Received: 30 March 2021 | Revised: 12 October 2021 | Accepted: 14 November 2021

DOI: 10.1111/vcp.13100

Veterinary Clinical Pathology
An International Journal of Laboratory Medicine
WILEY

REVIEW ARTICLE

Measurement and clinical applications of C-reactive protein in gastrointestinal diseases of dogs

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Abstract

C-reactive protein (CRP) is a positive acute-phase protein, serum concentrations of which increase nonspecifically in response to inflammatory processes of the dog. As such, it can aid in the identification of inflammatory disease and, maybe more importantly, the objective monitoring of disease progression. In dogs, CRP is frequently used to evaluate dogs with gastrointestinal diseases, such as chronic inflammatory enteropathies (also termed idiopathic inflammatory bowel disease), acute pancreatitis, canine parvovirus infection, hepatic disease, acute abdomen, and protein-losing enteropathy. The diversity of the assays available to measure CRP in dogs is nearly as numerous as the diseases in which serum concentrations of this protein are increased. Assay methodologies include laser nephelometric immunoassays, enzyme-linked immunosorbent assays, immunoturbidimetric assays, and time-resolved immunofluorometric assays. While many of these assays are acceptable for clinical use in the dog, the same assay and analyzer should be used to measure a patient's CRP concentration longitudinally. By looking at the uses of CRP in human gastroenterology, including reducing the duration of antibiotic therapy, the veterinary profession can gain insight into novel ways in which serum CRP concentration measurements might be applied in veterinary medicine in the future.

KEYWORDS
acute phase reactant, biomarker, canine, CRP, CRP assays, inflammatory disease

1 | BACKGROUND AND INTRODUCTION

C-reactive protein (CRP) is a positive acute-phase protein, which serves as a marker of the innate immune system's response in a variety of inflammatory processes.[1,2] CRP is synthesized in the liver and composed of five identical subunits that form a 120-kilodalton pentamer.[2] The concentration of circulating CRP in dogs rises rapidly in response to proinflammatory cytokines, and CRP is cleared by the liver upon resolution of the inciting cause, thereby allowing it to be used clinically as a sensitive marker of inflammation.[1] It should be noted that, as an indicator of inflammation, CRP is not a specific diagnostic test nor a marker for any specific disease entity. Rather, it is useful as a tool to objectively assess the degree of inflammation and, where evaluated, as a prognostic marker of disease severity and survival.

C-reactive protein is frequently used to evaluate dogs with gastrointestinal disease, as well as disease processes of other organ systems.[3-8] Although not the focus of this manuscript, it should be noted that CRP has been used extensively in Europe and Japan for the routine assessment of dogs with a wide variety of gastrointestinal

and nongastrointestinal diseases. The aim of this review is to draw from the past 20 years of literature and provide a brief overview of the potential uses for measuring serum CRP in dogs with gastrointestinal diseases. Some of the methods by which CRP is measured, including automated and point-of-care assays, will also be explored. Lastly, by outlining some of the ways in which CRP is used in human medicine, it is the hope of the authors that clinicians might be stimulated to explore other potential applications of CRP in the dog.

2 | CRP AND GASTROINTESTINAL DISEASE IN DOGS

Specific gastrointestinal diseases in dogs in which the measurement of serum CRP concentrations has been used include chronic inflammatory enteropathies (CIE; also referred to as idiopathic inflammatory bowel disease [IBD]),[3,4,9,10,11] acute pancreatitis (AP),[12-14] parvoviral infection,[15-17] hepatic disease,[5,18] acute abdomen,[19] and protein-losing enteropathy.[20]

