Is Progressive Chronic Kidney Disease a Slow Acute Kidney Injury?

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Key points

• Novel kidney biomarkers can expose subtle and subclinical kidney disease that may otherwise remain undiscovered with conventional diagnostic assessment.

• Active kidney injury biomarkers have the potential to establish a new understanding of traditional views of chronic kidney disease (CKD), including its early identification and possible mediators of its progression.

• Sensitive and specific biomarkers are likely to lead to insights into clinical kidney disease and facilitate new diagnostic and therapeutic approaches.

• Therapeutic approaches may be monitored to biomarker endpoints and logically adjusted or extended until the biomarker activity is nullified.

The 2015 IRIS Napa Meeting

In May, 2015 the International Renal Interest Society (IRIS), a veterinary society established to advance the scientific understanding of kidney disease in small animals, sanctioned the 2015 IRIS Napa Meeting. The meeting was conceived as a highly focused strategic planning forum composed of many of the profession's recognized leaders in nephrology[a] to direct recognition and innovative solutions to evolving critical issues in veterinary nephrology. The theme of the meeting was, "Re-evaluation and Understanding of IRIS Stage1 and Grade I Kidney Disease as Predictors of Progressive Kidney Disease." Since the inception of the IRIS CKD Staging and AKI Grading

systems to categorize and stratify kidney disease in animals[1,2], there has been confusion and misunderstanding of the value and clinical utility of these early (non azotemic) categories. The clinical relevance of these categories has been questioned, as has the justification to embed asymptomatic and non azotemic patients in the overarching understanding of kidney disease. A significant focus of the Napa Meeting reflected on the relevance of early kidney disease and the importance of its recognition to the subsequent fate and outcomes of kidney disease discovered by conventional practices and methodologies.

The recognition of early, asymptomatic kidney disease is generally established on the basis of directed screening of at risk animals or as an incidental observation from routine testing of animals for other purposes. What criteria define which animals to screen? What evaluations are most accurate and sensitive on a screening panel? Is there value to establish evidence-based risk assessment algorithms that generate a risk score providing cost-effective predictions to guide practice patterns for screening CKD Stage 1 and/or AKI Grade I? Answers to these questions will help to establish the diagnostic and clinical significance to Stage 1 and Grade I kidney disease.

Currently, kidney disease is documented and stratified by use of function markers that may reflect slow states of transition or relatively steady-state conditions. Conventional practice pattern rely on urine specific gravity, proteinuria, serum creatinine, and symmetrical dimethylated arginine (SDMA) to reflect chronic kidney disease (CKD) that may be static (non-progressive) or may be active and variably progressive. We have relied on serum creatinine for at least a century to predict the

adequacy or inadequacy of kidney function with relative utility.[3] More recently SDMA has become available to complement some of the short-comings of creatinine, but additional time is required to duly establish its role and acceptance. (See Relford et. al. and Yeramilli et. al. in this edition) Despite familiarity with creatinine for the diagnosis of kidney disease, its utility has been constrained by the misperception it is blinded to early and subclinical kidney dysfunction and its excessively broad reference range for dogs and cats.[3] IRIS CKD Stage 1 encompasses the normal reference range for creatinine which creates confusion identifying this stage as CKD. Documented CKD often is associated with the bias that the disease is advanced at the time of diagnosis, and the kidney fate is predetermined. However, a patient recognized in a more advanced stages of kidney disease, by logical extension, must have started from or passed through a lesser category of kidney disease prior to its recognition. Kidney disease that progresses from IRIS CKD Stage 1 to higher stages likely has undergone an episodic or ongoing active process promoting the progressive erosion of steady-state function.

A functional diagnostic marker may fail to detect the underlying active component if offset by renal reserve, or if it is matched by compensatory adaptation of the residual kidney mass. Only when active injury outpaces repair or compensatory adaptation will the progression become evident.[4] Equally important, many animals with recognized CKD maintain relatively "static" or non-progressive kidney function over extended periods of time.[40, 41] These patients may justify different diagnostic, monitoring, and therapeutic attention. An important outcome of the Napa Meeting was

establishment of a strategic hypothesis for the recognition and understanding of early (IRIS CKD Stage 1) kidney disease. Specifically, this hypothesis underscored the likelihood for underlying active kidney injury contributing to the risk for progression of CKD; or lack of an active component leading to stable kidney function.

