

## Basal Serum Cortisol Concentration as a Screening Test for Hypoadrenocorticism in Dogs

C. Bovens, K. Tennant, J. Reeve, and K.F. Murphy

**Background:** Measurement of basal serum or plasma cortisol concentration is used as a screening test for hypoadrenocorticism in dogs, but is not well characterized.

**Objectives:** To evaluate the sensitivity and specificity of basal serum cortisol to detect hypoadrenocorticism in a population of dogs with a clinical suspicion of hypoadrenocorticism.

**Animals:** Four hundred and fifty dogs with nonadrenal gland illness and 14 dogs with naturally occurring hypoadrenocorticism were included.

**Methods:** Retrospective case-control study. The records of all dogs having had an ACTH stimulation test performed between January 2005 and September 2011 at the University of Bristol were reviewed. Dogs were included if the test was performed as a screening for hypoadrenocorticism. The sensitivity and specificity of basal serum cortisol concentration to detect dogs with hypoadrenocorticism were calculated using 2 cut-offs and compared to the gold standard ACTH stimulation test.

**Results:** Using a cut-off of  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L), the sensitivity and specificity of basal cortisol to detect hypoadrenocorticism were 100% and 63.3%, respectively, whereas for a cut-off of  $\leq 1$   $\mu\text{g/dL}$  ( $\leq 28$  nmol/L), the sensitivity and specificity were 85.7% and 91.8%, respectively.

**Conclusions and Clinical Importance:** Measurement of basal serum cortisol is useful as a screening test for hypoadrenocorticism in dogs using a cut-off of  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L), and the disease is unlikely with a basal serum cortisol  $> 2$   $\mu\text{g/dL}$  ( $> 55$  nmol/L). A basal serum cortisol  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L) cannot be used to diagnose hypoadrenocorticism, and an ACTH stimulation test should be performed in these cases.

**Key words:** ACTH; Addison's disease; Canine; Endocrine.

Naturally occurring hypoadrenocorticism is defined as deficient production of glucocorticoids by the adrenal glands, with or without deficient production of mineralocorticoids.<sup>1,2</sup> As dogs with hypoadrenocorticism can present with a range of nonspecific clinical signs and clinicopathologic abnormalities and as the condition can become life-threatening without appropriate treatment, testing for hypoadrenocorticism is frequently performed despite the condition being uncommon. A definitive diagnosis of hypoadrenocorticism requires demonstration of inadequate adrenal reserve with a subnormal or absent serum or plasma cortisol increase after administration of exogenous ACTH.<sup>1–3</sup> A post-ACTH serum or plasma cortisol  $< 2$   $\mu\text{g/dL}$  ( $< 55$  nmol/L) confirms the diagnosis of hypoadrenocorticism, whereas a post-ACTH serum or plasma cortisol  $> 5$   $\mu\text{g/dL}$  ( $> 138$  nmol/L) excludes it.<sup>2</sup>

The cost of synthetic ACTH increased dramatically in the United States from 2004 and availability has also been an issue.<sup>4,5</sup> This prompted research for alternative screening tests for hypoadrenocorticism in dogs. Measurement of basal serum or plasma cortisol was reported to be useful as a screening test in 1 previous study, which included 13 dogs with hypoadrenocorticism and 110 dogs with nonadrenal illness.<sup>6</sup> Basal cortisol had a sensitivity of 100% for the detection of hypoadrenocorticism using cut-off concentrations of either  $\leq 1$   $\mu\text{g/dL}$  ( $\leq 28$  nmol/L) or  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L); the specificity of basal cortisol  $\leq 1$   $\mu\text{g/dL}$  ( $\leq 28$  nmol/L) was 98.2% and of basal cortisol  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L) was 78.2%.<sup>6</sup> That study showed that the test had excellent sensitivity and thus was good at identifying dogs with hypoadrenocorticism. However, the specificity, particularly using the cut-off of  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L), meant that the test would incorrectly identify some dogs as having hypoadrenocorticism, and thus dogs with a basal cortisol below the cut-off would require further testing.<sup>6</sup> The study recommended that a basal cortisol above a cut-off of 2  $\mu\text{g/dL}$  (55 nmol/L) should be used to exclude a diagnosis of hypoadrenocorticism.<sup>6</sup> However, the number of dogs without the condition included in that study was relatively low. Therefore, a study including a higher number of animals without the disease would improve the evidence for this test to be used as a screening test for hypoadrenocorticism as the clinical use of a diagnostic test is influenced by the prevalence of the disease in the population tested.

