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Journal Title: Journal of veterinary internal medicine

Volume: 7
Issue: 3
Month/Year: 1993

Article Author: Smiley LE, Peterson ME.
Article Title: Evaluation of a urine cortisol:creatinine ratio as a screening test for hyperadrenocorticism in dogs.
Pages: 163-168.

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Evaluation of a Urine Cortisol:Creatinine Ratio as a Screening Test for Hyperadrenocorticism in Dogs

Laura E. Smiley, DVM, and Mark E. Peterson, DVM

The authors collected urine specimens in 31 normal dogs, 25 dogs with hyperadrenocorticism, 21 dogs in which hyperadrenocorticism was suspected but was not present, and 28 dogs with a variety of severe, nonadrenal diseases. Cortisol and creatinine were measured in unextracted urine by radioimmunoassay and spectrophotometry, respectively, and the cortisol:creatinine ratio was calculated for each specimen. The mean ± SD urine cortisol:creatinine concentration ratio in the dogs with hyperadrenocorticism (103.1 ± 100.7) was significantly (P < 0.001) higher than that in the normal dogs (13.1 ± 7.0). The mean urine cortisol:creatinine ratio of dogs initially suspected of having hyperadrenocorticism (163.3 ± 7.0) was significantly (P < 0.001) lower than the ratio of dogs with hyperadrenocorticism, but was not significantly different than that in the normal dogs. The mean urinary cortisol:creatinine ratio in the dogs with nonadrenal disease (82.8 ± 197.7) was significantly (P < 0.001) higher than that in both the normal dogs and dogs in which hyperadrenocorticism was initially suspected, but was not different than the ratio in the dogs with hyperadrenocorticism. The sensitivity of the urine cortisol:creatinine ratio as a diagnostic test for hyperadrenocorticism was 0.92. The specificity was high in the normal dogs (0.97) and the dogs initially suspected of having hyperadrenocorticism (0.95), with ≤ 5% having false-positive results. However, the specificity was very low (0.21) in the dogs with moderate to severe nonadrenal disease, with 79% of the dogs having false-positive results. Similarly, both positive and negative predictive values and diagnostic efficiency were high in the normal dogs and dogs suspected of having hyperadrenocorticism but were low in the dogs with nonadrenal illnesses. When the results of the cortisol assay used in the study were compared to results obtained by two other commercially available cortisol radioimmunoassays, a high correlation between results was found. The urine cortisol:creatinine ratio is a sensitive screening test for the detection of hyperadrenocorticism in dogs. As with other pituitary-adrenal function tests, however, the urine cortisol:creatinine ratio cannot be used to diagnose hyperadrenocorticism in dogs that have moderate to severe nonadrenal disease. (Journal of Veterinary Internal Medicine 1993; 7:163–168)

HYPERADRENOCORTICISM is a common disorder in dogs, the diagnosis of which entails use of one or more of a number of pituitary-adrenal function tests. One of the most commonly used tests is the low-dose dexamethasone suppression test, which involves administration of exogenous dexamethasone followed by measurement of serum cortisol in blood collected over an 8-hour period. While this diagnostic test is sensitive and useful, it is time-consuming, somewhat costly, and not highly specific.1-3

Recently, investigators have described a screening test, which involves calculation of a cortisol:creatinine ratio by use of values obtained from a single urine sample, for dogs suspected of having hyperadrenocorticism.4-7 This test, which is comparatively convenient and inexpensive, appears to be highly sensitive for detecting hyperadrenocorticism in dogs and could prove useful in small animal practice.

This study determined the usefulness of the urine cortisol:creatinine ratio as a screening test for canine hyperadrenocorticism; determined the applicability of this test to a group of dogs with moderate to severe non-adrenal illnesses; and determined the ability of this test to distinguish...
dogs with hyperadrenocorticism from dogs with clinical signs similar to those of hyperadrenocorticism secondary to another disease; compared the sensitivity and specificity of this test to that of the low-dose dexamethasone suppression test; and compared the urine immunoreactive (IR) cortisol concentrations determined with three different radioimmunoassay (RIA) kits to determine if similar results would be obtained with commonly used commercially available assays.

**Materials and Methods**

We collected morning urine samples for determination of cortisol and creatinine concentrations in 105 dogs examined at The Animal Medical Center. The 105 dogs included four groups — normal dogs, dogs with documented spontaneous hyperadrenocorticism, dogs in which hyperadrenocorticism was initially suspected on the basis of clinical signs but were subsequently determined not to have the disorder, and dogs with a variety of severe, nonadrenal diseases in which hyperadrenocorticism was not suspected.