The Napa Meeting explored potential relationships between CKD and acute kidney injury (AKI). Both IRIS CKD Stages 1 and IRIS AKI Grade I represent early kidney disease states which may not be recognized until they proceed to a more advanced classifications. If progressive CKD is associated with active episodic or ongoing injury to the kidney, and if AKI is linked to progressive CKD (see below), could both these disease syndromes be explained by the same pathological process or processes progressing concurrently at different rates to establish both categories? Should early CKD and AKI be viewed as interconnected rather than separate clinical conditions? To this issue, the following questions relative to early CKD and early AKI were proposed by the participants:

1. Are IRIS CKD Stage 1 and IRIS AKI Grade I similar processes developing at different rates, or are they separate processes that should be viewed as interconnected? "Is progressive CKD a slow-moving AKI"?
2. Is IRIS CKD Stage 1 an active condition leading to progressive CKD or an inactive condition associated with stable kidney disease?
3. Is IRIS AKI Grade I a marker that could proceed to a rapidly progressive AKI or a slowly progressive CKD?
4. Are there better future definitions for IRIS CKD Stage 1?

The answers to these questions forecast the need to develop diagnostics to better distinguish active versues the absence of active kidney injury and progressive versus static CKD and to document the pattern and causes of progressive CKD. If early kidney disease (IRIS CKD Stage 1) could be identified as active or inactive, there would be potential to alter the management and monitoring of the patient to minimize its progression to more advanced stages.

Is there a need to distinguish two specific types of kidney disease: acute versus chronic?

Historically, kidney disease has been broadly defined into two seemingly distinct categories, chronic kidney disease (CKD) and acute kidney injury (AKI).[5, 6, 14] Each category of disease has distinctive features and has been defined by unique categorization schemes, the IRIS CKD Staging system for CKD and the IRIS AKI Grading system for AKI.[1, 2] Chronic kidney disease is perceived as slow in onset, characteristically progressive over time, and irreversible; whereas AKI develops rapidly and maintains the potential for repair and return of kidney function. Recently, these categories of kidney disease have been shown to interrelate, and their distinctions have become blurred at their interface. Chronic kidney disease is a known risk factor for the development of AKI, and AKI is recognized increasingly as a potential mediator for progressive CKD and end-stage kidney disease.[13, 14]

Chronic kidney disease is defined by sustained functional and/or structural damage to the kidneys over a course greater than 2 to 3 months.[1, 5] Decreasing kidney function recognized by reductions in glomerular filtration rate (GFR) or estimation of GFR by increasing serum creatinine or SDMA are the most common features identifying CKD in animals.[5, 7] (See Relford, et. al. in this issue) Chronic kidney disease is established initially by a singular or a combination of insults to the kidney that result in irreparable structural or functional damage to renal parenchyma that is repaired incompletely and promotes variable kidney dysfunction.

Progression, or sustained worsening of kidney function over time, is a hallmark of CKD in most animals, but its pathogenesis remains elusive and likely is multifactorial. Equally elusive is the explanation why some animals with CKD maintain relatively stable kidney function and apparently fail to progress. These differences in behavior may hold clues to explain the mechanisms of progressive CKD as well as therapeutic targets and intervention points to halt the process. Multiple risk factors for developing CKD have been documented including age, hypertension, proteinuria, infectious agents, endocrine disease, breed predilections, AKI, and heart disease among others. Progressive CKD is a natural consequence of an inability to resolve these risks or other co-morbidities in animals with established CKD. Identification of progressive CKD in the absence of these identifiable co-morbidities or following resolution of initial insults has been more intangible.

