Diagnostic efficacy of the leukogram and the chemiluminometric ACTH measurement to diagnose canine hypoadrenocorticism

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Key words
White blood cell count, neutrophil to lymphocyte ratio, sodium to potassium ratio, adrenocorticotropic hormone, Addison's disease, dog

Summary
Background and objective: The gold standard in the diagnosis of canine hypoadrenocorticism (HA) is the adrenocorticotropic hormone (ACTH) response test. As synthetic ACTH (tetracosactide [Synacthen®]) is currently not available in the European Union, the evaluation of other diagnostic tests seemed warranted. The diagnostic efficacy of electrolytes, the leukogram and endogenous ACTH concentrations to diagnose HA was investigated. Material and methods: The medical records of 145 dogs with clinical signs suspect for spontaneous HA were included in a retrospective study. HA was diagnosed (n = 38) or ruled out (n = 84) by using an ACTH response test. In 23 patients HA was excluded by basal cortisol measurement. The diagnostic performance of various variables was assessed based on receiver operating characteristic (ROC) curves and by calculating differential positive rates. A decision tree (IBM SPSS Decision Trees 20, IBM Corporation) was constructed with the variables neutrophil to lymphocyte ratio (N/LR) and sodium to potassium ratio (Na/KR) to illustrate the diagnostic efficacy of the respective test results. Results: The best single variables to diagnose HA were the endogenous ACTH concentration (area under the ROC curve [ROC AUC] 0.97; cutoff ≥ 50 pmol/l; sensitivity 96%, specificity 100%) and the Na/KR (ROC AUC 0.905; cutoff ≤ 22; sensitivity 92%, specificity 91%). The diagnostic performance of various variables of the leukogram was poor to moderate (ROC AUC ≤ 0.90). 68% of dogs with HA had a Na/KR ≤ 22 and a N/LR ≤ 2.3, a combination not observed in dogs with non-adrenal diseases. Conclusion and clinical relevance: As secondary HA is very rare, endogenous ACTH measurement is a very good alternative to the traditional ACTH response test. Data also suggest that the combination of a Na/KR ≤ 22 and a N/LR ≤ 2.3 is highly specific and can be used to rule in HA.

Schlüsselwörter
Weiβes Blutbild, Neutrophilen-Lymphozyten-Quotient, Natrium-Kalium-Quotient, adrenokortikotropes Hormon, Morbus Addison, Hund

Zusammenfassung
Introduction

Naturally occurring primary canine hypoadrenocorticism (HA) is a clinical syndrome caused by atrophy or destruction of the adrenal cortex resulting in glucocorticoid- and/or mineralocorticoid deficiency (14, 21). Isolated forms of either glucocorticoid deficiency, also called "atypical HA" (5, 28), or mineralocorticoid deficiency (19) have been described. Secondary HA is caused by a lack of adrenocorticotropic hormone (ACTH) production and is considered extremely rare (5, 20, 35). HA has been reported in dogs of all ages. Females are predisposed and the disease has a higher prevalence in breeds such as Standard Poodle, Bearded Collie, Portuguese Water Dog and Nova Scotia Duck Tolling Retriever (5, 25).

As clinical signs are non-specific, the disorder has also been called the "great pretender" (26). Commonly dogs with HA are presented with life threatening adrenal crisis requiring rapid initiation of therapy. Substitution therapy is usually started immediately based on the clinician’s index of suspicion. The most consistent biochemical abnormalities include azotemia, hyponatremia, hyperkalemia, low sodium to potassium ratio (Na/KR ≤ 27:1) and hypochloremia (1, 25). Their diagnostic utility is hampered by the fact that these changes can also occur in dogs with urogenital, cardiorespiratory and gastrointestinal diseases such as Trichuris vulpis infestation (22) and are usually absent in dogs with isolated glucocorticoid deficiency and secondary HA. Aside from electrolyte abnormalities, the absence of a stress leukogram including neutrophilia, lymphopenia and/or eosinopenia in a sick animal has long been postulated as suggestive of HA irrespective of cause (5, 10, 13). Only recently it was shown, that the combination of the NA/KR and the lymphocyte count provides a superior screening test for HA than these tests alone (33).

The gold standard to finally confirm HA was to date the ACTH stimulation test, where a synthetic ACTH-like compound namely tetracosactide (Synacthen*) is applied and the adrenal response is assessed by serum cortisol measurements. Unfortunately, Synacthen* has been removed from the market in the European Union. Suggested alternatives include basal cortisol measurement which can be used to "rule out" HA (17), the cortisol to ACTH ratio and the aldosterone to renin activity ratio (14). The latter two tests have yet to be tested in non-adrenal sick dogs with initially suspected, but later refuted HA.