Chronic inflammatory enteropathies encompass a wide variety of disorders, characterized by chronic gastrointestinal signs, mucosal inflammation identified on histopathologic evaluation, and the exclusion of other known systemic or gastrointestinal diseases.[3] As a nonspecific marker of inflammation, CRP is useful for evaluating disease progression and response to treatment over time in dogs with CIE.[9] However, due to the relatively high biological variability of serum CRP concentrations in dogs, clinicians should keep in mind that serum CRP concentrations must change by at least 2.7-fold for a change to be considered clinically significant.[21] Nonetheless, canine CRP remains a useful tool in not only the longitudinal evaluation of CIE treatment but also as an indicator of which clinical approach would be most useful at the onset of treatment.[9] For example, one prospective case-controlled study reported that a serum CRP concentration of ≥9.1 mg/L could differentiate dogs with CIE requiring anti-inflammatory or immunosuppressive treatment from those that would respond to an elimination diet or antibiotic therapy (sensitivity of 72%; specificity of 100%).[9]

Although still used by some clinicians, the term IBD may be less preferred over the term CIE, and some of the studies on the use of serum CRP concentrations in dogs with chronic enteropathies use the term IBD. These studies demonstrate the utility of CRP in guiding the treatment of dogs with IBD. For example, one prospective study, which used immunosuppressive drugs for the treatment of dogs with IBD, found that mean serum CRP concentrations decreased from a concentration of 10.4 mg/L (pretreatment; standard deviation [SD]: 2.5 mg/L) to 0.6 mg/L (posttreatment; SD: 0.1 mg/L).[4] Another, more recent, prospective study showed that plasma CRP concentrations were significantly higher in dogs with IBD compared with those of healthy control dogs.[10]

Serum CRP concentrations have also been suggested to play a role in predicting the prognosis of dogs with AP. However, the results reported are not conclusive. One retrospective study reported that, although there was no significant difference in serum CRP concentrations between surviving and nonsurviving dogs with AP at presentation (survivor median: 63 mg/L, range: 5.0-149.0 mg/L; nonsurvivor median: 100.0 mg/L, range: 10.0-176.0 mg/L), there was a significant difference in serum CRP concentrations between those two groups on the third (survivor median: 25.5 mg/L, range 3.0-63.0 mg/L; nonsurvivor: 68.0 mg/L, range: 12.0-188 mg/L) and fourth days (survivor median concentration: 16.0 mg/L, range: 3.0-47 mg/L; nonsurvivor 66.0 mg/L, range: 8.0-140 mg/L) of treatment.[14] The authors of this article did not discuss if the CRP concentrations were clinically significantly different or only statistically different between surviving and nonsurviving dogs on days 3 and 4 of AP treatment. Another prospective study by Holm et al showed that the mean CRP concentrations of 16 dogs with AP (56 mg/L, SD: 12.7 mg/L) were significantly higher compared with those of control dogs (2.8 mg/L, SD: 1.3 mg/L) on day 1 of diagnosis.[13] This study also demonstrated decreasing serum CRP concentrations in six of seven dogs over the 5 days of AP management compared with baseline.[13] All seven of the dogs in this study showed clinical improvement and were discharged from the hospital.[13] However, another prospective study failed to identify a significant difference in CRP concentrations between surviving (median: 53.1 mg/L, range: 2.6-98.4 mg/L) and nonsurviving (median: 58.1 mg/L, range: 22.3-94.3 mg/L) dogs with critical illnesses, including AP, sepsis, and severe trauma.[12] It should be noted, however, that these groups were evaluated together to determine survivability. Further research is needed to evaluate the clinical utility of CRP as a prognostic indicator for dogs with AP. It must also be noted that serum CRP concentration is not a diagnostic test for AP in dogs due to its nonspecific nature. Instead, the measurement of pancreatic lipase offers a more sensitive and specific marker of the disease.[22]

