Canine C-reactive protein in the 21st Century: Where are we now and where are we going?

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Abstract:

The acute phase response (APR) represents a reflex response to inflammation. Inflammation can be a result of infection, neoplasia as well as trauma. It comprises a series of sequential biochemical reactions resulting in the production of acute phase proteins (APP). This review article places focus on C-reactive protein (CRP) as a clinically relevant acute phase protein which forms the bedrock of acute phase reagents. The detection and quantification of CRP with sound interpretation provide valuable real-time information regarding the clinical state of the patient. In canines CRP is the major APP, with the most diagnostic relevance, likely the result of extrapolation from what is already known about CRP in humans.

C-reactive protein assays are becoming well-established, reliable and routine tests run daily in veterinary practices worldwide. The broad application of C-reactive protein for differing indications can be confusing and can threaten to overwhelm the practising veterinarian. The relative increase in CRP can be a helpful diagnostic tool. It has also been shown to be a valuable prognostic indicator and, in many diseases, a reliable method to monitor disease progression or response to treatment. This article categorises and summarises the available assays for CRP and how to apply them in daily practice in a manner accessible to the general veterinarian, ultimately allowing it to be leveraged maximally as a valuable and versatile diagnostic tool.

Introduction:

It is well known that the APR is an integral component of the innate immune system.[1] Medical academics have been investigating the APR since the early 19th century and its relevance began permeating through into veterinary medicine in the late 80's.[2] Since then our knowledge of the APR has expanded, but there are still many unknowns. Current knowledge suggests tissue impairment, or damage, induces the production and release of proinflammatory cytokines such as IL-1, IL-6 and TNF-α.[1] These cytokines have multiple roles including the activation of the liver, as well as other extrahepatic tissues[3], to produce acute phase proteins (APP).[2] These APPs are released into the bloodstream where they perform various roles, including immunomodulation and regulation of inflammation in the local tissues.[4]

There are several measurable APPs in canines, these can be categorized as either major, moderate, minor or negative APPs.[5] Major APPs are defined as: Proteins with negligible serum levels in a normal state, but will increase 10-1000 times (>25%) when stimulated.[6] Moderate APPs, haptoglobin and fibrinogen, only show a 2-10 times abnormal increase. Both major and moderate APP decrease at a proportionate rate, commensurately with the cessation of the inciting event.[4] Minor APPs demonstrate a small and slow increase followed by a delayed decrease in serum levels[2] and are neither sensitive nor specific. Negative APP, such as albumin are inhibited by cytokine-induced stimulation and subsequently decrease in response to tissue damage.[6] It is worth noting that the classification of APPs do vary in academic settings as the type of assay used can influence the detectability of the APP.[7] *Figure 1 demonstrates the acute phase proteins and how they respond to infection.*

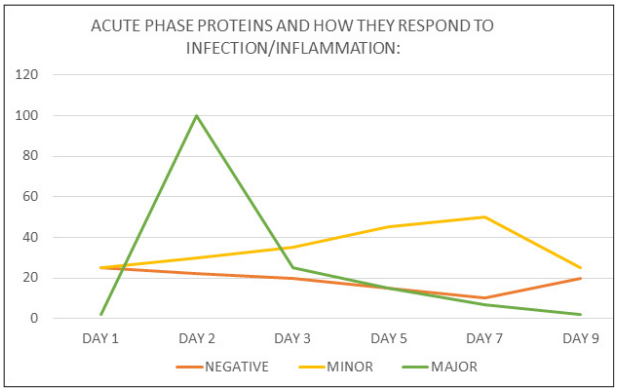

Figure 1

There are multiple variations in baseline levels and large variability between the differing APP's and their clinical importance.[8] The most comprehensively studied APP in canines are C-reactive protein (CRP), haptoglobin, α_1-acid glycoprotein (AGP) and serum amyloid A (SAA).[8] Canine CRP is the most widely tested APP in the clinical setting and forms the primary focus of this review. C-reactive protein is believed to play a role in meliorating opsonization, inducing complement and cytokines while impeding chemotaxis.[7] C-reactive protein production in response to cytokine induction, makes it highly sensitive to inflammatory conditions; however, given the non-discriminate nature of its production, the specificity is low.[4]

Body:

Where do we currently stand with CRP and its assays?
Immunoassays are the primary method used for detecting CRP in canines, with both species-specific antibodies and in some instances, heterologous antibodies with cross-reactivity amongst different species.[7, 9-13] A multiplicity of immunoassays have been employed to determine CRP in veterinary medicine namely; Immunoturbidimetry[9, 14], enzyme-linked immunoassay (ELISA)[10], magnetic binding immunoassay[11, 14], flow-type immunosensor assay[12-14] and dry immunochemistry (Catalyst)[15].