The aim of this retrospective study was to evaluate the sensitivity and specificity of basal serum cortisol measurement to detect hypoadrenocorticism using the same cut-offs used in the previous study<sup>6</sup> in a population of dogs with a clinical suspicion of hypoadrenocorticism.

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This study was performed at the University of Bristol, UK.

The preliminary results were presented as an abstract at the British Small Animal Veterinary Association annual congress in Birmingham, UK, on April 13, 2012.

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Submitted August 5, 2013; Revised March 9, 2014; Accepted June 17, 2014.

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DOI: 10.1111/jvim.12415

## Materials and Methods

The medical records of all dogs having had an ACTH stimulation test performed between January 2005 and September 2011 at the University of Bristol were reviewed retrospectively by either of 2 authors (C.B. or J.S.). During the study period, at this institution, the ACTH stimulation test was used as the screening test for hypoadrenocorticism in dogs. Dogs were excluded from the study if:

- There was any suspicion that the ACTH stimulation test was performed as a test for hyperadrenocorticism based on presenting clinical signs, clinical records, or referral letters
- The ACTH stimulation test was performed to monitor treatment of hyperadrenocorticism
- Polyuria, polydipsia, or both were the only clinical signs
- The clinical records or referral letter were not available
- The dog had received treatment in the 4 weeks before the ACTH stimulation test with oral or parenteral administration of steroids (including glucocorticoids and nandrolone laurate), topical steroids (cutaneous, external ear, ocular, or inhalation), or ketoconazole
- The dog had been treated with trilostane, mitotane, or fludrocortisone at any stage before the ACTH stimulation test
- The post-ACTH serum cortisol was equivocal (between 2 µg/dL [55 nmol/L] and 5 µg/dL [138 nmol/L]).

Dogs were included in the hypoadrenocorticism group if the post-ACTH serum cortisol was  $\leq 2$  µg/dL ( $\leq 55$  nmol/L) and a clinical diagnosis of naturally occurring hypoadrenocorticism was made. Other dogs for which hypoadrenocorticism was excluded based on the ACTH test results (post-ACTH serum cortisol  $> 5$  µg/dL [ $> 138$  nmol/L]) were included in the nonadrenal illness group.

The protocol for the ACTH stimulation test involved administration of 1 dose of synthetic ACTH<sup>a</sup> of 250 µg/dog (dogs  $> 10$  kg in weight) or 125 µg/dog (dogs  $< 10$  kg in weight) IV. Blood samples for measurement of serum cortisol were collected before the injection of ACTH and 1 hour later. The serum cortisol was measured by solid phase, competitive chemiluminescent immunoassay<sup>b</sup> with an Immulite analyzer. The assay had been validated for measuring canine cortisol with good precision, linearity, and recovery.<sup>7,8</sup> The Immulite analyzer was operated and maintained by clinical biochemists and biochemistry technicians, and the accuracy of its methodologies was assessed by continuous bimonthly participation in an external quality assurance program.<sup>c</sup> All calibrations and quality-control sample evaluations specified by the manufacturer were performed before sample analysis, with low, medium, and high value controls being measured with each analytical run. The samples were run following standard methodologies according to the manufacturer's instructions.

The sensitivity and specificity of basal cortisol concentration to detect dogs with hypoadrenocorticism were calculated using 2 cut-offs:  $\leq 1$  µg/dL ( $\leq 28$  nmol/L) and  $\leq 2$  µg/dL ( $\leq 55$  nmol/L). The positive and negative predictive values were calculated using the prevalence in this study and also the 2 prevalence values used in the previous study.<sup>6</sup>

## Results

Five hundred and eighty-six dogs were identified from the database search as having had an ACTH stimulation test performed because of a clinical