The primary objective of this retrospective study was to investigate the diagnostic performance of selected electrolytes, the leukogram and endogenous ACTH concentrations to diagnose HA.

Material and methods

Study design

In a retrospective study all records of dogs with suspected HA, presented between 2004 and 2010 at the local Clinic for Companion Animals were evaluated. Suspicion was based upon patient history as well as clinical and routine laboratory findings (5, 10). Search criteria used to identify these dogs in the computer based medical records were: HA listed as differential diagnosis during the diagnostic workup, the ACTH response test or basal cortisol measurement. Dogs which had been previously treated with glucocorticoids, mineralocorticoids or gestagens and dogs with suspected or treated hyperadrenocorticism were excluded.

The diagnosis of HA was considered confirmed in dogs with a serum cortisol concentration ≤ 55.2 nmol/l (≤ 2 µg/dl) one hour after the intravenous application of synthetic ACTH (tetracosactide; 250 µg in dogs > 5 kg and 125 µg in dogs ≤ 5 kg; Synacthen* 250 µg/ml, Defiante Farmaceutica S.A., Funchal, Portugal) and ruled out in dogs with a basal cortisol concentration > 55.2 nmol/l (> 2 µg/dl) (17) and/or a post-stimulation cortisol concentration > 138 nmol/l (5 µg/dl) (10) (33).

Endogenous ACTH (ACTH) was measured to differentiate between primary and secondary HA (normal electrolytes, low ACTH). ACTH measurement was performed prior to the ACTH stimulation test. Cortisol and ACTH were measured using a solid phase, competitive chemiluminescent enzyme immunoassay (Immulite 1000 ACTH, Siemens Medical Solutions Diagnostics, Vienna, Austria) validated for the use in dogs (27, 32). For ACTH determination plasma was collected in pre-cooled EDTA tubes (Vacuette, Greiner bio-one, Kremsmünster, Austria), immediately chilled to 4 °C and centrifuged at 5000 x g for 10 minutes. Plasma was separated and analyzed immediately or stored frozen at −20 °C until analysis.

Table 1  Demographic data, percentage of various clinical signs, as well as mean heart rate, and rectal temperature of dogs with (HA) and without hypoadrenocorticism (Sick non-HA).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypoadrenocorticism</th>
<th>Sick non-HA</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>38</td>
<td>107</td>
<td>0.303</td>
</tr>
<tr>
<td>Age (months)</td>
<td>72 ± 35</td>
<td>80 ± 51</td>
<td>0.084</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>20 ± 12</td>
<td>20 ± 15</td>
<td>0.009</td>
</tr>
<tr>
<td>Male</td>
<td>12 (32%)</td>
<td>60 (56%)</td>
<td>0.009</td>
</tr>
<tr>
<td>Female</td>
<td>26 (68%)</td>
<td>47 (44%)</td>
<td>0.009</td>
</tr>
<tr>
<td>Neutered</td>
<td>25 (66%)</td>
<td>34 (32%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Poor appetite/anorexia</td>
<td>91%</td>
<td>47%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lethargy/weakness</td>
<td>89%</td>
<td>54%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vomiting/regurgitation</td>
<td>80%</td>
<td>68%</td>
<td>0.017</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>37%</td>
<td>59%</td>
<td>0.028</td>
</tr>
<tr>
<td>Hypodipsia/adipsia</td>
<td>36%</td>
<td>12%</td>
<td>0.001</td>
</tr>
<tr>
<td>Polydipsia/polyuria</td>
<td>14%</td>
<td>26%</td>
<td>0.142</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>110 ± 38</td>
<td>119 ± 26</td>
<td>0.192</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>37.9 ± 1</td>
<td>38.4 ± 1</td>
<td>0.006</td>
</tr>
</tbody>
</table>
White cell blood count was obtained within 4 hours past sampling using an automated hematology analyzer (Advia 120®, Siemens Medical Solutions, Vienna, Austria) and a microscopic evaluation was performed, when either cell concentrations were more than 25% outside the reference interval and/or the respective scattergrams showed irregularities. Blood samples were stored at 4 °C and electrolyte concentrations were measured with ion selective electrodes integrated in the Cobas 6000/c501 analyzer (Roche Diagnostics, Vienna, Austria).