ELISAs have been validated for canine CRP measurement and are generally accepted as the standard for comparative studies, but there has not been an officially defined gold standard for CRP assays.[10, 13, 15, 16] Lack of a clearly and consistently defined gold standard leads to inconsistency with validation criteria when comparing different assays.[14] ELISA-based assays are expensive and time-consuming, taking up to 4 hours to process, this may limit their use in resource-constrained environments.[9, 15, 16] This has given rise to the development of a more accessible point-of-care assays (POCT) that require less turnaround time, simpler methodology and better economic benefit.[16, 17]

Human CRP immunoturbidometric assays have been refined over multiple years and adapted for veterinary use. Canine CRP is a glycosylated protein with variable molecular mass, in contradistinction to human CRP, which is non-glycosylated with a more predictable mass.[11] This could explain the poor cross-reactivity in some assays.

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Eckersall, Conner and Harvie developed an immunoturbidometric assay for canine CRP in 1991[18] which only became commercially available in the 2000s. In Japan, an immunoturbidometric assay for canine CRP was developed in 1998[19] and was made widely available for clinical use in Asia.[19, 20] Immunoturbidometric assays rely on introducing antiserum-containing antibodies to the sample.[18] The antiserum exhibits affinity for a selected canine CRP or to multispecies CRP.[18] The antibodies bind to available CRP forming an antibody/antigen complex which precipitates out to form a measurable change in the turbidity of the mixture that can be read by spectrophotometry.[18] This type of assay is rapid and can be used on automated chemistry analysers that handle multiple samples at a time making immunoturbidimetry the preferred testing method for most laboratories.[18]

The increasing relevance of real-time CRP measurements in a clinical setting has necessitated the development of several point-of-care assays.[11] Ibraimi et al. developed a magnetic binding immunoassay.[11] A magnetic nanoparticle is bound to a polyclonal anti-CRP antibody; CRP concentration is quantified by measuring the magnetic permeability of the precipitated antibody-antigen complex.[11]The study by Ibraimi and colleagues found that a cut-off of >20mg/L for detecting the presence of inflammation was 97% sensitive and yielded a specificity of 94%.[11]Flow-type immunoassays rely on the use of fluorescent labels that are bound to anti-CRP antibodies whose luminescence can be measured when exposed to ultra-violet light.[12, 13] A 2015 comparative study by Jasensky et al. compared an immunoturbidometric assay with a magnetic binding immunoassay and a flow-type immunoassay.[14] The findings showed that there were vast differences in the results between assays and each had differing reliability.[14] A more recent study in 2022 by Kubota et al. comparing a flow-type immunoassay to immunoturbidimetry and ELISA demonstrated equivocal performance for quantification of CRP.[12] A known limitation of flow-type immunoassays is their ability to detect very mild increases in CRP. When measuring CRP elevation in bodily fluids other than blood, a subtle rise in CRP can be significant, an example being cerebrospinal fluid (CSF).[21] Martinez-Subiela et al.[21] tested the performance of an immunofluorimetric assay, similar in concept to the flow-type immunoassay to detect subtle elevations in CRP in the CSF.[21] The study concluded that this technique showed superior sensitivity and accuracy when compared with ELISA and immunotuidometry.[21] The advent of dry slide assays on the Idexx Catalyst platform for CRP detection has substantially increased the accessibility of in-house CRP testing.[15] These assays use gold nanoparticles bound to species-specific anti-dog-CRP antibodies. The bound antigen-antibody complex then migrates in a predictable pattern and can then be measured using chromatographic technology.[17] Covin et al.[15] performed a comparative study of the IDEXX Catalyst CRP test where it was found to have comparative performance with immunoturbidometric assay, yielding satisfactory replicability and precision in measuring canine CRP.[15]

