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BRIEF COMMUNICATION

Evaluation of a point-of-care test for canine C-reactive protein

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Background: In veterinary medicine, there is increasing interest in measuring C-reactive protein (CRP) as a tool for diagnosis and monitoring of inflammatory diseases. Reported CRP concentrations for healthy dogs have ranged from 0 to 8.9 mg/L.
Objectives: The aims of this study were to evaluate a canine-specific point-of-care (POC) lateral flow immunoassay for qualitative CRP measurement in healthy and diseased dogs and to compare results with those obtained by a quantitative ELISA.
Methods: Blood samples from 73 client-owned dogs were available for testing: 16 healthy dogs and 57 dogs with a variety of infectious, inflammatory, or neoplastic diseases. CRP was measured in heparinized whole blood samples and serum with the TECOmedical Dog CRP-visual POC test. A red line develops in the POC device if CRP is $\geq$5 mg/L, and results are scored as negative or positive. An ELISA validated previously for canine serum was used as the reference method.
Results: For all dogs, serum CRP concentrations measured by the ELISA ranged from 0.1 to $\geq$350 mg/L (median = 38 mg/L). Percentages of the CRP POC test results that agreed with the ELISA results were 98.6% for whole blood and 97.3% for serum samples. For serum samples, sensitivity of the POC test was 96.4% and specificity was 81.3%. For whole blood, sensitivity was 94.7% and specificity was 93.8%.
Conclusions: The POC test had very good agreement with the ELISA test and had high sensitivity and specificity; therefore, it can be used as a qualitative test to screen for increases in CRP concentrations.

C-reactive protein (CRP) is an acute phase protein produced in the liver after stimulation by proinflammatory cytokines, eg, interleukin (IL)-1, IL-6, and tumor necrosis factor-α.[1–3] In dogs, increased CRP concentration has been demonstrated in several inflammatory processes[4,5] and might be useful for monitoring the course of inflammatory diseases in dogs undergoing treatment. An ELISA for quantitative measurement of CRP in dogs has been validated for clinical laboratory use[6,7]; reported values for healthy dogs range from 0 to 8.9 mg/L and median values range from 0.6 to 2.4 mg/L.[6–11] However, quantitative analysis of canine CRP by ELISA is time consuming and expensive. A qualitative point-of-care (POC) assay for human CRP has been evaluated for canine serum CRP (CRP Latex Slide Test, Randox Laboratories, Crumlin, County Antrim, UK),[12] but for qualitative analysis specific to canine CRP only one rapid assay is currently available (CRP Rapid Assay Pack, EVL, Woerden, the Netherlands), and, unfortunately, false-positive results have been reported with this assay.[13] A reliable qualitative POC test would offer the opportunity to detect dogs with low CRP concentrations and reduce the need for the more expensive quantitative ELISA. The aim of this study was to evaluate a POC canine-specific lateral flow immunoassay (TECOmedical Dog CRP-visual, TECOmedical AG, Sissach, Switzerland), subsequently referred to as CRP POC, for qualitative measurement of CRP in serum and whole blood of healthy and diseased dogs.

Blood samples from 73 client-owned dogs presented to the Small Animal Clinic at the Freie Universität Berlin were available for testing. Fifty-seven of the dogs had a variety of infectious, inflammatory, or neoplastic diseases, and 16 were healthy blood donors used as control dogs. Blood samples used for the study were collected for diagnostic purposes or for evaluation of health status; therefore, approval of the animal care and use committee was not required. Each

dog had a complete physical examination, CBC (Sysmex XT-2000iV, Sysmex Deutschland GmbH, Norderstedt, Germany), and biochemical profile (Konelab 30i, Thermo Electron GmbH, Dreieich, Germany). Clinical health of the control dogs was defined as absence of abnormalities on physical examination and laboratory tests.