**Normal Dogs**

Thirty-one clinically normal dogs were used as controls. These were pet dogs owned by employees of the hospital. They ranged in age from 1 to 14 years (mean ± SD = 6.9 ± 3.9 years). Fifteen of the dogs were male and sixteen were female; nine breeds were represented. All dogs were determined to be healthy on the basis of physical examinations and histories. In 24 dogs, urine was collected during a visit to the hospital, whereas urine was collected by the owners at home from the remaining 7 dogs.

**Dogs with Hyperadrenocorticism**

Twenty-five dogs had hyperadrenocorticism. These dogs ranged in age from 6 to 17 years (mean ± SD = 10.5 ± 2.7 years). Fifteen were female and ten were male; eleven breeds were represented. The clinical diagnosis of hyperadrenocorticism was made on the basis of clinical signs (e.g., polyuria, polydipsia, alopecia, pendulous abdomen, and weakness) and results of complete blood count, serum biochemical analysis, and urinalysis (e.g., leukocytosis, lymphopenia, eosinopenia, high alkaline phosphatase activity, and dilute urine specific gravity). The diagnosis of hyperadrenocorticism was confirmed by one or both of the following: 1) the finding of a mass in one adrenal gland or the pituitary gland by computed tomography (CAT scanning); or 2) a good to excellent response to treatment for hyperadrenocorticism. Of the 25 dogs, 21 (84%) had pituitary-dependent hyperadrenocorticism, whereas four had a unilateral adrenal gland tumor. In all dogs, an ACTH stimulation test and/or low-dose dexamethasone suppression test (0.015 mg/kg) was also performed as previously described, but the results of these tests were not used to diagnosis hyperadrenocorticism in this study.

**Dogs Suspected of Having Hyperadrenocorticism**

Twenty-one dogs were originally suspected of having hyperadrenocorticism on the basis of history and clinical signs. We excluded hyperadrenocorticism when an alternative diagnosis was found to explain the clinical signs, and the signs resolved either spontaneously or with treatment appropriate for the diagnosis. The dogs in this group ranged in age from 4 to 15 years (mean ± SD = 10.8 ± 3.3 years). Eleven were female and ten were male; twelve breeds were represented. The diseases represented included renal insufficiency (n = 6), mild liver disease (n = 4), urinary tract infection with pyelonephritis (n = 4), hypothyroidism (n = 3), chronic bronchitis (n = 2), and diabetes insipidus (n = 1). The diagnosis of renal insufficiency in the six dogs was based on abnormal creatinine clearance values despite normal serum concentrations of creatinine and BUN. Of the four dogs with liver disease, two had mild non-suppurative cholangiohepatitis, one had early cirrhosis, and one had a portal systemic shunt. In all of these 21 dogs, the severity of disease was mild, as evidenced by normal appetite and activity.

**Dogs with Nonadrenal Disease**

Twenty-eight dogs were hospitalized with a variety of moderate to severe, nonadrenal diseases. None were suspected of having hyperadrenocorticism. They ranged in age from 2 to 14 years (mean ± SD = 8.6 ± 3.8 years). Fifteen of the dogs were male and thirteen were female; seventeen breeds were represented. The disorders included gastrointestinal disease (n = 5), renal disease (n = 5), lower urinary tract disease (n = 3), liver disease (n = 3), neurologic disease (n = 3), immune-mediated disease (n = 3), cardiac disease with congestive heart failure (n = 2), trauma (i.e., hit by car; n = 1), respiratory disease (i.e., bacterial pneumonia; n = 1), infectious disease (i.e., Lyme disease with fever and polyarthropathy; n = 1), and disease with no diagnosis (n = 1). Of the five dogs with gastrointestinal disease, two had hemorrhagic gastroenteritis, and the other three had infiltrative bowel disease with hypoproteinemia, primary lymphangiectasia with hypoproteinemia, and parvo virus, respectively. Of the five dogs with renal disease and moderate to severe azotemia, three had glomerulonephritis, one had renal neoplasia, and one had renal dysplasia. Of the three dogs with lower urinary tract disease, two had cystic and urethral calculi causing urinary obstruction and one had prostatitis. Of the three dogs with liver disease, two had hepatic neoplasia and one had endstage cirrhosis. Of the three dogs with neurologic disease, one had seizures secondary to brain tumor, one had acute vestibulitis, and one had status epilepticus. Of the three dogs with im-
mune-mediated disease, two had thrombocytopenia with hemorrhage and one had hemolytic anemia.

