BIONOTE
Precise Diagnostics for Improved Care

Identification of Inflammation and Cardiac Disease in Everyday Practice: CRP, SAA and NT-proBNP

presented by dvm360

with **Kendal Harr, DVM, MS, DACVP**

Summary

In January of 2021 Kendal Harr, DVM, MS, DACVP along with dvm360's Adam Christman, DVM, MBA discussed the remarkable benefits of C-reactive protein (CRP), serum amyloid A (SAA), and N-terminal pro-brain natriuretic peptide (NT-proBNP) in-clinic testing. Below is the outline of the webinar presentation.

What is a Biomarker?

- A screening diagnostic analyte that indicates inflammation/infection, environmental exposure, or other disease.

Inflammation

- Cellular/Adaptive
  - Mediated by T cells the Communicators
  - Takes weeks for cellular mediated immunity
  - Has long term memory

Inflammation

- Innate immunity (rapid response)
- Innate proteins
  - Complements:
    - Classical (C1r/s, C2, C4, C4bp)
    - Alternative (C3, factor B)
    - Lectin (MBL, MASP1-3, Map19)
    - Terminal (C5, C6, C8, C9)
    - Soluble regulators - (factors I, H, C1 inhibitor)
  - Other Opsonins
    - CRP, SAA, SAP
  - Soluble factors for LPS signal
    - LBP, sCD14, sMD-2
  - Iron metabolism related proteins
    - Transferrin, Lipocalin-2
    - Hemopexin, Hepcidin
  - Others:
    - Fibrinogen, PGLYRP2

Functions

- Directly kills bacteria
- Acts as opsonins
- Enhances LPS signaling
- Blocks iron update and then kills bacteria
- Activates innate immunity by targeting liver non-parenchymal and immune cells

Acute Phase Proteins

All have functions that support inflammation and healing

Positive APPs	Negative APPs
C-reactive protein (CRP)	Albumin
Serum Amyloid A (SAA)	Transferrin
Haptoglobin (Hp)	Transthyretin
Ceruloplasmin	Retinol-binding protein
A2- Macroglobulin	***Note: Negative Acute Phase protein "decreases" in inflammation
A1- Acid glycoprotein (AGP)	
Fibrinogen	
Complement (C3, C4)	

Differing Concentration Changes

- Negative vs Positive
- Minor, Major and Classic patterns of increase
  - Dependent upon fold increase
- Better diagnostic with trace, baseline concentration and 10-to 100-fold or higher increase.
  - Classic response to bacterial pathogens

"Classic" Acute Phase Proteins

- Trace baseline to 100-fold increase
  - Dogs:
    - CRP (C Reactive Protein)
    - SAA - Major
  - Cats:
    - SAA (Serum Amyloid A)
  - Horses:
    - SAA (Serum Amyloid A)
  - Cows:
    - Hp (Haptoglobin)

These are interesting, but how do I use them in practice?

- Isn't this just more money for the same information?
  - Better information
  - Replaces CBC in a majority of cases
  - Complete blood count is often not needed

Maximize Diagnostic Sensitivity and Specificity– But How?

- Trace baseline concentration
- Minimal overlap
- In ER settings, WBC has repeatedly proven to have a sensitivity <50% i.e., More than half of severe inflammatory cases have a "normal" WBC concentration using good to excellent equipment.

C-Reactive Protein

- Named in the 1930s - isolated from human patient plasma because it reacted with Pneumococcal C-polysaccharide in the acute phases of pneumonia
- Functions as a signal for phagocytes and increases the rate of phagocytosis of bacteria
- Promotes binding of complement to bacteria, which initiates the complement cascade, induces cytokines

Is There Systemic Inflammation in the Animal?

- Is there real inflammation/infection?
  - AKA Do I need to give antibiotics?
- Stress induced? Or Low or Normal due to Consumption?

