Studies suggest that AKI and CKD are closely associated and connected with common risk factors and disease modifiers. This association between the two highlights the importance of having biomarkers that reflect functional and structural damage early to prevent further progression.

The most accurate assessment of renal function is measurement of GFR and it is the most sensitive method for early detection of kidney dysfunction, directly proportional to functional renal mass. These measurements are costly and time consuming and not suitable to be performed in most practices, although iohexol assays are now available.

Biomarkers are usually non-specific, since some protein markers are secreted by multiple tissues, and therefore it is important to target differences that are specific to a disease process in order to avoid false positives. Ideal biomarkers for detection of kidney disease need to be specific with known reference limits, non-invasive, accurate, and sensitive in order to detect disease early. They should allow the recognition of severity of disease, be suitable for monitoring, localise the injury, clinical outcome and prognosis, have a reasonable cost and be rapidly available from a reference laboratory or cage side.

**MARKERS OF GLOMERULAR DYSFUNCTION**

Serum creatinine is a standard test for kidney function although it is insensitive, since increases in serum creatinine usually remain within the reference range, until there is a reduction of 39%-68% in GFR. Usually, large changes in GFR early in the disease process result in no to minimal increases in creatinine, whereas in end stage disease small changes in GFR result in marked increases in creatinine. Serum creatinine is dependent on factors that are independent of kidney function. Since creatinine is a product of muscle metabolism, it varies with muscle mass, meaning that in young animals or those with poor muscle mass function is overestimated, whereas in mature or well-muscled animals it may result in false diagnosis.

There are several issues with the analytical measurement of creatinine. Haemolysed samples have an increased release of non-creatinine chromogens that cause an overestimation of creatinine, whereas lipemic and icteric samples can falsely lower the measurement of creatinine. It has been shown that several drugs, cephalosporins, aminoglycosides, trimethoprim and phenacemide, cause false increases in creatinine levels.

Events that can increase the creatinine value independently of GFR include the ingestion of cooked meat products. Biological processes increase creatinine concentrations independently of the kidney function i.e. hydration status, changes in tubular secretion and alterations in transport.

**Symmetric dimethylated arginine (SDMA)** and asymmetrical dimethylated arginine (ADMA) two isomers, related with a posttranslational modification of the protein arginine in mitochondria that are then released into the circulation. Approximately 80% of ADMA is used in enzymatic pathways and therefore is a poor marker for function. However, SDMA is eliminated by renal filtration and excretion, which makes the serum concentration dependent upon changes in GFR. An advantage of SDMA is that it is not influenced by muscle mass. A SDMA level above reference range corresponds to 40% loss of measured GFR. SDMA is an earlier marker of kidney dysfunction comparing to creatinine.
Serum SDMA concentration is independent of other processes or diseases. The IRIS recognized SDMA as a biomarker for kidney function in dogs and cats, and a clinical chemistry assay, developed by IDEXX, can be used for diagnosis.

A study showed no significant difference in the plasma concentration of SDMA between dogs with AKI and dogs with CKD, GFR is decreased in both AKI and CKD, and therefore cannot differentiate. Despite this, plasma SDMA concentration is an appropriate biomarker for recognizing AKI and CKD, since dogs with AKI or CKD have a markedly elevated SDMA concentration compared to healthy dogs.

**Cystatin C**: Is a non-glycosylated protein, which inhibits cysteine proteases, produced at a constant rate by nucleated cells, and it is filtered through the glomerulus and reabsorbed in the proximal tubules. The serum concentration of Cystatin C determined by GFR, because of its low weight and constant production rate, it increases when proximal tubular damage occurs. Studies have shown that higher urinary concentrations of Cystatin C can be due to massive proteinuria since it leads to inhibition of Cystatin C tubular reabsorption, and therefore measurements of total proteinuria is required. Limitations to the use of Cystatin C as a marker of GFR include thyroid disorders and glucocorticoid therapy, which may affect cystatin C independently of kidney function.

**Albumin**: Due to its size and the glomerular selective permeability, albumin is not usually present in large quantities in the glomerular filtrate. Since, it is almost completely reabsorbed by tubular epithelial cells, albuminuria frequently represents kidney dysfunction either by glomerular damage, which increases the leakage of albumin or by tubular damage, which decreases the ability of the nephron to degrade the albumin in the glomerular filtrate. The standard screening test to detect proteinuria is urine dipstick. Albuminuria is not affected by microscopic haematuria, and it is more likely to appear in dogs with pyuria and concurrent haematuria or bacteriuria. In critically ill dogs, microalbuminuria, which corresponds to a concentration of albumin of > 1mg/dl, is associated with shorter survival, while in cats, it is associated with the presence of an underlying condition e.g. neoplasia, infections, inflammatory or immune-mediated diseases and endocrine disorders. Microalbuminuria can appear in non-renal conditions, and therefore it is not specific for diagnosing renal conditions. Although marked albuminuria is typically related to glomerular disorders.

**Immunoglobulin G**: is a high weight protein, which has an important role in humoral responses and it is unable to pass an intact glomerular barrier. Therefore, detection of higher concentrations of IgG in urine indicates glomerular injury.

**C-reactive protein (CRP)**: Is an acute phase protein, and therefore its serum concentration increases in inflammatory conditions. Due to its size, it is not able to pass an intact glomerular barrier, so the presence of CRP in urine is the result of glomerular dysfunction. Studies have shown that for CRP to appear in urine, its serum concentration must be increased and the glomerular barrier must be sufficiently damaged to allow high weight protein filtration.