Urine Creatinine and Cortisol Assays

Morning urine samples were collected by cystocentesis, catheterization, or free catch and frozen at −70°C until the time of assay. Creatinine concentrations were determined by spectrophotometry (Jaffé reaction) on an autoanalyzer.* Immunoreactive (IR) cortisol concentrations were measured with a commercial RIA cortisol kit.† This assay was validated for use in dog urine by the following procedure. We assayed serial dilutions of four urine pools with initial IR-cortisol concentrations of approximately 750 nmol/L to 1300 nmol/L; the results yielded inhibition curves with slopes that paralleled the standard curve. Accuracy was determined by adding various quantities of cortisol§ to a canine urine pool containing a low concentration of IR-cortisol. Linear regression analysis of the resulting data (x = amount of cortisol added; y = amount of cortisol measured) resulted in the equation 1.11x + 24.3, with a correlation coefficient of 0.99. The sensitivity of the cortisol assay was 25 nmol/L; the intra- and interassay coefficients of variation were 10% (n = 6), and 9% (n = 6), respectively.

Comparison of Two Other Cortisol RIA Kits for Determination of IR-cortisol in Urine

In 69 and 33 dogs, respectively, enough urine was available to measure IR-cortisol concentrations by two other commonly used RIA cortisol kits.¶|| Results were compared with values determined by the first assay.† The purpose of this was to determine if similar results would be obtained with other commercially available assays.

Statistical Analysis and Calculations

All results are given as the mean ± SD. The structure of the data represented by box plots is defined as follows: the “box” in the box plot extends from approximately the 25th percentile to the 75th percentile, which represents the middle one-half of the data. The horizontal bar through the box is the median. The “whiskers” represent the main body of data, which in most cases is equal to the range. Outliers are represented by open circles.⁹

Urine IR-cortisol concentrations in the four groups of dogs were compared by analysis of variance and the Student's t-test with the Bonferroni adjustment.¹⁰ Correla-

ions between urine IR-cortisol concentrations determined by the three RIA kits were calculated by the Pearson Product-Moment Correlation and linear regression analysis.¹¹ For all statistical analyses, a P value of ≤0.05 was considered significant.

The sensitivity, specificity, diagnostic efficiency, and predictive value of the urine cortisol:creatinine ratio were calculated by the following equations.¹²

\[
\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}
\]

\[
\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}
\]

\[
\text{Efficiency} = \frac{\text{True Positives} + \text{True Negatives}}{\text{Total Number}}
\]

Positive Predictive Value

\[
= \frac{\text{True Positives}}{\text{True Positives} + \text{False Positives}}
\]

Negative Predictive Value

\[
= \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Negatives}}
\]

The sensitivity of a test is the ability of the test to identify individuals with a given disorder. Sensitivity is measured in a population of subjects known to have the disease. The optimal test has a sensitivity of 1.0 and a false negative value of zero. Specificity, on the other hand, is the ability of the test to correctly identify individuals that do not have a given disorder. Specificity is measured in a population of subjects known to be free of the disease. The optimal test has a specificity of 1.0 and a false positive value of zero. The efficiency of a test is the percentage of correct results regardless of whether they are positive or negative. The predictive value of a test is measured in a population that contains individuals with and without the disease. It is a measure of how accurately a positive or negative result will predict the presence (positive predictive value) or absence (negative predictive value), respectively, of the disease. It is dependent on the sensitivity and specificity of the test as well as the prevalence of the disease in the population.¹²