CRP in Dogs

- <10 mg/ml (trace concentration) in normal dogs
  - Some variation in literature on Ref Int likely due to different methods
- Markedly improved Diagnostic Specificity
  - If CRP is increased, there is inflammation.
- Half-life = around 6 hours
  - Can normalize from a 4 - 5 fold increase in a day
  - Usually indicates active inflammation

Concentration Change Varies with Disease

- Highest fold increases - gram neg bacteria
  - LPS – lipopolysaccharide is strong inducer
  - 100 to 1000-fold
- Viral does induce usually 5-to 20-fold change
  - Higher concentrations often due to secondary bacterial infection
- Neoplasia (especially hematopoietic and lymphoma) may induce mild increases.
- Significant tissue trauma (HBC, surgical, etc.)
  - May induce increased concentrations

CRP Cardiac Dz vs. Inflammation

- CRP - in human medicine
  - Linked to the formation of atherosclerotic plaques
- Markedly smaller changes in CRP concentration in cardiac disease than inflammation
- Cardiac assessment needs high sensitivity assay
  - -0 to 10mg/ml (trace)

Diagnostic Sensitivity and Specificity

Human Neonatal Septicemia after 3 days	CRP (88%/87%), WBC (74%/56%)
Adults with Acute Appendicitis	CRP (93%/87%), WBC (85/75%)
Human Adults with Acute Septic Peritonitis	CRP (74%/94%), WBC (52%/86%)

Canine CRP Diagnostic Sensitivity and Specificity

- In general, increased sensitivity and specificity in every inflammatory dz studiede
- DOES NOT diagnose and will not predict/ prognosticate noninflammatory disease
- Systemic inflammation – not peripheral or walled off.
  - Not great in assessment of GI tract or limb lesions.
- Canine Bacterial Cystitis CRP (92%/86%), TWBCC (not correlated)

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SAA - Serum Amyloid A

- Functions in healing and homeostasis
- Signal protein for numerous lipoproteins
- Allows passage of lipoproteins:
  - Into GI tract for removal
  - To inflamed lesions for healing, restructuring,and new cell formation
- Has a role in atherosclerotic plaques
- SAA can exchange between all lipoproteins
- In the absence of CETP, SAA [refers HDL]
- CETP activity increases SAA exchange to VLDL
- The presence of SAA increases lipoprotein binding to proteoglycans

SAA Immunomodulation in Horses

- Classic responder in horses
- Macrophage -> IL-6 -> Liver -> SAA -> T cell proliferation and adhesion/ Platelet aggression/ Neutrophil activation/ Immune cells migration/ Prostaglandin secretion

Is the Animal Anemic?

- PCV not HCT - most accurate across species
- Centrifuged not calculated.
- Cheap and a ton of good information
- Is there real inflammation/infection?
  - AKA Do I need to give antibiotics?
- Stress induced? Or Low or Normal due to Consumption?

Hematology Analyzers Give Misinformation

- Platelet Counts
  - False Thrombocytopenia
  - Need to look at feathered edge of slide to confirm
- Differential Cell Counts are not accurate
- Veterinary Technician Error

Error in Platelet Measurement

- Over 50% of animals at UF admitted for thrombocytopenia over two years had normal platelet counts at UF clin path
- Machines are Designed for Humans
- Dog and Cat Clotting Times are Faster
- Clumping on Smears = Decreased Cell Counts
  - Animal can likely clot well especially if counts are >100K
- Confirmation Before Referral!
- Must make slide immediately
- Must look at slide, preferably by a certified hematologist or boarded clinical pathologist

Differential Cell Counts

- Trusting automated differential cell counts is a bad idea.
  - Especially in sick animals
- Bands are erroneous if counted at all
- Total Error for Monocytes, Eosinophils and Basophils
  - 50%
  - 1000 cells/µl = 500-1500 cells/µl
  - That's when it's functioning well!

Bionote NT-proBNP vs. Imprecision

- The only company in the world to offer precise, quantitative results for Canine or Feline NT-proBNP in-clinic

NT-proBNP Instrument to Instrument Comparison

- Archived frozen EDTA plasma (Idexx) or serum (Bionote) samples were sent to referral laboratories on 12–to 24-hour FedEx.
  - Arrived frozen
- Most values were higher than the original values generated in an Idexx study.

NT- proBNP Real World Comparison

- Fresh EDTA plasma (Idexx) or serum (Bionote) samples were collected during the same venipuncture from dogs diagnosed with valvular disease or dilative cardiomyopathy by ECG.
- Practitioners analyzed sample in-house using Bionote assay or sent out to Idexx for NT-proBNP analysis at a reference lab.
- In all cases, manufacturer directions for sample handling, transport and analysis were followed.

NT- Marked Difference between Controlled Best-Handling Practice and Real World Analysis