CRP was measured within 2 hours after blood collection using the CRP POC test in accordance with the manufacturer's instructions. This is a standard double antibody sandwich lateral flow immunoassay (Figure 1). Ten microliters heparinized whole blood or 5 μL serum are pipetted onto the sample application zone and 2 drops of buffer dilution are added. The nitrocellulose membrane inhibits flow of cellular components toward the test line (T), while the fluid moves there by capillary action. Canine CRP is recognized by polyclonal rabbit anticanine CRP antibodies, which are linked to gold particles. CRP–antibody–gold conjugates are then captured by polyclonal anti-CRP–antibody at T, resulting in a red band (Figure 1). Excessive antibody–gold conjugates that were not bound at T diffuse within the membrane and are recognized by specific anti-rabbit IgG antibodies at the control line (C) to yield a red control band. Results are read after 5 minutes. Results were positive, ie, CRP is ≥5 mg/L, if a red band was visible at T. If a red band develops only at C, the result is negative or < 5 mg/L (Figure 1). Serum samples from the 73 dogs were then stored at −40°C for a maximum of 4 months to be assayed later using an ELISA (Tridelta Phase CRP, Tridelta Development Ltd., Maynooth, County Kildare, Ireland) validated for canine serum samples.[6,7]

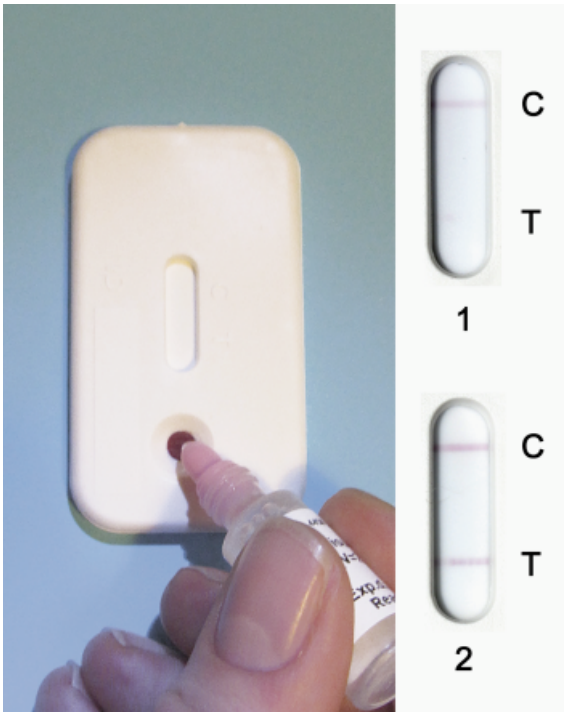

Figure 1. Point-of-care lateral flow immunoassay for canine C-reactive protein (CRP). (**Left**) After the sample (whole blood or serum) has been loaded onto the sample application zone, buffer is added. (**Right**) Negative result (1), CRP concentration < 5 mg/L; positive result (2), CRP concentration ≥5 mg/L. C, control line; T, test line.

Stability of CRP was determined using aliquots of serum and whole blood samples from 6 dogs (5 sick, 1 healthy) stored at room temperature (21°C) and 4°C. Samples stored at room temperature were tested over 4 hours (directly after sampling, and 2 and 4 hours after sampling), and samples stored at 4°C were tested over 24 hours (directly after sampling, and 4, 12, and 24 hours after sampling). Inter-assay variation was evaluated by testing 3 positive and 3 negative serum and whole blood samples each in a series of 6 measurements. To evaluate reading times suggested by the manufacturer, intensities of test bands were documented after 5, 15, and 30 minutes using 3 positive and 3 negative serum and whole blood samples each.

Statistical analysis was performed using PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA) and BiAS. für Windows v. 9.04 (epsilon-Verlag, Hochheim-Darmstadt, Germany).

Results were compared with those from the ELISA, and accuracy, defined as the proportion of results that agreed between the 2 methods, was reported as percent and 95% confidence interval (CI). Sensitivity, defined as the proportion of sick dogs correctly identified by the POC test, was reported as the percent positive results in sick dogs and 95% CI. Specificity, defined as the proportion of healthy dogs correctly identified by the POC test, was reported as the percent negative results in healthy dogs and 95% CI.