Results

The urine concentrations of IR-cortisol and creatinine and the cortisol:creatinine ratios in 31 normal dogs, 25 dogs with hyperadrenocorticism, 21 dogs in which hyperadrenocorticism was initially suspected and was determined to not be present, and 28 dogs with nonadrenal disease are given in Table 1. The mean urine IR-cortisol concentrations in the dogs with hyperadrenocorticism and the dogs with nonadrenal disease were significantly (P < 0.01) higher than normal. There was a great deal of
Table 1. Results of Urine Concentrations of IR-cortisol and Creatinine and Calculated Cortisol:Creatinine Ratios in 105 Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>IR-Cortisol (nmol/L)</th>
<th>Creatinine (nmol/L)</th>
<th>Cortisol:Creatinine Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 31)</td>
<td>175.5 ± 142.3 (3–565)</td>
<td>13.6 ± 6.9 (1.5–30)</td>
<td>13.1 ± 7.0 (0.1–31.2)</td>
</tr>
<tr>
<td>Hyperadrenocorticism (n = 25)</td>
<td>526.4 ± 493.6* (85–2252)</td>
<td>6.7 ± 5.0* (0.9–20.3)</td>
<td>103.1 ± 100.7* (21.3–432)</td>
</tr>
<tr>
<td>Suspect hyperadrenocorticism (n = 21)</td>
<td>127.0 ± 111.5 (9–475)</td>
<td>7.2 ± 4.7* (1.3–18.2)</td>
<td>16.3 ± 7.0 (6.9–36.1)</td>
</tr>
<tr>
<td>Nonadrenal disease (n = 28)</td>
<td>532.5 ± 600.7* (4–2965)</td>
<td>7.1 ± 4.6* (1.1–22.6)</td>
<td>82.8 ± 97.7* (2.7–524)</td>
</tr>
</tbody>
</table>

* P < 0.001 as compared with normal dogs.

The overlap in IR-cortisol concentrations between groups (Table 1); about half of the dogs with hyperadrenocorticism had IR-cortisol values in the normal range. The mean urine creatinine concentrations in the dogs with hyperadrenocorticism, dogs in which hyperadrenocorticism was initially suspected, and dogs with nonadrenal disease were significantly (P < 0.01) lower than normal.

The mean urine cortisol:creatinine concentration ratio in the dogs with hyperadrenocorticism was significantly (P < 0.001) higher than that in the normal dogs (Table 1; Fig. 1). The mean urinary cortisol:creatinine ratio in dogs originally suspected of having hyperadrenocorticism was significantly (P < 0.001) lower than the ratio in dogs with hyperadrenocorticism, but was not significantly different than that in the normal dogs (Table 1; Fig. 1). The mean urinary cortisol:creatinine ratio in the dogs with nonadrenal disease was significantly (P < 0.001) higher than that in both the normal dogs and dogs in which hyperadrenocorticism was initially suspected, but was not different than the ratio in the dogs with hyperadrenocorticism (Table 1; Fig. 1).

The sensitivity of the urine cortisol:creatinine ratio as a diagnostic test for hyperadrenocorticism was 0.92; the value of 30 was used as the upper limit of normal. In other words, the test was able to detect 92% of dogs with hyperadrenocorticism with only 8% false-negative results. The specificity of the urine cortisol:creatinine ratio varied according to the group analyzed. The specificity was high in the normal dogs (0.97) and the dogs initially suspected of having hyperadrenocorticism (0.95), with ≤5% having false-positive results. However, the specificity was very low (0.21) in the dogs with moderate to severe nonadrenal disease, with 79% having false-positive results. Both positive and negative predictive values were high in the normal dogs (0.96 and 0.94) and dogs suspected of having hyperadrenocorticism (0.91 and 0.96) but were low in the dogs with nonadrenal illness (0.51 and 0.75, respectively).

Of the dogs with hyperadrenocorticism, results of low-dose dexamethasone suppression tests were diagnostic for hyperadrenocorticism in 21 of the 23 dogs in which the test was performed. Thus, the sensitivity of the test was 0.92. The two dogs that had normal results of low-dose dexamethasone suppression tests both had high urine cortisol:creatinine ratios. Of the dogs suspected of having hyperadrenocorticism, low-dose dexamethasone suppression tests were performed in 13 dogs, all of which had normal cortisol suppression as well as normal results for the urine cortisol:creatinine ratio. Therefore, as with the urine cortisol:creatinine ratio, the specificity of the low-dose dexamethasone suppression in the group of dogs suspected of having hyperadrenocorticism was very high (1.0).

Figure 2 shows the regression analysis of urine IR-cortisol concentrations measured by the three different assays. When results of measuring specimens by the ICN Biomedicals Inc. and Diagnostic Products Corporation as well as the one used in the present study were compared, the correlation was high (R² = 0.96). The correlation coefficient between the two assays was 0.95.