### Statistical analysis

Statistical analysis was performed with the statistical software package SPPS for Windows (IBM SPSS Statistics 20, IBM Corporation, Chicago, USA) and the statistics add in software for Micro-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference range</th>
<th>Dogs with HA1</th>
<th>Controls</th>
<th>P (Z)</th>
<th>AUC (95%CI)</th>
<th>Accuracy</th>
<th>Best cutoff</th>
<th>Sens/Spec</th>
<th>DPR</th>
<th>+LR</th>
<th>–LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>37–55</td>
<td>49 ± 11</td>
<td>43 ± 13</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6–7.5</td>
<td>6.8 ± 1.2</td>
<td>6.2 ± 1.3</td>
<td>0.020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.58–4.73</td>
<td>3.3 (1.8–4.4)</td>
<td>3.3 (0.8–4.7)</td>
<td>0.664 (−0.435)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>55–90</td>
<td>85 (18–296)</td>
<td>96 (26–1239)</td>
<td>0.240 (−2.25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionized calcium (mmol/l)</td>
<td>1.12–1.42</td>
<td>1.28 (1.11–1.94)</td>
<td>1.27 (0.51–1.84)</td>
<td>0.368 (−0.899)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>140–152</td>
<td>128 (107–143)</td>
<td>145 (100–155)</td>
<td>&lt; 0.001 (−7.298)</td>
<td>0.903 (0.853–0.953)</td>
<td>high</td>
<td>≤ 140</td>
<td>97/76</td>
<td>0.73</td>
<td>4.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>3.6–5.6</td>
<td>7 (3.1–10.8)</td>
<td>4.2 (2.3–8.7)</td>
<td>&lt; 0.001 (−7.339)</td>
<td>0.902 (0.834–0.971)</td>
<td>high</td>
<td>≥ 5.7</td>
<td>92/87</td>
<td>0.79</td>
<td>7.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Sodium/potassium ratio</td>
<td>&gt;27:1</td>
<td>18 (13–45)</td>
<td>34 (14–59)</td>
<td>&lt; 0.001 (−7.318)</td>
<td>0.905 (0.836–0.973)</td>
<td>high</td>
<td>≤ 22</td>
<td>92/91</td>
<td>0.84</td>
<td>10.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Eosinophils (cells/µl)</td>
<td>&lt; 800</td>
<td>654 (39–2648)</td>
<td>216 (0.1–3538)</td>
<td>&lt; 0.001 (−4.699)</td>
<td>0.800 (0.704–0.896)</td>
<td>moderate</td>
<td>≥ 398</td>
<td>81/72</td>
<td>0.53</td>
<td>2.89</td>
<td>0.26</td>
</tr>
<tr>
<td>Relative eosinophils (%)</td>
<td>&lt; 4%</td>
<td>5.7 (0.3–15)</td>
<td>1.6 (0.1–9)</td>
<td>&lt; 0.001 (−4.687)</td>
<td>0.804 (0.706–0.902)</td>
<td>moderate</td>
<td>≥ 5.5</td>
<td>60/90</td>
<td>0.51</td>
<td>5.94</td>
<td>0.45</td>
</tr>
<tr>
<td>Neutrophil/eosinophil ratio</td>
<td>n.a.</td>
<td>9.5 (3.6–264.7)</td>
<td>46.6 (2.53–118000)</td>
<td>&lt; 0.001 (−5.051)</td>
<td>0.828 (0.733–0.922)</td>
<td>moderate</td>
<td>≤ 12.5</td>
<td>68/87</td>
<td>0.56</td>
<td>5.23</td>
<td>0.37</td>
</tr>
<tr>
<td>Lymphocytes (cells/µl)</td>
<td>780–4500</td>
<td>3344 (786–7749)</td>
<td>2056 (687–9400)</td>
<td>&lt; 0.001 (−4.050)</td>
<td>0.732 (0.636–0.827)</td>
<td>moderate</td>
<td>≥ 2456</td>
<td>79/61</td>
<td>0.40</td>
<td>2.03</td>
<td>0.34</td>
</tr>
<tr>
<td>Relative lymphocytes (%)</td>
<td>&lt; 30%</td>
<td>28.4 (12–57.7)</td>
<td>18.09 (2.1–53.01)</td>
<td>&lt; 0.001 (−4.764)</td>
<td>0.775 (0.687–0.864)</td>
<td>moderate</td>
<td>≥ 23</td>
<td>76/71</td>
<td>0.47</td>
<td>2.62</td>
<td>0.34</td>
</tr>
<tr>
<td>Neutrophil/lymphocyte ratio</td>
<td>n.a.</td>
<td>2.1 (0.5–6.1)</td>
<td>4.1 (0.8–45.4)</td>
<td>&lt; 0.001 (−4.914)</td>
<td>0.784 (0.669–0.869)</td>
<td>moderate</td>
<td>≤ 2.3</td>
<td>67/82</td>
<td>0.48</td>
<td>3.68</td>
<td>0.41</td>
</tr>
<tr>
<td>Neutrophils (cells/µl)</td>
<td>3300–11250</td>
<td>6958 (3408–23097)</td>
<td>9229 (771–71048)</td>
<td>0.025 (−2.236)</td>
<td>0.629 (0.533–0.725)</td>
<td>low</td>
<td>≤ 10375</td>
<td>88/45</td>
<td>0.33</td>
<td>1.6</td>
<td>0.27</td>
</tr>
</tbody>
</table>
| Cortisol basal (nmol/l)         | 27.6–110.4      | 24.8 (24.8–33.7) | 99.4 (24.8–1382.8) | < 0.001 (−6.702) | n.c. | n.c. (< 55.2)
| ACTH (pmol/l)                   | n.a.            | 244 (2.2–275)  | 3.1 (2.2–27.3) | < 0.001 (−6.582) | 0.970 (0–1) | high | ≥ 50  | 96/100 | 0.96 | 0.04 |