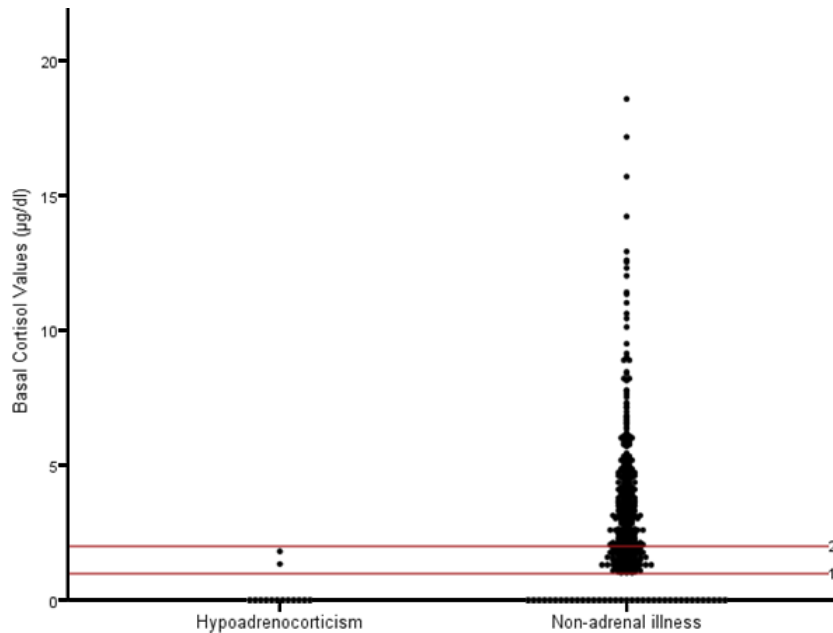
suspicion of hypoadrenocorticism. One hundred and fourteen dogs were excluded because of administration of systemic and topical steroids during the 4 weeks before the ACTH stimulation test, including 5 dogs diagnosed with hypoadrenocorticism and 109 dogs with nonadrenal illness. One dog diagnosed with hypoadrenocorticism was excluded because of prior treatment with mitotane. Seven dogs were excluded because of equivocal ACTH stimulation test results with a post-ACTH serum cortisol between 3.82 and 4.86 µg/dL (between 106 and 135 nmol/L). Four hundred and sixty-four dogs were included in the study, including 14 dogs with hypoadrenocorticism and 450 dogs with nonadrenal illness.

Five dogs in the hypoadrenocorticism group were presented with acute and severe clinical signs consistent with an Addisonian crisis, whereas the other 9 cases had chronic clinical signs. Two dogs with hypoadrenocorticism had concurrent disease: 1 dog was diagnosed with concurrent primary immune-mediated polyarthritis and the other dog presented with acute tetraparesis suspected to be because of a cerebrovascular event.

In the hypoadrenocorticism group, the basal serum cortisol was  $< 1$  µg/dL ( $< 28$  nmol/L) in 12 cases and between 1 and 2 µg/dL (between 28 and 55 nmol/L) in the other 2 cases (Table 1, Fig 1). The post-ACTH serum cortisol was  $< 2$  µg/dL ( $< 55$  nmol/L) in all 14 cases, confirming the diagnosis of hypoadrenocorticism. In the dogs with nonadrenal illness, basal serum cortisol varied between  $< 1$  and 18.58 µg/dL (between  $< 28$  and 516 nmol/L). Basal serum cortisol was  $\leq 1$  µg/dL ( $\leq 28$  nmol/L) in 37 dogs,  $\leq 2$  µg/dL ( $\leq 55$  nmol/L) in 165 dogs, and  $> 2$  µg/dL ( $> 55$  nmol/L) in the remaining cases (Fig 1). Based on these results, the sensitivity of a basal serum cortisol concentration  $\leq 1$  µg/dL ( $\leq 28$  nmol/L) for detecting hypoadrenocorticism was 85.7% with a specificity of 91.8%. Using a cut-off value of  $\leq 2$  µg/dL ( $\leq 55$  nmol/L), the sensitivity was 100% with a specificity of 63.3%. The prevalence rate of hypoadrenocorticism in this study was 3.02%; at this prevalence, and using a cut-off value of  $\leq 2$  µg/dL ( $\leq 55$  nmol/L), the positive predictive value was 7.82% and the negative predictive value was 100%. Using a prevalence of 0.5% and the same cut-off value, the positive predictive value was 1.3% and negative predictive value was 100%. Using a prevalence of 15% and the same cut-off value, the positive predictive