To date, the timely recognition of animals with progressive CKD and those with static CKD has been constrained by the relative lack of early diagnostic predictors. Serum creatinine and SDMA are relatively static markers of kidney mass reflecting steady-state predictions of kidney function which may include renal reserve, compensatory adaptations to ongoing nephron loss in addition to discrete reductions of functional kidney mass. Neither of these "static" (functional) kidney markers are sufficiently sensitive to signal early, subtle, and potentially ongoing kidney injury which may evolve slowly with a resultant decrease in functional renal mass. Other predictors of progression need discovery and validation. Proteinuria has been associated consistently with risks for progression in both human and animal patients with CKD. Urinary protein excretion has been shown to correlate with progressive CKD in cats and dogs, but urine protein:creatinine ratio has low specificity for CKD progression, and it is unclear if proteinuria is a marker for progression or participates in it development.[8] Currently, there are few criteria in veterinary patients to project the risk for progression of CKD. Prediction models in human patients are similarly problematic and subject to bias.[9]

Sustained and overt damage to the glomerulus or tubulointerstitium from primary diseases like hypertension, glomerulonephritis, or chronic pyelonephritis which are not fully resolved promote an expected progressive damage and an expected loss of functional kidney mass. The risks, patterns, and mechanisms for progression from seemingly occult CKD remain unknown. Progression of CKD to end-stage

kidney disease could occur step-wise from sporadic active insults which are either overt or subclinical and of similar or differing etiologies. These episodic injuries could promote cumulative damage resulting in further dysfunction and concurrent reduction of GFR and GFR markers. (Figure 1) Alternatively, metabolic or signaling disruptions to kidney structures intrinsically associated with the establishment of CKD or AKI could promote ongoing stresses or disordered metabolism within the residual renal mass which perpetuates sustained injury with a varying time course and further loss of kidney parenchyma and function. The respective prevalence and influences of these alternative pathways is unknown. Similarly, the underlying mechanisms participating in progression have been hypothesized widely but remain largely undefined.[9, 10] Regardless of which pattern or mechanism of CKD progression prevails in an individual patient, all appear to share a common feature of active stress or injury to the residual structures of the kidney.

There is accumulating evidence from a variety of models of AKI proposing a sequence of effective adaptive or maladaptive events in cellular repair that likely influence the prevention or predisposition to progressive CKD.[4, 11-18, 20] Following an acute insult, injured tubular epithelial cells may become fatally injured and undergo necrosis or apoptosis, proliferate and regenerate the damaged epithelium, or undergo failed regeneration but survive cell death in a state of cell cycle G2/M arrest.[4, 13, 14, 15, 16, 17, 27] Arrested cells reprieved from apoptosis, however, fail to participate in regenerative repair and upregulate maladaptive signaling pathways for myofibroblast

proliferation and fibrosis in the interstitium predisposing to progression of CKD. Tubular epithelia subjected to more severe or repeated injury, sustained or ongoing injury, or epithelia that are more senescent also are more susceptible to cell cycle arrest.[13, 15, 16, 18]

These observations provide a speculative foundation for progression of CKD that may involve recurrent or sustained injury to the kidneys which promotes active interstitial inflammation and fibrosis. These events remain clinically occult and undetected until there is a quantum decrement in functional renal mass ultimately detectable by traditional functional clinical markers. Triggers for sustained or active injury could include unresolved primary disease (e.g., glomerulonephritis), co-morbid conditions (e.g., systemic hypertension, heart disease, and regional ischemia), chronic medications (e.g., ACEI, diuretics, antibiotics, NSAIDs), chronic inflammation, chronic immune stimulation, recurrent infection, and proteinuria among others.

Independent of the nature of the insult to the kidney, a common theme for CKD progression appears to be active and ongoing stress, metabolic dysregulation, and loss of morphologic and functional integrity of the tubular epithelium leading to interstitial inflammation and fibrosis. The tubular epithelial focus prevails whether the insult is pre-glomerular hemodynamic changes predisposing to subtle hypoxia; sustained glomerular disease promoting vascular rarefaction, proteinuria and reduced ultrafiltrate; tubular stress or inflammation; or post renal events associated with outflow obstruction.[10, 13, 16, 19]

A prevailing stress to tubular epithelia is persistent and severe exposure to protein escaping glomerular permselectivity. An excessive protein load can dysregulate the normal cubilin and megalin receptor-mediated endocytosis of protein by proximal tubular cells and predispose these cells to atrophy and apoptosis.[19] In addition, excessive reabsorption of protein and protein-bound substances including fatty acids may promote cellular stress responses that activate a variety of genes promoting proinflammatory cytokines, autophagy, and activated immune responses. Consequently, there is compelling evidence that proteinuria *per se* propagates a maladaptive cycle of tubular injury, epithelial degeneration, and scaring in the adjacent interstitium.[19]