![Box plots of the urine cortisol:creatinine ratios found in normal dogs, dogs with hyperadrenocorticism (HAC), dogs in which hyperadrenocorticism was initially suspected but did not have the disease (suspect HAC), and dogs with a variety of severe, nonadrenal diseases. The number of dogs in each group is shown in parentheses. The "box" represents the interquartile range from the 25th to 75th percentile (represents the middle one-half of the data). The horizontal bar through the box is the median. The "whiskers" represent the main body of data, which in most cases is equal to the range. Outlying data points are represented by open circles (exact value is given for these cases).](image-url)
The urine cortisol:creatinine ratio is valuable in distinguishing dogs with hyperadrenocorticism from normal dogs and from dogs in which hyperadrenocorticism is suspected clinically but in which normal pituitary-adrenal gland function is present. As with other pituitary-adrenal function tests, however, the urine cortisol:creatinine ratio cannot be used to diagnose hyperadrenocorticism in dogs that have moderate to severe nonadrenal disease.

The most commonly used screening tests for hyperadrenocorticism in dogs are the low-dose dexamethasone suppression test and ACTH stimulation test. The low-dose dexamethasone suppression test is a useful test for hyperadrenocorticism, with a reported sensitivity of 0.85 to 0.94; however, performing the test requires collection of three to five blood samples over an 8-hour period.\textsuperscript{1,3,5,13} The ACTH stimulation test requires only two blood samples over a 1- to 2-hour period, but is slightly less sensitive than the low-dose dexamethasone suppression test (0.81 to 0.83).\textsuperscript{1,3,13}

The sensitivity of the urine cortisol:creatinine ratio determined in this study (0.92) was similar to that reported by other investigators (0.99–1.0).\textsuperscript{3,5,7} Advantages of the urine cortisol:creatinine ratio over the low-dose dexamethasone suppression and ACTH stimulation tests include lower cost and greater convenience. Only a single urine specimen is required, and no special precautions are needed for urine collection.

Because none of the commonly used screening tests for hyperadrenocorticism have a perfect sensitivity (i.e., 1.0), the disorder will be missed in some dogs if only one test is completed. Therefore, if one suspects hyperadrenocorticism on the basis of clinical signs in a dog with negative or borderline results of the low-dose dexamethasone suppression test, ACTH stimulation test, or urine cortisol:creatinine ratio, an alternate screening test should be done.

The specificity of the urine cortisol:creatinine ratio is extremely low in dogs with moderate to severe nonadrenal disease. In our dogs with nonadrenal disease, 79% had a false-positive urine cortisol:creatinine ratio. In this respect, the urine cortisol:creatinine ratio offers no advantage over the low-dose dexamethasone suppression and ACTH stimulation tests, both of which are also nonspecific.\textsuperscript{1-3} Thus, dogs hospitalized with moderate to severe nonadrenal disease are not appropriate patients for one of these screening tests, and ideally should not be tested for hyperadrenocorticism until their nonadrenal disease has resolved. At the very least, one must interpret the results of any of these screening tests in light of all other clinical findings.

The predictive value and efficiency of a test is believed to be a greater indication of the clinical value of the test than the sensitivity and specificity alone.\textsuperscript{11} In this study, both the positive and negative predictive values and efficiency of the urine cortisol:creatinine ratio were extremely high in the group of dogs with hyperadrenocorticism plus normal dogs, and the group of dogs with hy-
peradrenocorticism plus dogs initially suspected of having hyperadrenocorticism. However, as with the specificity, values for predictive value and efficiency were very low in the group of dogs with hyperadrenocorticism plus dogs with moderate to severe nonadrenal disease.

A high correlation between results was found when IR-cortisol values, determined by three different assay kits, were compared. On the basis of this, most commercially available cortisol kits can be used to measure IR-cortisol in urine. However, because the assay results were not always in agreement, normal values must be established for each individual laboratory.

As with other screening tests for hyperadrenocorticism, the urine cortisol:creatinine ratio can not differentiate dogs with pituitary-dependent hyperadrenocorticism from those with hyperadrenocorticism caused by an adrenal gland tumor. Further tests such as the high-dose dexamethasone suppression test, endogenous plasma ACTH determination, and diagnostic imaging (i.e., radiography, ultrasonography, and computed tomography) are helpful in making this distinction.1,3,8

References

The XVIII World Congress of the World Small Animal Veterinary Association (WSAVA) will be held in Berlin, Germany, October 6-9, 1993. There will be simultaneous translation in German, English, and French. For further information please contact:

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