Canine parvoviral enteritis is an acute disease caused by the highly contagious canine parvovirus type-2 (CPV-2) and is characterized by mucoid to hemorrhagic diarrhea, vomiting, fever, profound leukopenia, weakness, and, in some dogs, multiorgan failure and death.[15] Serum CRP concentrations can be used to provide prognostic information in clinically ill dogs with CPV-2 infection. One prospective study by Kocaturk et al found that mean CRP concentrations were significantly higher in dogs with parvoviral enteritis compared with healthy control dogs and was higher in nonsurviving dogs (mean: 180 mg/L) compared with surviving dogs (mean: 130 mg/L).[16] Additionally, this study also reported that CRP outperformed ceruloplasmin, haptoglobin, or albumin in distinguishing surviving from nonsurviving dogs. Serum CRP concentrations above 92.4 mg/L predicted patient mortality with a 91% sensitivity.[16] It should be mentioned that the specificity, using this cut-off value, is suboptimal. A prospective observational study by McClure et al found that CRP was moderately accurate (sensitivity: 86.7%; specificity 78.7%) in differentiating between survivors and nonsurvivors of naturally acquired canine parvoviral enteritis in a group of 79 client-owned puppies at 24 hours after admission.[17] This accuracy is based on a cut-off value of 97.3 mg/L at 24 hours after admission and was calculated using receiver operating characteristic curve analysis and Youden index calculations. While this study demonstrated that the

CRP concentration was associated with outcome in puppies with canine parvoviral enteritis, the discriminative ability of CRP alone was found to be suboptimal in predicting survival in puppies.[17]

Serum CRP concentrations in dogs with hepatic disease have been evaluated. In one prospective observational study of 46 dogs with congenital portosystemic shunts (cPSS), chronic hepatitis, or hepatic neoplasia, a positive correlation was found between serum CRP concentrations and hepatic necro-inflammatory scores as seen on histology ($r = 0.428$).[5] In this study, no significant difference was found between serum CRP concentrations and disease type.[5] Nonetheless, canine CRP could still provide useful ancillary information in dogs with hepatic disorders, especially when combined with signalment, history, and clinical signs. This point was demonstrated by another prospective study that found a significant difference in serum CRP concentrations between dogs with a cPSS that had hepatic encephalopathy and those with a cPSS that did not have hepatic encephalopathy.[18] A retrospective study by Tivers et al found that CRP was a useful indicator of successful surgical attenuation of cPSS in dogs, as the median CRP concentration was found to decrease significantly (presurgical attenuation median: 3.4 mg/L, range 0.7-12.7 mg/L; postsurgical attenuation median: 1.4 mg/L, range 0.6-3.1 mg/L) after shunt attenuation.[23]

CRP has been found to be useful as a prognostic indicator in dogs with acute abdomen.[19] Acute abdomen is characterized by the acute onset of abdominal pain caused by a variety of etiologies, ranging in severity. Common underlying disorders associated with this syndrome include gastric dilatation and volvulus, small bowel obstruction, peritonitis, neoplasia, and pancreatitis, among others.[24] One prospective study evaluated serum CRP concentrations in 32 dogs with acute abdomen.[19] This study found that dogs that died had higher initial (median: 140 mg/L; range 74-202.5 mg/L) and 48-72 hour posthospitalization (median: 47.6 mg/L; range 22.4-91 mg/L) CRP concentrations compared with dogs that survived (initial median CRP concentration: 18.5 mg/L; initial range: 0-146 mg/L; 48-72 hour median CRP concentration: 13.6 mg/L; 48-72 hour range: 0-50.2 mg/L).[19] However, it should be noted that since this study only included three dogs that died as a result of an acute abdomen, it did not provide sufficient data to determine the prognostic utility of serum CRP concentration measurements in dogs with an acute abdomen.

Protein-losing enteropathy (PLE) is a syndrome in which an excess of protein is lost through the gastrointestinal mucosa.[25] Many disorders can lead to PLE in dogs, including lymphangiectasia, CIE, lymphoma, chronic intussusception, and even hookworm infestation.[25] One prospective study reported that serum CRP concentrations were significantly higher in dogs with PLE (median 13.0 mg/L; range 0.1-101.3 mg/L) than in those who had food-responsive diarrhea (median 1.4 mg/L; range 0.1-23.0 mg/L).[20] This study also reported that a mild to moderately increased serum CRP concentration was a negative prognostic indicator in dogs with PLE and was associated with an increased risk of death or euthanasia.[20] It should be noted that the authors of this study did not numerically define these mildly to moderately increased serum CRP concentrations. Thus, clinicians might be able to use serum CRP concentrations as an ancillary diagnostic test or as a marker of prognosis in dogs with PLE.