Recent experimental studies using both ischemia-reperfusion and toxic models of acute kidney injury, have provided an enhanced understanding how active, potentially sustained, and ongoing stresses to tubular epithelia can promote progressive cellular maladaptation and inflammatory and fibrotic consequences in the tubulointerstitium. Of further note, these active cellular events are temporally disassociated from worsening GFR or markers of kidney function.[17, 21] Despite its high metabolic activity and oxygen requirements, the inner cortex and outer medullary segments of the kidney exist in a state of tenuous oxygenation which is highly regulated in health but subject to profound inadequacy with vascular compromise, hypoperfusion, and relative hypoxia. With either subtle or profound tubulointerstitial injury, this tenuous vasculature can be secondarily compromised further disrupting oxygen delivery and the balance between

tubular energy demands and oxygen availability. Kidney injury can be directed specifically to the vasculature promoting endothelial cell activation, leukocyte and platelet aggregation, and compromised perfusion and oxygen delivery to the tubular epithelium. More importantly, tubular epithelial injury subsequent to oxidative stress activates vasoconstrictive signals promoting a vicious cycle of heightened ischemia, progressive vascular rarefaction, and stimulation of growth factors which signal interstitial fibrosis and progressive hypoxia.[10, 13, 15, 16, 17, 18]

Cellular stress of various causes also stimulates a diverse spectrum of directed pathways leading to dysregulated apoptosis, local inflammatory responses, and/or interstitial fibrosis which have been associated with progressive CKD. [10, 13, 15, 16, 17, 18] One of these maladaptive cellular responses is associated with accumulation of inappropriately processed or unfolded proteins in the endoplasmic reticulum (ER).[17, 22] Cellular adaptations to ER dysregulation are initiated to adjust the translation, translocation, folding, and degradation of ER proteins as protective mechanisms, but they simultaneously promote cellular autophagy.[17, 22] Autophagy is a cellular "cleanup" process in which damaged cytosolic constituents and organelles are encapsulated in autophagosomes and degraded for reutilization in cytosolic lysosomes. Autophagy is a protective adaptation to protect the cell from death. However, in experimental models of kidney diseases, the misfolded protein-induced dysfunction of the ER and mitochondria can stimulate pro-inflammatory responses via nuclear factor-κB (NF-κB) upregulation and mediation of transcription of target genes for

inflammatory interleukins, tumor necrosis factor-α, and adhesion molecules.[17, 22] Sustained or severe cellular stresses over stimulate these adaptive responses and activate downstream signaling pathways for apoptosis of the disrupted cell.

These observations illustrate that diverse and seemingly uncoordinated events or reactions often are channeled through common metabolic junctions to promote a common or universal cellular response. Similarly, the kidney responds to many overt stresses and injuries with a series of adaptive reactions fundamentally intended to reestablish cellular integrity and promote cell survival. However, when the stress or injury is sustained or insurmountable, these same cellular responses may become maladaptive or the cell is programmed to die.[4, 15] These latter responses are expressed clinically as acute kidney injury with variable recovery, kidney death, or more subtly as progressive chronic kidney disease. If the kidney recovers, many of these adaptive cellular responses become pathways for the transition of overt acute kidney injury to progressive chronic kidney disease. The majority of models highlighting this progression have focused on the continuum from AKI to CKD.[4, 11-18, 20, 21, 27] As an overview, in many circumstances, the apparent resolution of AKI, which by all clinical indications is resolved, leaves behind pathophysiologic embers that smolder asymptomatically to sustain tubular and vascular injury which slowly and progressively erode functional renal mass. The erosion is perpetuated by pro-inflammatory messengers that at first are deemed adaptive but ultimately may become maladaptive.