Table 1 summarises some of the advantages and disadvantages of available assays for CRP:

Assay type	Advantages	Disadvantages
ELISA	**Reliable and repeatable** **Large numbers run simultaneously** **Used as a benchmark for comparing CRP assays**	**Long time for results** **Expensive to run** **Requires dedicated lab** **Requires a high skill level**
Immunoturbidometric	**Rapid test time** **Multiple samples run in unison**	**Variations in available antibodies** **Variable reliability** **Calibration required between batches** **Reference labs only**
Magnetic binding immunoassay	**Rapid test time** **Suitable for POCT**	**Reliable at CRP >20mg/L but less reliable at lower levels**
Flow-type immunoassay	**Rapid test time** **Suitable for POCT**	**Smaller volumes** **High manual input required increasing the risk of processing error**
Immunochromatographic assay (including Catalyst)	**Rapid test time** **Available to already existing chemistry analysis machines** **Suitable for POCT**	**Reliable at moderate to high CRP levels but less so at low concentrations**

Table 1 has been compiled by the author based on the references listed in the reference list.

How can the measuring of APP be helpful to the general practitioner veterinarian?

The broad application and rapid development of CRP assays have led to an increased interest in its use as a diagnostic tool.[22, 23] Moreover, its use in prognostication and disease monitoring adds to its appeal in the armamentarium of the general veterinarian.[19, 22-24] Recent studies demonstrating how CRP can be exploited in general veterinary practice are discussed below. Keshet et al. found that in humans, CRP was substantially elevated in cases of bacterial infection, especially where sepsis was present.[25] In human medicine CRP >100mg/L has been used as an indicator of bacterial infection and may warrant initiation of antibiotic therapy.[26] C-reactive protein has become a regular diagnostic tool when screening for infection of hospitalised patients in human medicine.[4, 26]

Hindenberg, Bauer and Moritz conducted a study to investigate the relevance of a CRP >100mg/L in canines.[26] This study showed that there were multiple non-bacterial and even non-infectious causes for CRP >100mg/L including; immune-mediated disease, malignant neoplasia and trauma.[26] The study concluded that CRP cannot be used in isolation as an indicator for the initiation of antibiotic therapy in canines.[26]

In canines diagnosed with parvovirus enteritis (CPV), CRP levels have been shown to be a valuable prognostic indicator of mortality.[5, 27] In a single-centre study by Kocaturk et al. a CRP of >92.4mg/L had a sensitivity of 91% when predicting the likelihood of death at the time of admission.[27] C-reactive protein was measured using a clinically validated, human immunoturbidometric assay. The sensitivity is substantially superior to that of white blood cell counts (often suggested as a prognostic indicator for CPV) which had a sensitivity of only 52% in this same study.[27] A study by McClure et al. used serial measurements of CRP to determine the outcome of patients and found that values >97.3mg/L at 24 hours after admission had a sensitivity of 86.7% for predicting death.[5] This study concluded that consecutive CRP measurements proved more reliable for monitoring disease progression.[5] The value of CRP when treating parvovirus patients is twofold.

Firstly, CPV treatment can be labour-intensive and expensive, determining the prognosis assists in decision-making for both the veterinarian and the pet owner. Secondly, having an idea of how aggressively to manage a CPV patient, for example, when to initiate antibiotic therapy could potentially improve the chances of survival.