AUC = area under the curve, CI = confidence intervals, DPR = differential positive rate, HA = hypoadrenocorticism, +LR = positive likelihood ratio, –LR = negative likelihood ratio, n.a. = not available, n.c. = not calculated, Sens. = sensitivity, Spec. = specificity.

1 Primary and secondary HA; 2 As suggested by Javadi et al. (18)
soft Excel Analyse-it (analyse-it®, version 2.03, Leeds, UK). Continuous variables were assessed for normal distribution using the Shapiro-Wilk test. Statistical analysis included the t-test or Welch t-test for normally distributed data and the Kruskal-Wallis and Mann-Whitney U-test (unpaired data) for non-normally distributed data. Categorical variables were described with percentages and compared with the Pearson's Chi-squared test. A value of p < 0.05 was considered significant.

The cutoff for various variables to discriminate between dogs with and without HA was determined by two different methods, namely the differential positive rates (cutoff with the highest sensitivity and specificity) and the positive/negative likelihood ratios (+LR/–LR) based on receiver operating characteristic (ROC) curves. A +LR/–LR > 5/0.2 was considered to be of high diagnostic evidence. The diagnostic efficacy was additionally assessed by calculating the area under the ROC curve (AUC ROC plus 95% confidence interval [CI]). An AUC ROC of 0.5–0.7, 0.7–0.9 and 0.9–1.0, was considered a test with low (useless test), moderate and high diagnostic accuracy, respectively (34).

For statistical analysis ACTH concentrations below the detection limit of 2.2 pmol/l (10 pg/ml) and above 275 pmol/l (1250 pg/ml) are given as 2.18 pmol/l (9.9 pg/ml) and 275.2 pmol/l (1251 pg/ml), respectively. Cortisol concentrations below the detection limit of 27.6 nmol/l (1 µg/dl) are given as 24.8 nmol/l (0.9 µg/dl).

A decision tree (IBM SPSS Decision Trees 20, IBM Corporation, Chicago, USA) was constructed with the variables N/LR and Na/KR.

Results

Following record review 145 dogs met the inclusion criteria. Most of the patients had been presented in the emergency and primary care ambulance.

Primary and secondary HA were diagnosed in 36 (25%) and 1 (<1%) patients, respectively. In all but one patient with primary HA the Na/KR was < 27. In one dog with hyponatremia, hypokalemia and a Na/KR of 45, differentiation between primary and secondary HA was not possible, since ACTH had not been measured.

16 (42%) of the dogs with HA were mixed breeds, 2 (5%) were Jack Russell Terriers, 2 (5%) were Cocker Spaniels and 2 (5%) were German Shepherd Dogs. The remaining dogs comprised 16 other breeds, including one Standard Poodle (vs. one Poodle in the sick non-HA group) and one Bearded Collie (not represented in the sick non-HA group).