Identification of Progressive CKD: The Search for Active Injury Markers

In distinction to the AKI to CKD scenario (outlined above), progressive CKD is recognized in the majority of animals in the absence of an overt AKI. The progression is variable over time and recognized primarily by worsening changes in markers of steady-state kidney function like serum creatinine or SDMA resulting in increased CKD Stage over time. An animal with a serum creatinine of 1.8 mg/dL previously classified as IRIS CKD Stage 2 who subsequently is recognized with a serum creatinine of 3.5 mg/dL and classified as IRIS CKD Stage 3 must have experienced interim kidney injury resulting in the progression. Where there is less attention to trending kidney function markers or staging of CKD, the progressive nature of CKD may be signaled by the appearance and worsening of overt clinical features of kidney dysfunction including inappetence, weight loss, polydipsia, polyuria, micturition disorders, lethargy, and vomiting. In further distinction to the AKI to CKD scenario, many animals are identified with CKD of unknown etiology and no precedent acute injury in which kidney function and health appears stable for extended periods of time. To date it is unknown why some animals manifest progression and some do not, and in the absence of overt and persistent kidney disease (e.g., pyelonephritis, glomerulonephritis, nephrolithiasis, hypertension, nephrotoxic drugs), there are no diagnostic features of CKD that forecast which individual animals are predisposed to progression and which are not.

Also unknown is the pattern or nature of the injury promoting progression. For some animals, progression may be provoked by sequential episodic bouts of kidney damage that remain subclinical and undetected. (Figure 1) These episodes may escape functional detection and remain unrecognized clinically until the episodic damage exceeds the renal reserve capacity or compensatory adaptations of the kidneys.[4, 23] At this stage there will be evident worsening of steady-state function markers and progressive increases in CKD Stage. Alternatively or in combination with episodic damage, underlying mechanisms promoted by the existing CKD or an ongoing but low-grade AKI may perpetuate cellular stresses or insults that direct sustained injury to the kidney. Again, this ongoing damage may remain undetectable until parenchymal loss exceeds functional compensations. Either of these potential patterns of progression may be too subtle to be recognized by clinical features or evidence of ill health. Similarly, our current definitions of AKI Grade and CKD Stage may be too insensitive to reveal ongoing or episodic active kidney injury until there has been a finite change in static kidney function predicted with currently available test. Unfortunately, static kidney function tests only detect the impact of these active processes after substantial functional or structural damage has occurred.[4, 24]

A sensitive and specific predictor that discriminates whether a patient is likely to progress or remain stable with CKD would provide tremendous diagnostic and therapeutic advantage.[4, 17, 23] For patients with identified progressive CKD, this could provide earlier opportunity to seek subclinical conditions which might be injuring the

kidneys and institute more timely monitoring of the disease. It also would provide an opportunity to initiate therapies that might ameliorate progression or development of clinical signs as well as indicate the effectiveness of therapeutic interventions. If a therapeutic regimen failed to convert the patient to a non-progressive status, there might be justification to modify the therapy before additional loss of kidney function occurred.

There has been growing interest and research in human nephrology directed at discovering biomarkers that would predict the early onset of AKI.[4, 24, 25, 26] Similar efforts are underway in veterinary medicine and show great promise.[28-36] (See also, Yerramilli in this issue) An AKI biomarker should be detectable in urine and/or plasma such that it can be assessed routinely and serve as an indicator of kidney function or dysfunction or response to injury. The ideal marker should reflect kidney specific events, be unique and specific to the kidney, and reflect very early and potentially sustained phases of the pathogenesis and repair processes. Additionally, the ideal marker should reflect the extent of these ongoing processes, their location, changes in these processes in response to therapeutic intervention over time, and potentially the etiological insult. A singular biomarker is highly unlikely to be able to distinguish both processes associated with induction of the AKI and also events associated with repair of the injury. However, the absence of a biomarker associated with active injury may predict resolution of the active phase. More realistically, a panel of biomarkers predictive of differing phases of induction, maintenance, and repair of AKI might serve these ideal goals.