**Table 1.** ACTH stimulation test results at initial presentation for all dogs diagnosed with hypoadrenocorticism.

Number of Dogs with Hypoadrenocorticism	Basal Cortisol µg/dL (nmol/L)	Cortisol after ACTH µg/dL (nmol/L)
12	$< 1$ ( $< 28$ )	$< 1$ ( $< 28$ )
1	1.33 (37)	1.84 (51)
1	1.80 (50)	1.87 (52)
Reference interval	1.80–9.0 (50–250)	5.40–19.80 (150–550)



**Fig 1.** Basal serum cortisol concentrations in 14 dogs with hypoadrenocorticism and 450 dogs with nonadrenal gland illness. Samples with no detectable cortisol ( $<1.0$   $\mu\text{g/dL}$ ) were plotted with a value of 0.

value was 32% and negative predictive value was 100%.

### Discussion

Results of this study indicate that a basal serum cortisol concentration  $>2$   $\mu\text{g/dL}$  ( $>55$   $\text{nmol/L}$ ) is useful in excluding hypoadrenocorticism in dogs, as the sensitivity of this test using this cut-off value was 100%; this finding was in agreement with the previous study.<sup>6</sup> The negative predictive value for all the prevalence rates assessed in the present study remained 100%, confirming the utility of this test for ruling out hypoadrenocorticism. Analysis of our data also revealed that a basal serum cortisol concentration cut-off of  $>1$   $\mu\text{g/dL}$  ( $>28$   $\text{nmol/L}$ ) should not be used to exclude hypoadrenocorticism, as when using this cut-off value, the sensitivity was only 85.7%; this finding was in contrast to the findings reported by Lennon et al,<sup>6</sup> where the use of this cut-off value in a similarly sized group of dogs with hypoadrenocorticism resulted in a sensitivity of 100%. There are potentially serious consequences of missing a diagnosis of hypoadrenocorticism and the present study therefore emphasizes the importance of using the higher cut-off of  $>2$   $\mu\text{g/dL}$  ( $>55$   $\text{nmol/L}$ ) to exclude the disease.

The present study showed that using a basal serum cortisol concentration cut-off of  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$   $\text{nmol/L}$ ) to detect hypoadrenocorticism resulted in a specificity of 63.3%, which was lower than the specificity reported in a previous study<sup>6</sup> (78.8%) using the same cut-off value. The difference between the 2 specificities may be explained by the higher number of dogs with nonadrenal disease included in the present study (450 dogs versus 110 dogs).

In the previous study,<sup>6</sup> the positive and negative predictive values were 2.3% and 100% respectively for a disease prevalence of 0.5%, and 45% and 100% respectively for a prevalence of 15%. These results were similar to those in the present study (positive predictive and negative predictive values of 1.3% and 100% respectively for a prevalence of 0.5%, and 32% and 100% respectively for a prevalence of 15%). These results confirm that the positive predictive value of basal cortisol is low at the low prevalence rates seen in both studies. Predictive values of positive and negative test results are, in essence, the clinical applications of sensitivity and specificity, and are influenced by the true prevalence rates of the disease in the population tested. The positive predictive value of a test is influenced by the specificity of the test, whereas the negative predictive value is influenced by the sensitivity. A test with a high sensitivity and high negative predictive value is useful as a screening test to exclude a diagnosis. Our study confirms the utility of basal serum cortisol measurement as a screening test to exclude hypoadrenocorticism. However, because the test has a low specificity and a low positive predictive value, it is not suitable to diagnose the disease and thus an ACTH stimulation test must be performed to obtain a definitive diagnosis for the condition. In the present study, if basal serum cortisol measurement had been used as the screening test for hypoadrenocorticism, an ACTH stimulation test would have been required to exclude the disease in 165 (36.6%) dogs with nonadrenal disease. This was a higher percentage of dogs compared to the previous study,<sup>6</sup> where 21.8% of dogs with nonadrenal disease would have required further testing. Performing a basal serum cortisol measurement followed by an ACTH stimulation test represents

an increased cost to the client. Further evaluation of the cost/benefit ratio of using basal serum cortisol measurement as a screening test for hypoadrenocorticism may be warranted in populations with a low prevalence of disease.