3 | MEASUREMENT OF CANINE CRP

There are a multitude of assays available for the measurement of canine CRP, both as patient-side point-of-care tests (POCTs) and those performed in a commercial laboratory setting. It should be noted that using a specific assay in dogs requires two validation stages, analytical validation and clinical validation.[26] Analytical validation evaluates whether an assay is technologically sound—in other words, does the assay give the same result upon repeated measurements, and is there dilutional parallelism and spiking recovery. Each assay also needs to be clinically validated in that the assay needs to be able to differentiate groups of patients.[26] For example, a CRP assay intended to measure CRP in serum samples from humans may be analytically valid in dogs, but cross-immunoreactivity may be too low to show distinguishing results between different groups of dogs. Analytical and clinical validation studies are complementary but also not equivalent; each requires a different set of experiments, statistical analysis, and interpretation.[26] It should be noted that, as is the case for any study methodology, the trustworthiness of clinical validation studies is dependent upon its design and conduct; likewise, the results of analytical validation studies might not apply to other patient groups, settings, and analyzers.[27,28] Finally, if an assay fails analytical validation, clinical validation studies are not meaningful.

Prior to measuring canine CRP, careful thought should be given to preanalytical factors which could affect the accuracy of the sample. These factors may include withholding food from the animal overnight to avoid postprandial lipemia, using the appropriate blood collection tube as outlined in the manufacturer's package insert, and ensuring the careful and timely storage and transportation of samples to the laboratory.[28] The sample type (serum vs lithium heparinized plasma) and volume required to measure canine CRP vary widely across individual assays and should be reviewed using the manufacturer's package insert.

Point-of-care tests offer clinicians the ability to evaluate canine CRP in an emergency setting or as a rapid ancillary test to track the progression of an inflammatory disease process. There are many species-specific POCTs available that have been evaluated for the measurement of CRP in dogs, including the TECOdogCRP-quant (TECO; TECOmedical AG, Sissach, Switzerland),[29] EURO-Lyser solo cCRP test (EURO; EUROLyser, Salzburg, Austria),[29] LifeAssays canine CRP test (LifeAssay; LIfeAssays, Lund, Sweden),[29] and the Point Strip canine CRP assay (Point Strip; Point Strip Canine CRP Kit, USHIO Europe BV, BC Oude Meer, The Netherlands).[30] The TECO, EURO, and LifeAssay were found to have interassay coefficients of variation (CVs) of 20.7%, 7.0%, and 7.4%, respectively. The inter-run imprecision expressed by the interassay CV was unacceptable for the TECO assay. The bias of these assays calculated using Bland-Altman plots (the relative difference in CRP concentrations between the reference ELISA and each of the POCTs) was