Candidate Biomarkers for Active Kidney Injury

Novel AKI biomarkers have been screened and selected for their prediction of early and sensitive alterations of normal cellular processes in the kidney and historically have been exploited for their potential to detect early acute kidney injury.[4, 24, 25, 26] However, this restricted focus to AKI constrains their broader and potentially important application to CKD. Kidney-specific biomarkers that localize to functional renal tubular epithelia (or other kidney-specific loci) and respond to diverse stresses or disruption of normal cellular function have potential to signal the early, specific, and sensitive existence of kidney injury and are perhaps better termed "active kidney injury" biomarkers. An "active kidney injury" biomarker could expose ongoing or progressive kidney injury in advance of conventional diagnostic methods that document consequent alterations in glomerular filtration rate or substantive loss of functional parenchyma over time.

With this perspective, static CKD may be redefined as a stable, unchanging state of long-standing and irreversible loss of varying degrees of kidney parenchyma and function associated with the absence of detectable active kidney injury. It would be characterized by stable biochemical markers of kidney function (e.g., serum creatinine and/or SDMA concentration) over a prolonged but variable period of time of perhaps greater than 5 to 6 months. Progressive CKD, on the other hand, is a state of long-standing and irreversible loss of variable degrees of kidney parenchyma and function likely associated with persistent or intermittent active kidney injury over an arbitrary interval of 2 to 4 month interval. The implied "active injury", albeit subtle or occult, secondarily generates

cumulative damage to the kidney and an ongoing loss of structural kidney parenchyma and function. These ongoing pathologic processes ultimately are detected by conventional biochemical markers of kidney function and increases in CKD Stage. Evidence of serum creatinine and/or SDMA trending upward is the current standard for identifying progressive CKD, but these changes can be relatively slow to develop and detect injury after it has already reduced kidney function. (Figure 2) Detection of biomarkers associated with "active kidney injury" in animals with CKD has the potential to predict or identify those patients whose underlying kidney disease is ongoing and likely to progress in advance of biochemical markers whose short-term values reflect relatively static kidney function.

Many candidate serum and urinary biomarkers have been assessed in human medicine[7, 18, 23, 24, 26, 27,], and many of the promising markers are now being evaluated and validated in animals.[28, 36] (See also, Yerramilli in this issue) Some of the most promising candidates include urinary proteins that reflect functions or cellular processes specific to the kidney that are disrupted by pathophysiologic events secondary to injury or cellular stress. Retinol binding protein (RBP), cystatin C, cystatin B, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18, liver-type fatty acid-binding protein L-FABP), tissue inhibitor metalloproteinase-2 (TIMP-2), and IGF-binding protein 7 are among the most actively pursued.[7, 18, 23, 24, 26, 28, 36] (See also, Yerramilli in this issue)

Retinal binding protein is a 21 kDa protein produced in the liver and serves as the principal carrier of vitamin A in the circulation. When not complexed to larger plasma proteins, free RBP is freely filtered by the glomerulus and subsequently reabsorbed by megalin-mediated endocytosis and catabolized by the proximal tubular epithelium. With proximal tubular dysfunction RBP can escape reabsorption and appear abnormally in urine.[28, 37] This linkage to tubular dysfunction has established RBP as a candidate for early identification of kidney injury. Retinal binding protein has been evaluated in dogs associated with a variety AKI models, and its appearance precedes routine clinical predictors as an early marker of AKI. However, urinary RBP is only loosely associated with proximal tubular dysfunction and is nonspecific for active injury to the tubular epithelium.[28, 37]

Cystatin C is a 13 kDA cysteine protease inhibitor constitutively produced by most cells and subsequently circulated in blood after release from the cells. It is freely filtered across the glomerulus and subject to proximal tubular reabsorption from the filtrate and degradation in the proximal epithelia. Like RBP, dysfunction of megalin-mediated endocytosis from acute injury or decreased reabsorptive capacity results in urinary detection of cystatin C, and its increased renal excretion.[28] Cystatin C has been proposed as an early predictor of kidney dysfunction. However, it also lacks specificity for active kidney injury, is not uniquely produced by kidney cells, and, as for any freely filtered protein, is confounded by excessive proteinuria which might promote its urinary excretion without substantive active or ongoing tubular injury.