The poor specificity of basal serum cortisol to detect hypoadrenocorticism is most likely because of the normal episodic secretion of cortisol in dogs. In this species, cortisol concentrations can become intermittently low or undetectable over a 24-hour period.<sup>9,10</sup> Dogs homozygous for the ABCB1 genetic mutation (previously called MDR1) also have a lower basal cortisol than dogs that do not carry the mutation.<sup>11</sup> A lower basal cortisol can also occur with critical illness, inflammation, or damage to the adrenal glands such as hemorrhage or infarction secondary to the main disease process.<sup>12</sup> However, cases with critical illness were rare in the dogs with nonadrenal illness in this study, including the dogs with a basal cortisol  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L). All the cases in the nonadrenal illness group with a low basal cortisol also had an appropriate response to exogenous ACTH administration, making significant damage to the adrenal glands as a cause for the low basal cortisol very unlikely.

Suppression of the endogenous production of cortisol can last for several weeks after administration of systemic or topical glucocorticoids, which is why dogs having received such treatments before the ACTH stimulation test were excluded from this study.<sup>13–16</sup> Measurement of basal cortisol should be performed and ideally the results obtained before administration of glucocorticoids as if the basal cortisol is  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L), an ACTH stimulation test will be required. In cases suspected to be suffering from an acute hypoadrenocorticism crisis, or when administration of steroids cannot be delayed, an ACTH stimulation test should be performed rather than a basal cortisol measurement, so that administration of glucocorticoids, mineralocorticoids, or both can be started immediately after collection of the blood samples. If it is clinically necessary to administer corticosteroids before performing the ACTH stimulation test, dexamethasone should be used as it is not detected by cortisol assays and a single administration of dexamethasone only causes a minor decrease in post-ACTH cortisol concentration.<sup>17</sup>

Our study had several limitations. It was a retrospective study and therefore it is possible that some dogs were not identified correctly by the database search and were thus not included. In addition, we cannot completely exclude that incomplete recording of information could have resulted in the inclusion of dogs that should have been excluded from the study, such as dogs having received treatment with corticosteroids before admission. The authors have minimized the likelihood of these occurrences by utilizing the database in the referral hospital as well as the laboratory database and by reviewing not only the summary referral letter but also the complete patient record including the history before referral. Another limitation is that this population of referred dogs may not be representative of cases tested for hypoadrenocorti-

cism in first opinion practice. The number of dogs with hypoadrenocorticism and the nature of this study were similar to those in the study by Lennon et al,<sup>6</sup> which is relatively low, whereas the higher number of dogs with nonadrenal illness included in our study allowed further evaluation of the specificity of basal cortisol measurement. Further studies with a larger number of dogs with hypoadrenocorticism would allow more precise evaluation of the sensitivity and negative predictive value of basal cortisol measurement.

In conclusion, measurement of basal serum cortisol is useful as a screening test for hypoadrenocorticism in dogs using a cut-off of  $>2$   $\mu\text{g/dL}$  ( $>55$  nmol/L), and the disease is unlikely to be present if the basal serum cortisol is  $>2$   $\mu\text{g/dL}$  ( $>55$  nmol/L). A basal serum cortisol  $\leq 2$   $\mu\text{g/dL}$  ( $\leq 55$  nmol/L) cannot be used to diagnose hypoadrenocorticism and an ACTH stimulation test should always be performed in these cases.

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## Footnotes

<sup>a</sup> Tetracosactide, Synacthen®; Alliance Pharmaceuticals, Chippenham, Wiltshire, United Kingdom

<sup>b</sup> Immulite/Immulite 1000 Cortisol; Siemens, Camberley, Surrey, United Kingdom

<sup>c</sup> RIQAS; Randox, Crumlin, Co. Antrim, United Kingdom

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## Acknowledgments

The authors thank Dr E.N. Barker and Dr K. Pappasoulotis for their help with this study.

The study was not supported by a grant. Catherine Bovens' position was sponsored by MSD Animal Health while the study was performed.

*Conflict of Interest Disclosure:* Catherine Bovens' small animal residency was sponsored by MSD Animal Health, which produces the dexamethasone-containing drugs Dexadron and Dexafort, which can be used in the treatment of hypoadrenocorticism in dogs.

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