27.6% (TECO), −14.2% (EURO), and −15.7% (LifeAssay), and the total error (TE) of each assay, calculated as a function of each assay's intraassay CV and bias, was 69.0% (TECO), 28.2% (EURO), and 30.5% (LifeAssay).[29] These data indicate that each assay should be used with a specific reference interval established for the particular assay that is being used; however, the EURO assay was the only assay with a TE below the authors' acceptable TE of 29.6%.[29] While all three assays were able to measure CRP in dogs, the precision and accuracy of each assays varied, and not all of them had interassay CVs or TEs that were acceptable. It should also be noted that while the TECO, EURO, and LifeAssay tests were generally able to distinguish between groups of dogs with CRP concentrations above and below 10 mg/L as measured by the reference ELISA, the results of their analytical validation was variable across each assay.[29] The Point Strip assay was found to reliably measure CRP concentrations above 50 mg/L, with intraassay and interassay CVs of ≤8.0% and ≤11.0%, respectively. For samples with CRP concentrations below 50 mg/L, the intraassay and interassay CVs were substantially higher at ≤22.0% and ≤28%, respectively.[30] Although the acceptable and ideal CVs have not been universally adopted, CVs for most commercial assays are ideally <10% with suboptimal, but acceptable, variabilities of ≤20%.[31] This assay has a high degree of variability which limits its clinical usefulness. According to the authors' knowledge, clinical validation of this assay has yet to be reported in the primary literature. It should be noted, however, that clinical validation of each new CRP assay might not be necessary in situations where a thorough method comparison with a validated canine-specific CRP assay shows acceptable agreement. Recently, we have analytically validated an additional dog-specific POCT, the IDEXX Catalyst One CRP assay (Catalyst; IDEXX Laboratories, Inc, Westbrook, ME, USA).[32] The Catalyst CRP assay was found to have intraassay and interassay CVs between 6.4%-9.5% and 3.8%-18.2%, respectively. The Catalyst had an observed-to-expected ratio for recovery between 85.6% and 110.7% and a coefficient of determination, or R^2, of 0.98 when compared with the previously validated Gentian canine CRP assay.[32] Thus, this particular assay was found to be sufficiently accurate and precise for clinical use. One study which served as a clinical validation of the Catalyst CRP assay found that it could reliably differentiate between the CRP concentrations of dogs that were pre- and postmedial patellar luxation surgery.[33]

There is also a laser nephelometric immunoassay available (Laser CRP-2; Laser CRP-2, Arrows Co., Ltd., Osaka, Japan).[34] This POCT measures the scattering of light from a laser and its interaction with CRP and anti-canine CRP antibody complexes. The assay is commercially available and has been used in a number of studies in dogs with various diseases.[34,35] In one study, the Laser CRP-2 assay was able to distinguish between dogs with various infectious, inflammatory, and traumatic diseases and those who had noninflammatory, focal, or chronic inflammatory diseases.[36] While the above study would suggest that the Laser CRP-2 assay has been clinically validated, to the authors' knowledge, a report of the analytical validation of this assay has yet to be reported in the primary literature.

Commercial laboratory assays allow for the evaluation of CRP at a much greater scale than that of POCTs. Multiple canine-specific ELISAs are commercially available for the measurement of CRP, including the Phase Range canine CRP ELISA[37] and one recently developed by Waritani et al, which is, to the authors' knowledge, not yet commercially available.[38] The Phase Range canine CRP ELISA (Tridelta; Tridelta Development Ltd, Kildare, UK) was shown in one study to have an intraassay CV between 6.9% and 10.1% and an interassay CV between 7.5% and 29.0%.[37] While this interassay CV is considered unacceptable, this is not unusual for assays when a sample is evaluated that is close to the detection limit of the assay (mean result: 54.9 mg/L; working range: 3.7-60 mg/L when sample diluted 1:500). This is most often observed for measurements close to the lower limit of the working range of the assay, but as seen in this case, it can also be observed at the upper end of the working range. However, this loss of precision close to the limits of the working range is tolerated as this lack of precision has no impact on clinical interpretation. Alternatively, the working range of the assay could be adjusted to obtain acceptable interassay variability across the entirety of the working range of the assay. The Tridelta assay was also found to accurately distinguish between dogs with infectious or inflammatory processes and healthy control dogs.[37] Additionally, this study found that the Tridelta assay could detect the expected changes in CRP concentration during and after the cessation of an acute inflammatory stimulus in two clinical cases.[37] The Tridelta assay has been used in a number of studies.[29,32] The ELISA developed by Waritani et al was shown to have an intraassay CV between 0.7% and 10.0% and an interassay CV between 6.0% and 9.0%.[38] When spiked with purified canine CRP, this assay also demonstrated a recovery between 105% and 109%.[38] Given these performance parameters and the assay's good correlation with the previously validated Laser CRP-2,[34] it appears sufficiently accurate for clinical use. To the authors' knowledge, clinical validation of this assay has not yet been reported in the primary literature.