HA was excluded in 107 dogs (sick non-HA). This group consisted of 41 breeds. Golden Retrievers (n = 12 vs. 0 in dogs with HA), Labrador Retrievers (n = 8 vs. 1 in dogs with HA), Yorkshire Terriers (n = 5 vs. 1 in dogs with HA) and Cocker Spaniels (n = 4) were over-represented. In these dogs HA had primarily been suspected because of gastrointestinal problems (n = 69), azotemia (n = 15), hypoglycemia (n = 8), megaesophagus (n = 7), lethargy or weakness (n = 4), hypercalcemia (n = 3) and a low Na/KR (n = 1). An ACTH stimulation test was performed in all dogs with HA (100%) and in 84 (79%) sick non-HA dogs. In 23 dogs (21%) HA was excluded by measuring basal cortisol concentrations. ACTH was measured in 27 dogs with and 39 dogs without HA, respectively. There were 26 females (68%, female/male ratio: 2:2:1) in the HA group and 47 females (44%, female/male ratio: 1:1:3; see Table 1) in the sick non-HA group.

For clinical and epidemiologic characteristics, patient history, as well as mean heart rate, and rectal temperature of the study population see Table 1.

Hematocrit, total protein, sodium, potassium, Na/KR, eosinophils, lymphocytes, neutrophils, N/LR, neutrophil to eosinophil ratio (N/ER), basal cortisol and ACTH were all significantly different between dogs with and without HA. No differences were found in the variables glucose, albumin and ionized calcium (Table 2).

ACTH concentrations and calculation of the Na/KR, followed by sodium and potassium concentration were the best single-

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Laboratory tests to diagnose HA (▶ Fig. 1, ▶ Fig. 2, ▶ Table 2). Their diagnostic efficacy was further improved by their combination with various hematologic tests (for Na/KR plus N/LR see ▶ Fig. 3). Only dogs with HA had the combination of a low Na/KR (≤ 22) and a low N/LR (≤ 2.3). This combination identified 26 (68%) of 38 dogs with HA and had a specificity of 100%. The diagnostic performance of the leukogram alone was low to moderate (▶ Table 2).

In 13 (46%) of 28 patients with HA in which ACTH had been measured, the ACTH concentration was > 275.2 pmol/l (1251 pg/ml), the upper limit of detection of the assay. Basal cortisol concentration was measured in 32 patients with HA and was below the detection limit of the assay (27.59 nmol/l [1 µg/dl]) in all but one sample (33.7 nmol/l [1.22 µg/dl]).

Discussion

This study corroborates the results of Seth et al. (33) who showed that the absolute lymphocyte count and the N/LR are useful additional tests in the initial diagnostic workup of dogs with suspected HA. In contrast to the earlier study, the eosinophil concentration as well as the N/ER also proved useful to discriminate between sick dogs with and without HA. To the knowledge of the authors this is the second study in which various components of the leukogram were compared between dogs with untreated HA and dogs with initially suspected but later refuted HA. As a consequence this is the second study where the calculation of test specificities was possible.

The rationale behind the use of lymphocyte or eosinophil concentrations or their relation to neutrophils is that cortisol is an important regulator of peripheral leukocyte distribution and is released under various physical, emotional and chemical stressors. The typical “stress leukogram” as part of the stress response consists of one or more of the following: neutrophilia, monocytosis, lymphopenia and eosinopenia (12) and is mediated specifically by glucocorticoid signaling (8). Even minor stressors such as transportation to the veterinary practice increase cortisol secretion and effect leucocyte trafficking in dogs (4). It is generally accepted that cortisol deficiency “results in the inability to mount an adequate stress response” (18) and that the lack of indicators of cortisol induced changes in the leukogram should prompt the clinician to consider HA (15, 16). The results of this study are in line with this concept. Dogs with HA had significantly lower neutrophil, higher lymphocyte and higher eosinophil concentrations, and as a consequence lower N/L and the N/E ratios. The N/LR is of special interest as neutrophil and lymphocyte concentrations can be reliably measured in the veterinary office. It has also been shown in dogs that this ratio is a sensitive marker for the peripheral blood response to ACTH and accurate determination is possible by smear preparation (24).
Although the performance of the N/LR alone was only moderate (AUC 0.784), the diagnostic efficacy in combination with the per se already very good Na/KR (AUC 0.905) was excellent (▶Fig. 3). This is in line with the results of Seth et al. (33) who integrated the Na/KR and absolute lymphocyte count into a forwarded stepwise binary logistic regression model. The combination of both tests increased the AUC from 0.873 (Na/KR) and 0.847 (lymphocyte count) to 0.927 (Na/KR and lymphocyte count). In the present study two of three dogs with HA had a Na/KR ≤ 22 and a N/LR ≤ 2.3, a combination not seen in any control dog. Accordingly these tests can be used to rule in primary HA in dogs with appropriate clinical signs.

