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C-REACTIVE PROTEIN AND THE COMPLETE BLOOD COUNT: HOW TO MAXIMIZE THEIR VALUE IN CHARACTERIZING INFLAMMATORY DISEASE

Inflammatory disease is one of the most common clinical presentation seen in the veterinary practice; it may be associated with any organ system and may be caused by infectious and non-infectious diseases as well as trauma and immune-mediated disease. Inflammation may vary in presentation from very mild to extremely severe in degree. Although common, inflammatory disease is many times difficult to recognize because of various complicated conditions typically seen in conjunction with inflammation, namely, glucocorticoid influence (stress) and epinephrine influence (excitement). There are three primary changes that will assist the veterinarian in identifying inflammatory disease including recognition of significant clinical signs, observation of various complete blood count (CBC) changes and measurement of acute phase protein levels in the plasma/serum.

Clinical signs can be anywhere from completely occult in nature to extremely severe observations. Evolutionarily, our domestic animals have learned not to show any evidence of deviation from health; the weak appearing animal historically has been more vulnerable to removal from the pack. Classic signs associated with inflammatory disease may include any or all of the following: redness, swelling, heat and pain. These signs oftentimes prove helpful in localization of the inflammatory condition. For example, a dog presenting with fever and localized pain in the region of the pancreas during abdominal palpation has a high probability of pancreatitis or inflammation to any condition in that region. If clinical signs are not evident or are extremely subtle as is commonly the case with many inflammatory conditions, there are no findings to aid the veterinarian in identifying the inflammatory process.

Changes in the CBC are something the veterinarian believes is quite common, especially with more severe active inflammation, but interpretation of the leukogram (the white blood cell compartment) can be quite confusing since there is no single leukogram picture that supports inflammatory disease. Most veterinarians look for either an increase in the total white blood cell (WBC) count or the absolute neutrophil count. There are two primary problems with using this leukogram picture to identify active inflammation. First, more than 50% of the CBCs from animals with active inflammation will have total WBC and neutrophil counts within reference interval limits. Second, both simple glucocorticoid influence (stress) and epinephrine influence (excitement) can result in increases in both total WBC and neutrophil counts. The neutrophil is the key cellular element in identifying active inflammation, but the most significant change is not numeric but morphologic in nature. The presence of immature and/or toxic neutrophils are the hallmark of inflammation. These may be seen with decreased, normal and increased total neutrophil counts. Because most reference laboratory and in-clinic hematology analyzers cannot accurately identify these forms of neutrophils, the primary way in which they are identified is through microscopic evaluation of a peripheral blood film. Since the vast majority of CBCs in veterinary medicine do not have a blood film review, there is no way to identify the active inflammatory processes when there is no increase in neutrophil numbers.

However, remember also that there are non-inflammatory conditions that can result in increased neutrophil numbers; therefore, simple identification of a neutrophilia (neutrophil counts above the upper reference interval limit) is not adequate to identify inflammation. There are many false positive identifications of inflammation based merely on neutrophil numbers. There is a need for a more objective indicator of immature and/or toxic neutrophils and with a relatively new in-clinic hematology analyser (IDEXX ProCyte Dx™) and a reference laboratory hematology analyser (Sysmex XT-v) there are cytogram (dot plot) changes that provide this insight. Evaluation of cytograms is something that most veterinarians and veterinary nurses have not been trained on; however, once these professionals are exposed to the features of the cytogram pattern associated with active inflammation, recognition of these patterns is relatively simple. Below is an example of a normal dog dot plot from a ProCyte Dx run as well as two samples from dogs with mild to moderate active inflammation. Note one of the obvious pattern changes related to the cluster of digitized events representing the neutrophil population. The pale purple cluster of dots are neutrophils, which in the normal dot are extremely similar to one another and therefore in a tight cluster. The y-axis of the dot plot represents cellular nucleic acid (DNA and RNA) and the primary

difference between the three images is the location of the neutrophil cluster on this axis. The greater the severity of inflammatory disease, the higher the digitized events are on the y-axis because they represent increasing numbers of immature and/or toxic neutrophils as well as increase immaturity. Immature and/or toxic neutrophils have more cytoplasmic RNA than mature neutrophils.

There is clearly more information in these cytograms, but the focus in this discussion is the identification of immature and/or toxic neutrophils.

Measurement of acute phase proteins provides even greater objectivity in characterizing the active inflammatory process in the dog. Acute phase proteins are proteins that either produced in either increased or decreased amounts during the active phase of inflammation. In many cases, the induction of increase production of these proteins is stimulated by various inflammatory cytokines released in the inflammatory process. The acute phase proteins can rapidly drop or increase in amount in the plasma/serum as well as return to normal extremely rapidly as the inflammatory process subsides. Most veterinarians are aware of fibrinogen as an acute phase protein; however, this protein has several problems as an indicator of active inflammation. First, decreases as well as increases can occur during inflammatory disease and the time to response during the initial phase of inflammation or during the resolution of the inflammatory process is retarded compared to the more classic acute phase proteins. The classic proteins include C-reactive protein, serum amyloid A, haptoglobin and several others that are less commonly used in the dog.

Measurement of acute phase proteins routinely in veterinary medicine was limited because of the unavailability of validated assays and originally, these were only available at the commercial or academic reference laboratories. In recent years, several in-clinic methods have become available and the interest in acute phase proteins is increasing. C-reactive protein (CRP) has taken the lead in canine medicine while serum amyloid A is used in feline medicine. Patterns of response (increases and decreases) is different for different acute phase proteins with different species.

The key with defining the presence or absence of active inflammation as well as to characterized the severity of the inflammatory response itself is to not use only one of the three primary means of detecting inflammation. Each of the three (clinical signs, CBC changes and acute phase protein measurement) means of detecting inflammation should be used together to maximize the characterization of inflammation.