There are a number of automated immunoturbidimetric assays available for the measurement of CRP in dogs, including the Gentian canine CRP assay (Gentian; Gentian AS, Moss, Norway)[39,40] and Turbovet canine CRP assay (Turbovet; Acuvet Biotech, Zaragoza, Spain),[41] as well as a time-resolved immunofluorometric assay (TR-IMFA) developed by Parra et al[42] The Gentian canine CRP assay is a canine-specific and a commercially available immunoturbidimetric assay that is based on polyclonal chicken anti-canine CRP antibodies. When anti-CRP-immunoparticles bind with canine CRP, these complexes are quantified by turbidometry, and the canine CRP concentration is determined using a calibration curve.[39] One study found that the Gentian assay was reliable, accurate, and precise; the assay had a CV of <2.4% for all tested samples, lower limit of quantification (LoQ) of 6.8 mg/L, and recovery of 123% and 116% when spiked with two different concentrations of purified canine CRP.[40] Another study confirmed the utility of the Gentian assay, demonstrating a recovery between 90% and 105%, intraassay CV between 0.7% and 12.1%, interassay CV between 0.9% and 7.8%, and Spearman's rank correlation coefficient of $r = 0.98$ on method

comparison with the previously validated Randox canine CRP assay (High Linearity CRP, Randox Laboratories Ltd., Crumlin, UK).[39] It should be noted that the Randox assay was originally developed as an immunoturbidimetric assay intended for use in people, but was calibrated with canine-specific control calibration material (Canine CRP Life Diagnostics, Inc, West Chester, PA, USA) for use in this study.[39] An analytical validation of the Gentian assay demonstrated similar results.[32] Furthermore, the Gentian assay has been clinically validated in that it has been shown to differentiate between healthy dogs and those with inflammatory conditions, such as leishmaniasis and pyometra.[43] The Turbovet assay was found to be similarly accurate and precise for the measurement of CRP in dogs, with an intraassay CV of <1.7%, interassay variation of 4.2%, LoQ of 1.4 mg/L, and very good agreement with the Gentian assay.[41] Recently, the Turbovet assay was clinically validated and shown to detect a statistically significant difference in CRP concentrations in dogs that had pyometra compared with a control group of healthy dogs.[44]

Recently, a high-sensitivity CRP (hs-CRP) assay was developed by modifying the previously discussed Gentian CRP assay.[45] Depending upon the methodology used, the LoQ may vary. While some routine automated assays for the measurement of CRP have reported LoQs of 3.8 mg/L, other hs-CRP assays have been reported to be linear down to concentrations of 0.3 mg/L.[39,46] The LoQ for the hs-CRP assay was 0.5 mg/L, allowing the increase in CRP concentration postovariohysterectomy to be detected earlier in dogs when compared with the original Gentian assay.[45] The new hs-CRP assay was found to have acceptable analytical performance, with an intraassay CV of ≤2.7%, interassay CV of ≤3.0%, and acceptable linearity.[45] Furthermore, this assay has been clinically validated in that it has been shown to differentiate between groups of dogs with congestive heart failure due to myxomatous mitral valve disease (MMVD) and dogs with less advanced stages of MMVD.[47]

It should be noted that, when evaluating serum CRP concentrations in patients with severe systemic inflammation where markedly increased CRP concentrations are expected, it might be unnecessary or even contraindicated to use an hs-CRP assay due to the possibility of a prozone effect causing falsely low results.[39] In such cases, it could be acceptable to use a validated CRP assay with an LoQ above the upper limit of the reference interval. However, when investigating a low-grade inflammatory condition, using a hs-CRP assay that accurately measures low concentrations may be necessary. Also, while factors such as subclinical disease can largely be ignored when interpreting CRP concentrations from dogs with severe systemic inflammation, they might need to be considered when evaluating low-grade inflammatory conditions.[48]