Seventy-three dogs of 31 different breeds were enrolled in this study and included 18 mixed-breed dogs. There were 38 males, 7 of which were neutered, and 35 females, 15 of which were spayed. Blood donors comprised a group of 16 dogs with no clinical and laboratory abnormalities. These 16 dogs were used as healthy control dogs and had a median age of 4 years (range 1–9 years) and median body weight of 29.2 kg (range 20–65 kg). The 57 diseased had a median age of 5 years (range 1.2 months–15 years) and median body weight of 22 kg (range 1–55 kg). These dogs had a variety of conditions, including infectious disease/sepsis (20), gastroenteritis (10), wounds (8), malignant neoplasia (5), immune-mediated polyarthritis (2), and other conditions such as nephropathy or pancreatitis (12).

The CRP quantitative test results (ELISA) for all 73 dogs ranged from 0.1 to ≥350 mg/L (Tables 1 and

Table 1. CRP concentrations in samples from 16 healthy dogs using the serum ELISA and a POC lateral flow immunoassay* for canine CRP.

		POC	
Dog	ELISA (mg/L)	S	B
1	0.1	−	−
2	0.4	−	−
3	0.4	−	−
4	0.8	−	−
5	0.9	−	−
6	1.1	−	−
7	1.4	−	−
8	1.5	−	−
9	1.5	−	−
10	1.6	−	−
11	1.7	−	−
12	2.3	+	−
13	2.5	−	−
14	3.4	+	−
15	4.5	−	−
16	9.4	+	+

*The TECOmedical Dog CRP-visual test, developed and produced by TECOmedical, has also been commercialized as FASTest CRP canine (MEGACOR Diagnostik GmbH, Hörbranz, Austria).
POC, point-of-care; S, serum; B, whole blood; −, negative; +, positive; CRP, C-reactive protein.

2). Compared with the ELISA results, the CRP POC assay results using serum had 100% agreement for negative results (15/15; 95% CI 81.9–100%) and 96.6% agreement for positives (56/58; 95% CI 88.1–99.6%). Using whole blood, there was 94.4% agreement for negatives (17/18; 95% CI 72.7–99.9%) and 100% for positives (55/55; 95% CI 94.7–100%). Overall agreement for serum samples was 97.3% (71/73; 95% CI 90.5–99.7%) and for whole blood was 98.6% (72/73; 95% CI 92.6–100%) (Figure 2).

Using serum, sensitivity of the CRP POC test was 96.4% (55/57; 95% CI 87.9–99.6%) and specificity was 81.3% (13/16; 95% CI 54.4–96%). Using whole blood, sensitivity was 94.7% (54/57; 95% CI 85.4–98.9%) and specificity was 93.8% (15/16; 95% CI 69.8–99.8%). Different conditions and durations of storage for serum and whole blood samples did not influence test results. Interassay variation was not detected. Color intensity of bands over 30 minutes revealed a slight increase in intensity of positive bands for the 6 positive samples (3 serum and 3 whole blood samples). In 4 of 6 samples (2 serum and 2 whole blood samples) initially evaluated as negative, a very light red band appeared at T after 30 minutes, but intensity was still less than at C.

Several methods for measuring CRP concentrations in canine serum and plasma have been developed

Table 2. CRP concentrations in samples from 57 sick dogs using the serum ELISA and a POC lateral flow immunoassay for canine CRP.

