hyperthyroidism
Vector-borne disease
Systemic hypertension
Cardiorenal syndrome
Lower urinary obstruction
Sepsis
Cancer
Drug toxicity

Sources:
4. Data on file at IDEXX Laboratories, Inc. Westbrook, Maine USA.
What are their kidneys telling you? Listen closer with IDEXX SDMA®

**Detects**

diseases of the kidney sooner\(^1\)–\(^3\)

- Chronic kidney disease
- Acute kidney injury
- Pyelonephritis
- Upper urinary obstruction
- Kidney stones
- Congenital disease

**Reflects**

other disease processes affecting the kidneys\(^4\)

- Hyperthyroidism
- Systemic hypertension
- Lower urinary obstruction
- Drug toxicity

**Sources:**

4. Data on file at IDEXX Laboratories, Inc. Westbrook, Maine USA.
What are their kidneys telling you?

Reflects
other disease processes
affecting the kidneys

Hyperthyroidism
Systemic hypertension
Lower urinary obstruction
Drug toxicity

Sources:
4. Data on file at IDEXX Laboratories, Inc. Westbrook, Maine USA.
Feline Hyperthyroidism

- Prevalence varies based on geography with senior cats at diagnosed at approximately 10-12% and geriatric cats closer to 25%
- 15-49% of cats with hyperthyroidism have renal dysfunction
- Reduced muscle mass is a hallmark of feline hyperthyroidism (FHT).

SDMA is less affected by extrarenal factors – more consistent marker than creatinine in FHT.

Elevated SDMA with appropriate clinical picture should elicit concern for underlying renal disease – should encourage action.
Feline Hyperthyroidism and Renal Disease

- Hyperthyroidism is the most common endocrine disease of older cats.

- CKD is estimated to affect 1-3% of all cats, and > 30% of cats older than 15 years of age.\(^1\)\(^-\)\(^5\)

- Prevalence of hyperthyroidism and renal disease occurring together reported at approximately 14%.\(^5\)\(^,\)\(^6\)
  - Masking of underlying kidney disease is common due to increased GFR during disease state and reduction of GFR post-therapy.\(^7\)

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Cats with iatrogenic hypothyroidism have a greater incidence of increased creatinine and SDMA.¹

- Likely due to decreased GFR
- Worsening underlying kidney disease ²,³,⁴
- Development of azotemia negatively impacts survival time.¹
- Restoring euthyroidism appears to reduce the occurrence of azotemia.¹

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4) Peterson ME, Nicholas R, Rison M. Serum thyroxine and thyroid-stimulating hormone concentration in hyperthyroid cats that develop azotemia after radiodine therapy. J Small Anim Pract. 2017:58(9) 519-530
Hypertension occurs when there is smooth muscle contraction in the small arterioles.
Systemic Hypertension

Defined as:

- Normotensive (minimal TOD risk) SBP <140 mm Hg
- Prehypertensive (low TOD risk) SBP 140-159 mm Hg
- Hypertensive (moderate TOD risk) SBP 160-179 mm Hg
- Severely hypertensive (high TOD risk) SBP ≥180 mm Hg

Since blood pressures > 160 are associated with a moderate risk of pathologic changes treatment is usually recommended." or some such rather than writing usually:

- Hypertension can occur with any severity of kidney disease.

- Blood pressure should be part of the routine follow-up for all cats with kidney disease.

Clinical Case Example
### Chemistry

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>72-1.75 mg/dL</td>
<td></td>
</tr>
<tr>
<td>IDOD ASMA</td>
<td>17 pg/dL</td>
<td>0-14 pg/dL</td>
</tr>
<tr>
<td>Creatinine</td>
<td>70-203 mmol/L</td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>15 mg/dL</td>
<td>16-27 mg/dL</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.9-6.3 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>8.2-11.2 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>147-157 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>3.7-5.2 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Na:K Ratio</td>
<td>38-42</td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>114-120 mmol/L</td>
<td></td>
</tr>
<tr>
<td>TCO2 (Bicarbonate)</td>
<td>12-22 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Anion Gap</td>
<td>12-25 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Total Protein</td>
<td>6.6 g/dL</td>
<td>6.3-8.8 g/dL</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.6-3.9 g/dL</td>
<td></td>
</tr>
<tr>
<td>Globulin</td>
<td>3.0-5.9 g/dL</td>
<td></td>
</tr>
<tr>
<td>Albumin:Globulin</td>
<td>0.8 0.5-1.2</td>
<td></td>
</tr>
</tbody>
</table>

#### Differentials:

**Primary vs Secondary kidney disease**

**Hyperthyroidism**

**Primary liver disease**
Questions?