There is an in-house TR-IMFA that uses lanthanide chelate labels and polyclonal goat anti-canine CRP antibodies to measure CRP in dog serum.[42] To the authors' knowledge, this assay is not yet commercially available. One study found this assay to be precise, accurate, and sensitive, with an intraassay CV between 5.3% and 7.1%, interassay CV between 4.8% and 13.3%, and recovery of 99.9% and 106.8% after spiking serum with 2 or 10 mg/L of pure CRP, respectively.[42] Another study clinically validated this TR-IMFA assay and found that it was able to detect differences in cerebrospinal fluid CRP concentrations in dogs with inflammatory disorders compared with dogs with spinal cord compression or idiopathy epilepsy.[49]

It should also be noted that various CRP assays designed for use in human medicine have been evaluated for use in the dog.[50-52] There are differing opinions regarding the utility of these assays in a veterinary setting, and their clinical utility in dogs is dependent upon which assay is evaluated and which study is referenced. One study evaluated three automated immunoturbidimetric assays developed for use in humans (ie, Randox, Thermo, and Wako assays) and found that only the Randox and Wako (CRP-HS, Wako Chemicals GmbH, Neuss, Germany) assays were able to reliably distinguish between healthy dogs and those with marked inflammatory disease; the Thermo assay (KonelabTM CRP; Thermo Clinical Labsystems Oy, Vantaa, Finland) had a low cross-reactivity with canine CRP.[51] The Randox assay used in this study is the same as the previously cited Randox assay,[39] although test-specific calibration material was used rather than canine-specific calibration material.[51] The Randox and Wako assays mildly overestimated canine CRP concentrations in the range of 10-30 mg/L.[51] Another study used the Randox assay as a comparison assay in a validation study of a different automated immunoturbidimetric assay, the Biotecnica assay (Biotecnica Instrument S.p.A., Rome, Italy).[50] In this study, an evaluation of 91 serum samples from dogs found that the Biotecnica assay could reliably measure canine CRP with an intraassay CV between 3.3% and 7.6% and an interassay CV between 7.4% and 10.3%. However, interferents such as hemoglobin, triglycerides, and bilirubin resulted in an unacceptable bias (>10%) and thus are a limiting factor in the utility of this assay.[50] It should also be noted that, to the authors' knowledge, clinical validation of the Biotecnica assay for use in dogs has yet to be reported in the literature. An older study, which evaluated two other CRP assays intended for use in people, found that neither was suitable for use in dogs. One of these assays failed to detect any canine CRP in plasma, while the other assay measured canine CRP values that were significantly different from those measured by the reference ELISA.[52] Overall, while certain human CRP assays have been shown to be suitable for use in dogs, a careful review of the literature and an awareness of each assay's limitations should be considered prior to clinical use. Preferably, canine-specific calibrators should be used when using a CRP assay developed for use in people. The Quality Assurance and Laboratory Standards Committee of the American Society for Veterinary Clinical Pathology (ASVCP) has previously published guidelines for method validation studies.[28] It is not uncommon for method validation studies to fail to comply with all of the ASVCP recommendations. The latest version of the ASVCP guidelines states that method validation studies may include the following parameters: reportable range/linearity, repeatability (intraassay variability), reproducibility (interassay variability), method comparison, interference, recovery, reference interval determination, detection limit, and quality control (QC) validation.[28] Many of the above studies do not include all of these parameters in their study design. For example, a number of these studies failed to compare methods,[34,37,42]

study sample interference,[29,32,34,37,42,51] study recovery,[34,37,40,41,51] determine reference intervals,[29,34,39,40,41,42,45,50] and determine detection limits.[29,32,34,39,51] None of the CRP assay validation studies cited included a QC validation as outlined in the ASVCP guidelines. With regard to the determination of a limit of detection, it should be noted that many CRP assay validation studies do not include this parameter due to its lack of clinical significance, as there is no clinical relevance of a decreased serum CRP concentration.