		POC	
Dog	ELISA (mg/L)	S	B
1	2.4	−	−
2	4.1	−	−
3	10.2	+	+
4	12.5	+	+
5	13.4	+	+
6	16.1	+	+
7	16.1	+	+
8	17.8	+	+
9	18.7	+	+
10	20.2	+	+
11	21.8	+	+
12	22.0	+	+
13	22.3	+	+
14	26.5	+	−
15	31.9	+	+
16	35.6	+	+
17	36.7	+	+
18	36.7	+	+
19	39.2	+	+
20	41.3	+	+
21	43.6	+	+
22	44.3	+	+
23	46.8	+	+
24	47.3	+	+
25	49.8	+	+
26	62.7	+	+
27	67.5	+	+
28	69.3	+	+
29	83.5	+	+
30	85.5	+	+
31	90.5	+	+
32	104.5	+	+
33	105.9	+	+
34	109.5	+	+
35	119.8	+	+
36	121.8	+	+
37	135.0	+	+
38	136.8	+	+
39	141.3	+	+
40	157.5	+	+
41	157.8	+	+
42	158.3	+	+
43	163.8	+	+
44	166.3	+	+
45	167.0	+	+
46	175.5	+	+
47	177.0	+	+
48	214.3	+	+
49	221.8	+	+
50	286.8	+	+
51	288.5	+	+
52	316.8	+	+
53	350.0	+	+
54	350.0	+	+
55	350.0	+	+
56	350.0	+	+
57	350.0	+	+

POC, point-of-care; S, serum; B, whole blood; −, negative; +, positive; CRP, C-reactive protein.

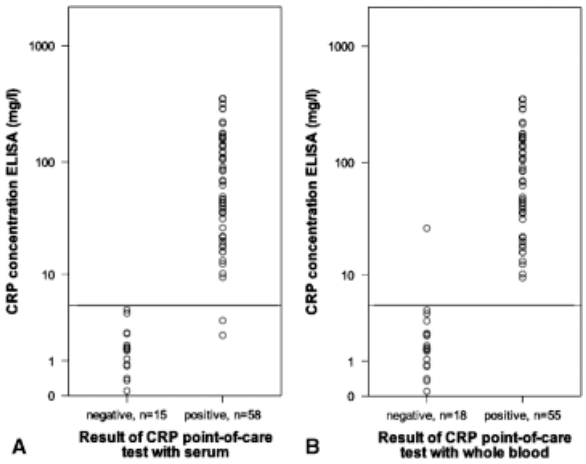

Figure 2. Scattergrams of canine C-reactive protein (CRP) concentrations for 73 serum (**A**) and whole blood (**B**) samples, comparing negative and positive results for the CRP point-of-care test (*x*-axis) with results from the ELISA (*y*-axis). The horizontal line marks the cut-off for a positive result by the CRP point-of-care test (5 mg/L).

for use in different studies,[14–20] but only a few tests are commercially available.[12,13,21,22] Most test systems are only suitable for laboratory settings and technical requirements must be met. Use of 2 POC CRP tests for semiquantitative and qualitative determination of canine CRP has been reported. Evaluation of a canine CRP immunochromatographic test (EVL) was based on 32 serum samples.[13] Sensitivity of 100% and specificity of 6.3% were reported when interpreted according to the manufacturer's instructions, ie, positive when CRP concentrations were > 5 mg/L, weak positive at 3–5 mg/L, and negative at < 3 mg/L. Specificity improved to 93.8% when weak positive results (3–5 mg/L) were reclassified as negative. A slide-reversed passive latex agglutination test (Randox Laboratories), based on an antibody reaction against human CRP, was reported to have high analytical specificity for canine CRP.[12] The clinical cut-off was 35 mg/L. Prediluted serum samples (1:8) were able to discriminate between positive and negative samples with reference to an automated immunoturbidimetric method. The CRP POC test evaluated in our study is based on a polyclonal antibody reaction against canine CRP. The results are not dependent on special sample preparations, like predilutions, which may be advantageous for use in clinical settings and may prevent preanalytical errors. The low cut-off of 5 mg/L may reduce the proportion of false-negative results. Also, this CRP POC test is applicable on-site in any clinical setting with no need for additional equipment. Test results should be read at exactly 5 minutes, following the manufacturer's instructions as an increase in color intensity in some samples over time might lead to false-positive readings. The main disadvantage of the test is the current restriction to qualitative analyses. During therapeutic monitoring, if results for a specific patient change from negative to positive with the POC test, CRP concentrations should be measured using the quantitative assay.

In conclusion, agreement between the POC test and the ELISA was high for both whole blood and serum samples. Furthermore, the POC test was able to differentiate between healthy and sick dogs with adequate sensitivity and specificity for use as a qualitative screening test for increased CRP concentrations.

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