In cases of bacterial pneumonia, Viitanen et al.[28] found that CRP was predictably elevated in all cases, but more specifically the rate of decrease in CRP values correlated well with recovery.[28] Additionally, cessation of antibiotic therapy once CRP had normalised (mean of 21 days) did not increase the probability of relapse.[28] The importance of this study shows that the conventional recommendation of 35 days of antibiotic treatment[28] can be substantially reduced if CRP normalisation is used to guide treatment response. This limits long-term antibiotic side effects and unnecessary administration of potentially expensive medication. It is worth noting that this study used the magnetic binding immunoassay validated by Ibraimi et al. discussed earlier, which has a maximum reading of 210mg/L.[11, 28] This prevented the determination of prognostic cut-offs for disease severity in this study as many patients recorded CRP levels of >210mg/L at presentation.[11] Pancreatitis is a common clinical condition seen by veterinarians but marked variability in severity and the subsequent intensity of treatment makes clinical decision-making very challenging.[29] In humans with pancreatitis, CRP is used for prognostication.[29] Sato et al. found that CRP levels remained persistently high in the non-surviving patients even after five days of treatment.[29] The inclusion of CRP measurement when monitoring pancreatitis could help provide clinicians with a tool for clinical decision-making as it relates to hospitalization, treatment and recovery.[29] The utility of CRP to monitor response to treatment in patients with idiopathic polyarthritis (IPA) was demonstrated by Ohno et al. in 2006.[19] Symptoms for IPA are broad and non-specific.[19] This study showed that very high levels of CRP could help differentiate IPA from milder diseases with the same symptoms.[19] Moreover, CRP levels decreased dramatically 6-13 days after the initiation of corticosteroids making it a valuable indicator of response to treatment.[19] Steroid-responsive meningitis arteritis has been shown to induce a substantial acute phase response.[23] CRP will be elevated in both the serum and the CSF.[22, 23]

Classification	CRP (mg/L)	Pathology
Normal	0-12	
Mild increase	12-20	Mild inflammation Uncomplicated gastrointestinal disease Uncomplicated nasal disease
Moderate increase	20-39	Uncomplicated viral disease Inflammatory bowel disease
Marked increase	40-100	Parvovirus survivors (<100.3mg/L) Demodicosis Aspiration pneumonia Discospondylitis Pancreatitis
Severe increase	>100	Parvovirus non-survivors (>146.3mg/L McClure, >92.4mg/L - Kocaturk) Neoplasia (especially if malignant) Trauma Immune-mediated disease (IMHA, SRMA, IPA) Babesia Pyometra

Table 2 has been compiled by the author based on the references listed in the reference list.

Lowrie et al.[23] and Bathe-Noethen et al.[22] demonstrated that CRP has a very high sensitivity for detection of SRMA but, similar to other studies,[20] lacks specificity.[22, 23] The value of this knowledge is that a patient showing signs of neurologic disease and no evidence of increased CRP, in either serum or CSF, is highly unlikely to have SRMA.[22, 23] Both studies also showed a marked decline in CRP after initiation of corticosteroid treatment and this correlated well with patient recovery.[22] Serial CRP measurements for the early detection of post-operative complications have been suggested. Löfqvist, Kjelgaard-Hansen and Nielsen conducted a study to determine the significance of CRP in patients undergoing tibial plateau levelling osteotomy for the treatment of cranial cruciate ligament rupture.[24] This study found, with 100% sensitivity, that patients with normal CRP six days after surgery did not have infectious post-operative complications.[24] This knowledge should help guide the initiation of early post-operative intervention, such as antibiotic therapy when clinically appropriate. A different study attempted to determine if ovariohysterectomy resulted in a higher surgical stress than ovariectomy and included CRP measurements to represent surgical stress.[30] Both these studies eloquently demonstrate current and potential uses of CRP for post-operative patient monitoring as well as for the potential development of evidence-driven surgical technique standards of practice. Nakamara et al.[20] did a comparative study where they measured CRP in 928 canines with varying diseases and compared the CRP results with other inflammatory markers. The study demonstrated that CRP levels were highest with immune-mediated disease, haematopoietic neoplasia and those with severe metastasis.[20] *Table 2 shows some of the applications of CRP in a clinical setting.*

What does the future hold for APPs and CRP?

Harmonisation of standard calibration using a clearly defined gold-reference standard will enhance future validation of assays and increase the diagnostic accuracy of CRP.[14] It is the view of the author that increased awareness of the importance of CRP as a diagnostic adjunct will propel research and development, ultimately increasing its diagnostic utility. The use of human CRP antibodies has presented a limitation in the accuracy of canine CRP assays.[31] Evolving knowledge and the increasing clinical application of CRP as an APP will hopefully translate into increased accessibility and affordability of commercially available reliable assays.[31] The development of more sensitive assays, especially at mildly raised CRP levels, will afford us the opportunity to test for the presence of this inflammatory marker in bodily fluids other than blood, thereby furthering its diagnostic potential.[3, 22] Harnessing other acute phase reactants in conjunction with CRP has the potential to add specificity to a clinically suspected disease process.[31] This is exemplified in a study by Caldin et al.[32] which found that an increase in the APP haptoglobin alone, greatly increased the probability of hyperadrenocorticism as a diagnosis, compared to when haptoglobin and CRP were both raised.[32] It is with this knowledge that the development of multiplex assays, and testing for the presence of multiple APRs simultaneously could be of immense clinical utility.[31]