To the authors' knowledge, there are relatively few studies evaluating the stability of canine CRP in storage. One study by Hillström et al evaluated the stability of canine CRP at different temperatures over 14 days.[40] This study found that canine CRP was acceptably stable (<10% deviation in concentration) when serum samples were stored for 14 days at approximately 22°C or 4°C.[40] It was also determined that the serum CRP concentrations detected after four freeze-thaw cycles were 97%-102% of the initial concentrations.[40] Another study by Hillström et al found that canine CRP was stable in serum samples stored at −80°C for up to 3 months.[45] However, there is reason to believe that canine CRP could remain stable when stored for greater periods of time. A study of high-sensitivity human CRP found that, after 30 human serum samples were stored at −80°C for an average of 11 years, there was no significant change in hs-CRP concentrations (baseline median: 1.3 mg/L, range: 0.1-13.4 mg/L; poststorage median: 1.4 mg/L, range: 0.3-13.5 mg/L).[53] Additional studies are warranted to further evaluate the stability of CRP in canine serum samples after long-term storage.

Given the wide assortment of assays available for the measurement of CRP in dogs, it is important that the same assay and instrument be used to measure CRP when repeat measurements are being taken.

4 | APPLICATIONS OF CRP IN HUMAN MEDICINE

Looking at the uses of CRP in human gastroenterology provides the veterinary profession insight into how the measurement of serum CRP concentrations might be applied in the future. One of the uses in human medicine includes reducing the duration of antibiotic therapy.[54-57]

C-reactive protein has been used to reduce inappropriate antibiotic use in people. One prospective study by Elsing et al sought to evaluate CRP as a biomarker to reduce unnecessary antibiotic therapy in people with gastrointestinal infections.[57] It was found that, among 88 patients with acute gastroenteritis, CRP concentrations were significantly higher in patients with bacterial-induced gastroenteritis (mean ± SD: 104 ± 96 mg/L) compared with those with viral or nonspecific gastroenteritis (mean ± SD: 38 ± 55 mg/L), as determined by negative stool cultures.[57] The authors of this study used receiver operator characteristic analyses to determine a cut-off CRP concentration of 17 mg/L, which could differentiate bacterial-induced gastroenteritis from viral or nonspecific gastroenteritis, with a sensitivity of 82% and specificity of 55%.[57] While this sensitivity and specificity is suboptimal, the authors concluded that with a cut-off value of 17 mg/L, antibiotic therapy could have been avoided in 7 of 66 (11%) patients who had viral gastroenteritis.[57] Similar studies have been performed in veterinary medicine in recent years,[58] although few studies pertained specifically to the duration of antibiotic use for gastrointestinal disease in dogs.[59] Overall, the use of CRP to guide antibiotic therapy in canine gastrointestinal disease has not been as well-described and requires further research.

5 | CONCLUSIONS

In conclusion, CRP is widely used as a sensitive, nonspecific marker of inflammation both within the veterinary and human medical fields. Being that CRP is not specific for any particular disease, it should not be considered as a diagnostic marker for any specific disease but rather as a tool to objectively assess disease severity of inflammatory diseases in dogs. A multitude of canine-specific and human-specific CRP assays is available for the measurement of CRP in dogs, but the use of assays specifically developed for use in dogs is preferred. Also, each assay should only be used with its own reference interval, and when using the measurement of CRP longitudinally, the same assay must be used for each measurement. Lastly, when considering the uses of CRP in human medicine, a number of novel applications for this biomarker are promising, most notably reducing the duration of antibiotic therapy in dogs.

DISCLOSURE

The authors of this manuscript are affiliated with the Gastrointestinal Laboratory at Texas A&M University, which offers measurement of serum concentrations of CRP in dogs on a fee-for-service basis. Dr Steiner also serves as a paid consultant for IDEXX Laboratories, however, unrelated to CRP assays, which are offered by IDEXX Laboratories.

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How to cite this article: Covin MA, Steiner JM. Measurement and clinical applications of C-reactive protein in gastrointestinal diseases of dogs. *Vet Clin Pathol*. 2022;50(Suppl. 1):29–36. doi:10.1111/vcp.13100