**MARKERS OF TUBULAR INJURY**

**Urinary enzymes**

**Gamma-glutamyl transpeptidase (GGT)**: A proximal tubular enzyme. Is influenced by urine pH and therefore that it is also influenced by gender in dogs.

**N-acetyl-β-D-glucosaminidase (NAG)**: A lysosomal enzyme, present in the renal proximal tubule cells. Studies show that NAG has some important characteristics that could lead to it having value for use as a biomarker, including no circadian variations in urinary NAG (uNAG) excretion in dogs and cats, no significant difference in uNAG in young and older healthy dogs, no differences in gender in dogs and cats, it is not affected by urine pH in dogs, and its urinary concentration is similar whether it was a collected by cystocentesis or free-catch.

**Alkaline phosphatase**: An enzyme located in the brush border of the proximal tubular cell, increases in
urine has been associated with proximal tubular damage in dogs.

LOW MOLECULAR WEIGHT PROTEINS

Retinol binding protein (RBP): A low molecular weight protein synthesized in the liver and circulating in plasma transporting retinol, where 90% of the complex is bound to transthyretin, preventing the passage of this complex through the glomerulus. The free fraction is filtered through the glomeruli and is reabsorbed in the proximal tubules and then catabolized, so increases in urinary RBP may occur in dogs with proximal tubule dysfunction.

Microglobulins: Are proteins that are filtered through the glomerulus and reabsorbed by the proximal tubules. An increase in alpha1- microglobulin urine concentrations, occurs when reabsorption is reduced, due to disturbance in tubular function. This microglobulin is stable at different urine pH values and at room temperature. Studies show that urine beta2-microglobulin : creatinine ratio increases prior to azotaemia, being an independent predictor of GFR. However, this microglobulin has a limited utility due to poor thermic stability and instability at acid pH.

TUBULAR PROTEINS

Clusterin: A glycoprotein. Is expressed by multiple tissues and it part of several physiologic processes, such as sperm maturation, lipid transportation, complement inhibition, tissue remodelling, membrane recycling, stabilization of stressed proteins, and is an inhibitor of apoptosis.

Clusterin is expressed in urine at low levels and these levels increase significantly if injury occurs. Studies show that urinary clusterin levels decrease upon recovery. Contamination of a urine samples with blood can lead to false positives. This contamination brings nonspecific clusterin isoforms into the sample, and therefore it is not possible to measure kidney specific clusterin. Contamination, even small, is almost impossible to avoid, it can occur due to infection, trauma, neoplasia, inflammation and contamination during catheterization and cystocentesis. Despite, these issues, urinary clusterin can be a sensitive and specific marker for active injury, when specific clusterin is measured.

Inosine: Renal hemodynamics may depend on the adenosine concentrations in the interstitium, when the adenosine concentrations increase due to an inhibition of cellular uptake of adenosine and the circulating concentrations decrease, this leads to a decrease in renal blood flow and GFR. Usually the adenosine concentration in the interstitium is low, but during hypoxia and inflammation it increases due to release from injured or apoptotic cells. Studies show that in dogs the interstitial adenosine is converted into inosine during hypoxia. Results suggest that inosine is a sensitive biomarker for injury and also response to injury restoration of the normal circulating concentrations, correlate with recovery.

Neutrophil gelatinase-associated lipocalin (NGAL): A glycoprotein that binds siderophores, which are iron containing ligands. It was initially purified from neutrophils during infection and inflammation and is found in several tissues, such as skin, alveolar and oral mucosa, adipose tissue, and proximal and distal tubules. In injured organs, including kidney, stomach, colon, liver, trachea and lung, and in neoplasia this protein is induced, its function is to bind extracellular iron, thereby inhibiting bacterial growth. This protein can bind siderophores from prokaryotes, functioning as a bacteriostatic, and eukaryotes by helping carry iron across cellular membranes for cellular proliferation and differentiation, and is also involved in the attenuation of apoptosis.

In humans, plasma and urine NGAL concentrations are used as a marker of AKI, even in people with underlying CKD, since these concentrations increase with renal damage. NGAL is one of the earliest biomarkers in ischemic and nephrotoxic animal models of AKI.

Studies have shown that in dogs NGAL concentration increases earlier than serum creatinine in AKI. It increases dramatically when the renal insult occurs, and then gradually decreases with time, measurable only hours after the injury occurs.
An increase in urinary NGAL concentration may predict kidney injury in dogs after surgery, in comparison to serum creatinine for the diagnosis of AKI at 48h post-surgery. A study conducted by Steinbach et al revealed that dogs with renal azotaemia had higher plasma and urine NGAL concentrations compared with healthy dogs. Other results, from this study were that plasma NGAL seems to be less sensitive compared with urinary NGAL. In this study the increase in plasma NGAL in dogs with AKI could be due to 2 mechanisms, the up-regulation of inflammatory genes, one of them which codes for NGAL, and the reduced filtration rate which leads to reduced clearance of NGAL and therefore systemic accumulation. NGAL circulating concentrations may be influenced by coexisting conditions, such as CKD, chronic hypertension, systemic infections, inflammation, anaemia, and hypoxia. Urinary NGAL concentrations correlate with serum creatinine concentrations, GFR and proteinuria.

Sepsis increases NGAL expression in the kidney, leukocytes and liver, and therefore urine and blood concentrations increase, in the absence of AKI. Cortellini et al confirmed that sNGAL is increased in dogs with sepsis. The inability of NGAL to distinguish between the presence of AKI and systemic inflammation in septic dogs, suggest it is a poor marker of AKI in these patients. Further studies are required to assess if NGAL can be used as a predictor of AKI in cases nephrotoxicant ingestion. Any questions CVS stand 124!

REFERENCES