The good diagnostic performance of the Na/KR (alone or in combination with the N/LR) in the present study is possibly a consequence of the very rare occurrence of Trichuris vulpis infections in the local dog population. Nematode-associated so called “pseudo-HA” is an important differential diagnosis in dogs with clinicopathologic findings resembling HA (9, 30). Only 0.2% of fecal samples examined at the University of Veterinary Medicine Vienna are currently positive for Trichuris vulpis [Dr. Silbermayr, personal communication]. This fact might also explain the good performance of the eosinophil concentration in the present study, which was considered a “not useful discriminatory test” by other authors (33).

A remarkable finding in many sick non-HA dogs was the lack of neutrophilia and/or lymphocytopenia and/or eosinopenia, reducing the specificity of the white cell blood count to rule in HA. As these changes are part of the so called “stress leukogram”, it is tempting to speculate that some of these dogs suffered from glucocorticoid receptor resistance, causing a blunted reaction of the immune system. Several studies in human patients have shown that stressful life experiences and chronic stress can cause glucocorticoid receptor resistance and leucocyte desensitization (6, 7).

With an AUC of 0.97, chemiluminometric ACTH measurement was the best single test to diagnose HA in this study (▶Fig. 2). This test is currently used in dogs to differentiate between isolated glucocorticoid deficiency and secondary HA (5, 20) and between adrenal and pituitary hyperadrenocorticism (29, 36). ACTH concentrations were significantly higher in dogs with...
HA (p < 0.001) and the “cutoff” 50 pmol/l detected HA with 96% sensitivity and 100% specificity. The ACTH concentration was < 28 pmol/l in all controls and > 50 pmol/l in all but one dog with HA. This dog had no electrolyte abnormalities and was diagnosed as having secondary HA. The promising results of this study are in line with the findings of Javadi et al. (14), who measured ACTH in 22 dogs with primary HA and 60 healthy controls. The highest concentration in healthy controls was 21 pmol/l and only one dog with HA was misclassified. In humans an ACTH concentration > 22 pmol/l is consistent with primary HA and most patients have a concentration > 45 pmol/l (2). In summary the results of the two studies in dogs suggest that, as in humans (2, 3, 23), ACTH measurement is a sensitive and highly specific test to diagnose primary HA associated with (classical HA) or without (atypical HA) electrolyte abnormalities.

The main drawback of ACTH measurement in practice is the instability of this peptide hormone. To avoid enzymatic degradation, blood should be collected in pre-cooled EDTA plastic (not glass) tubes and plasma frozen at < 10 °C until dispatch. With the use of the proteinase inhibitor aprotinin that helps preservation, a negligible estimated negative bias of about 7 pmol/l has to be taken into consideration. This applies to the commonly used Immulite ACTH assay (32) and not automatically to other assays (31).

In case of clinical suspicion for HA, we recommend to initially measure electrolytes and to perform a white blood cell and differential count. If the Na/KR is > 22, especially in the presence of a N/LR > 2.3, HA is unlikely to be present (Fig. 3). HA is also implausible in dogs “without” inappetence/anorexia and lethargy/weakness (decision tree, data not shown). The most straightforward approach to exclude HA in this group of dogs is to measure the basal cortisol concentration (17). If the concentration is > 55.2 nmol/l, HA can be reliably excluded (17). If the basal cortisol concentration is < 55.2 nmol/l little information regarding the adrenocortical function is gained. The specificity of basal cortisol at this cutoff to rule in HA was 72% in the present versus 78% in an earlier study [17]. Accordingly, confirmatory tests with a high specificity, such as ACTH measurement are advocated. If ACTH is > 50 pmol/l, HA can be confirmed.

If the Na/KR is ≤ 22 and the N/LR ≤ 2.3, HA is extremely likely. Although the specificity of this combination was 100% in this study, we recommend additional ACTH-measurement until the results have been corroborated in a larger number of patients.

**Relevance for clinical practice**

ACTH measurement is a sensitive and highly specific test to diagnose canine primary hypoadrenocorticism. As secondary hypoadrenocorticism is extremely rare, it is an excellent alternative to the ACTH stimulation test which relies on the availability of synthetic ACTH. Hypoadrenocorticism is also very likely in dogs with a sodium to potassium ratio (Na/KR) ≤ 22 in combination with a neutrophil to lymphocyte ratio (N/LR) ≤ 2.3.
Conflict of interest
The authors confirm that they do not have any conflict of interest.

References


