Cutoff values of urinary corticoid:creatinine ratio for the diagnosis of Cushing's syndrome in dogs using automated chemiluminescent immunoassay (CLIA)

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INTRODUCTION

Cushing's syndrome is one of the most common endocrinopathies in dogs.[1,2] Hormone tests for verification should only be performed in case of strong suspicion due to clinical signs, routine blood work, and urinalysis.[3] The current diagnostic test of choice is the low-dose dexamethasone suppression test (LDDST, 85-100% sensitivity, 44-73% specificity).[2-15]

The urinary corticoid:creatinine ratio (UCCR)-test is an alternative noninvasive test (75-100% sensitivity, 21-100% specificity)[12,16-20] with diagnostic value still under discussion. Established by use of radioimmunoassays (RIA) with some adaptions, UCCR-measurements are currently performed using automated chemiluminescent immunoassays (CLIA). Diagnostic thresholds and cut-off values have not been adapted though.[20] The American College of Veterinary Internal Medicine proposed a need for re-evaluation based on this in a 2013 consensus statement.[3]

AIMS OF THE STUDY

Aim of this study was to establish updated diagnostic cutoff values for the UCCR-test using CLIA as test method. UCCR served as index test with LDDST as the clinical reference standard. Samples submitted to a commercial diagnostic laboratory served as test material. Bayesian latent class models (BLCM) were used to calculate sensitivity and specificity of the LDDST and UCCR-test with updated diagnostic thresholds independently from each other taking the lack of gold standard in diagnosing Cushing's syndrome into consideration.

Table 1: UCCR values (x 10^{-6}) in dogs classified as LDDST negative or positive in three groups depending on time interval between both tests (LABOKLIN, Bad Kissingen, Germany)

Study group (days)	Dogs tested LDDST positive			Dogs tested LDDST negative			*P*[1]
	UCCR	Range UCCR	Median Z	UCCR	Range UCCR	Median Z	
I (0-7)	78.4	11.7 - 840.2	-0.04	31.1	3.8-290.4	-0.41	*P* < 0.001, Z -7.8
II (8-14)	73.7	15.7 - 927.5	-0.05	32.4	11.5-282.2	-0.41	*P* < 0.001, Z -5.9
I+II (0-14)	76	11.7 - 927.5	-0.04	31.5	3.8-290.4	-0.42	*P* < 0.001, Z -9.8

[1]Mann-Whitney-U-test: UCCR vs result LDDST; If two urine samples were sent in from an individual dog, median was calculated; level of significance *P* < 0.05

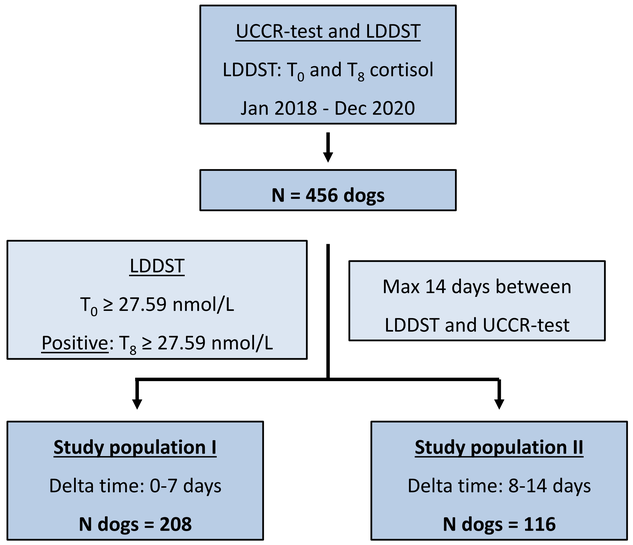

Figure 1: Dogs tested by the LDDST and UCCR-test by Laboklin (Bad Kissingen, Germany) between 2018 and 2020; inclusion criteria of the study are demonstrated

MATERIAL AND METHODS

Data from year 2018 to 2020 was obtained retrospectively from a commercial laboratory (Figure 1, Table 1). Cortisol for LDDST was measured with COBAS e602 (Roche Diagnostics). UCCR testing utilized CLIA to cortisol measurement (ADIVA Centaur XPT, Siemens Healthineers). For urinary creatinine COBAS 8000 (Roche Diagnostics) with CREJ2-creatinine Jaffé Gen.2 assay was performed. Delta time between both cortisol measurements was 14 days maximum.