Discussion:

Understanding the physiological dynamics of CRP as an important APP is essential for the responsible interpretation of the analyte. Equally, comprehension of the unknowns regarding CRP response in differing disease processes is paramount to cautious analysis of the protein. The assay used to determine the CRP value can greatly influence the result obtained.[12-16] The cognisance of variability between the different available CRP assays underpins the importance of consistency in clinical practice.[12-16] Using the same assay when obtaining serial CRP measurements will help prevent inaccurately interpreting CRP trends, thereby facilitating the ability to reliably marry the clinical picture of the patient to the biochemical status.[12-16] This suggests that a single assay should be used for serial measurements to prevent biases inherent between different assays, additionally reference ranges and cut off values should be assay specific.[12-16] The author predicts that increased usage and awareness of CRP as a diagnostic tool as well as its inherent limitations will prompt further research and development into CRP and other APPs. The establishment of a universally accepted gold standard assay for CRP will help to homogenize research and raise both the sensitivity and specificity of these tests.

References available on request.

QUESTION 1

Which of the following are considered the primary functions of acute phase proteins in the innate immune response:

a. Activation of the liver to produce APPS
b. Regulation of inflammation
c. Immunomodulation
d. B + C
e. A + B + C

QUESTION 2

What happens to major, moderate and minor acute phase proteins after tissue damage?

a. They decrease at a proportionate rate
b. They increase at a proportionate rate
c. The remain stable
d. None of the above
e. All of the above

QUESTION 3

Why can the classification of acute phase proteins (APPs) vary in academic settings?

a. Because of differences in statistical analysis
b. Because of differences in research design
c. Because of differences in disease models
d. Because the type of assay used can influence the detectability of the APP
e. Because different laboratories use different assays

QUESTION 4

Which of these is not recognised as a function of C-reactive protein (CRP)?

a. Melioration of opsonization,
b. Induction of complement
c. Downregulation of immune response
d. Impeding chemotaxis
e. Induction of cytokines

QUESTION 5

Which of these immunoassays are considered point-of-care (POC) assays:

a. Magnetic binding immunoassay
b. Immunochromatographic assay
c. Immunoturbidometric assay
d. Flow-type immunoassay
e. A + B + D

QUESTION 6

Which of these endings would make the sentence incorrect: CRP is most helpful as a diagnostic tool when used to:

a. Monitor disease progression
b. Prognosticate a patient's likelihood of survival
c. Increase the sensitivity of your diagnostics
d. Monitor response to treatment
e. Help guide clinical decisions

QUESTION 7

If you are presented with a young patient with diarrhoea; which differential would be less likely if the CRP level was 90mg/L:

a. Parvovirus infection
b. Intestinal neoplasia
c. Pancreatitis
d. Inflammatory bowel disease
e. Bacterial enteritis

QUESTION 8

A canine presents with hindlimb ataxia and delayed proprioception. There is no history of trauma and you run a CRP and get a serum level of 200mg/L. Which of these differential diagnoses should be highest on your list:

a. Discospondylitis
b. Intervertebral disc disease
c. Steroid-responsive meningitis arteritis
d. Wobblers disease
e. A + B

QUESTION 9

What is predicted to be a very important step for future development and validation of CRP assays?

a. Increased use of human CRP assays on canine samples
b. The establishment of a universally accepted gold-reference standard assay for CRP
c. The establishment of a human-specific anti-CRP antibody
d. The development of assays that can measure extremely high levels of CRP
e. C + D

QUESTION 10

Which of these statements is incorrect:

a. CRP is a highly sensitive diagnostic tool
b. CRP is a highly specific diagnostic tool
c. CRP gives real-time information regarding the clinical state of a patient
d. CRP helps to detect and quantify the presence of inflammation
e. CRP is both sensitive and specific

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