The optimal cut-off value for UCCR-testing was calculated by Youden index. Sensitivity and specificity of these cutoff values for the UCCR-test and LDDST were assessed by Bayesian latent class models (BLCM) using the software OpenBUGS for windows (version 3.2.3; OpenBUGS Foundation) (Table 2).

Table 2: Posterior inferences with informative priors in the BLCM in 324 dogs

	Median (50th percentile)	2.5th percentile	97.5th percentile
UCCR test (sensitivity)	0.86	0.73	0.94
LDDST (sensitivity)	0.91	0.85	0.96
Difference sensitivity (DSe)	-0.05	-0.19	0.05
Prob (DSe>0)	0.172		
UCCR test (specificity)	0.63	0.58	0.68
LDDST (specificity)	0.54	0.49	0.59
Difference specificity (DSp)	0.09	0.04	0.15
Prob (DSp>0)	0.999		

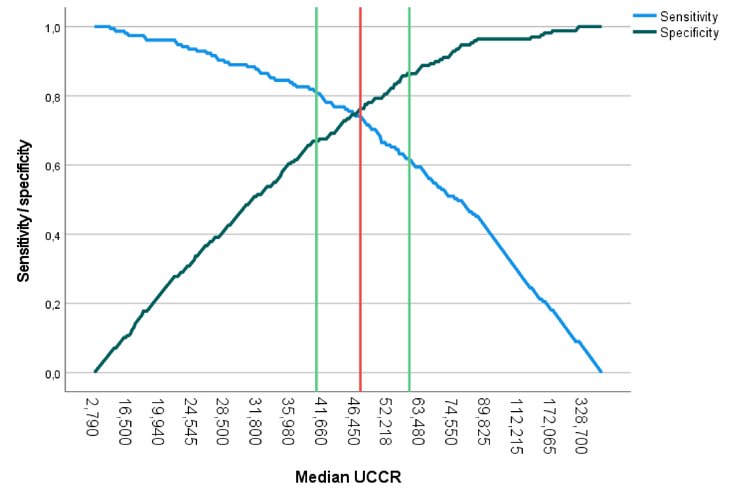

Figure 2: Sensitivity and specificity of the UCCR-test (index test) compared to the LDDST-results (clinical reference standard). Red line: optimal cut-off calculated by Youden index, green lines: cut-off values for optimized sensitivity and specificity creating a grey zone (in case of two UCCR-values the median was used for statistical approaches)

RESULTS

This study included 324 dogs with both UCCR-test and LDDST results (Figure 1). The optimal UCCR cut-off value as calculated by Youden index was 47.44 x 10^{-6} (Figure 2). Any UCCR < 40 x 10^{-6} was interpreted as a negative result, 40-60 x 10^{-6} as grey zone and > 60 x 10^{-6} as positive. Using the cutoff of 60 x 10^{-6}, BLCM showed 91% (LDDST) and 86% (UCCR-test) sensitivity and 54% (LDDST) and 63% (UCCR-test) specificity (Table 2).

LIMITATIONS

Background information including history, clinical presentation, blood work, and response to treatment was not available. No control group of healthy dogs was included. Animals with negative LDDST results were considered to be free of Cushing's syndrome. This group will include a proportion of falsely negative classified animals resulting in underestimating the sensitivity of the UCCR test. Although standard protocol for LDDST and UCCR-test was recommended, deviation by the practitioners may have happened with unknown effect.

DISCUSSION AND CONCLUSIONS

Considering an 86% sensitivity and a 63% specificity of the UCCR test by use of CLIA this test can be considered as first-line tool to rule out Cushing's syndrome. Urine samples can be collected noninvasively at home by the owner, minimizing stress and its potential effect.

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