Despite the interest and efforts to validate KIM-1 as an AKI biomarker in human patients,[7, 18, 23, 24, 26, 27] in preliminary investigations, there has been no success (working with the developers) to detect KIM-1 in dogs with any of the available human, primate, or rodent assays for KIM-1. (Personal communication, Drs. Carrie Palm and Larry D. Cowgill)

Neutrophil gelatinase-associated lipocalin (NGAL) is a 24-kDa protein initially identified bound to gelatinase in specific granules of neutrophils. Subsequently, NGAL expression has been demonstrated by a variety of epithelia and specifically is up-regulated more than tenfold in renal tubular epithelia within the first few hours following ischemic, obstructive, and toxic kidney injuries in human patients with AKI, naturally acquired kidney disease in dogs, and experimental models of AKI in dogs.[24, 25, 27, 28-36, 38] Recent studies in dogs with experimental gentamicin-induced AKI and in dogs with naturally acquired AKI, demonstrated NGAL sensitivities and specificities approaching 95% or greater for the early detection of AKI.[30, 31] A commercially available assay for detection of canine NGAL is available[b] and has been validated for early detection of AKI. While urinary NGAL currently is promising, it lacks uniqueness with regard to its cellular origins and specificity for kidney injury, and it can be influenced by co-morbid diseases.

Other promising biomarkers include serum inosine, urinary clusterin, and urinary cystatin B which are under active evaluation in a variety of dog and cat models of AKI, naturally acquired active kidney injury, and progressive CKD. (See Yerramilli in this issue) The attraction of these novel biomarkers is their exclusive origins to renal tubular epithelia, integral association with cellular activities coupled to stress or damage, and their highly

specific analytical evaluation. As such, these markers offer the potential sensitivity and specificity to forecast active and sustained disruption of normal cellular processes as harbingers of subsequent loss of steady-state kidney functions or residual renal mass. Although the clinical directive for these kidney-specific biomarkers is to facilitate the diagnosis of early AKI, preliminary studies have documented their sensitivity for kidney disease beyond AKI. Their potential extends to document subclinical yet active and ongoing kidney injury associated with concurrent urinary diseases or other systemic illness that may impact the kidney secondarily. For future diagnostics, individual markers or a panel of novel markers could provide the potential to recognize subtle and often subclinical kidney disease that would otherwise remain undiscovered until more substantial injury and dysfunction triggers alterations in conventional diagnostics. Even more importantly, "active kidney injury" biomarkers have potential to establish a new understanding of our traditional views of CKD including early identification and possible mediators of its progression. Novel, kidney-specific biomarkers will likely establish a completely new and sophisticated paradigm in our approach to the understanding, diagnostic evaluation, and treatment of kidney disease in dogs and cats. However attractive initially, the value and clinical utility of any novel kidney biomarker to forecast outcomes or direct treatment must be founded on the basis of well-designed and prospective controlled longitudinal studies of patients with CKD.

Potential Application of Novel Active Injury Kidney Biomarkers to Forecast Early Kidney Injury

Early recognition of AKI: Urinary NGAL has been shown to be a sensitive and early predictor of AKI in dogs with both experimentally-induced and naturally occurring AKI and may precede detection of kidney disease by serum creatinine or SDMA by more than a week.[29, 30, 31, 34] (Figure 3) In dogs with naturally acquired urinary tract disease, urinary NGAL was a highly sensitive and specific predictor of AKI including dogs categorized as IRIS AKI Grade I.[30]

In collaboration with IDEXX Laboratories, the one of the authors[c] similarly has evaluated the diagnostic performance of serum inosine, urinary clusterin, and urinary cystatin B in a gentamicin-induced model of AKI in dogs. (See Yeramilli in this issue) In this model, each of these novel biomarkers demonstrated a robust response or change within 72 to 120 hours from the induction of gentamicin documenting early and ongoing active injury. Each marker forecast the active kidney injury 7 to 10 days before any identifiable change in serum creatinine or SDMA. (See Yeramilli in this issue) These preliminary observations have provided optimism they may serve as unique diagnostic tools to disclose kidney injury at a time when it is otherwise diagnostically camouflaged.

Active kidney injury in urinary tract infection: In a previous study evaluating the specificity of urinary NGAL as a predictor of AKI in dogs, a significant increase in its urinary expression was observed in dogs with lower urinary tract disease (infection, neoplasia, urolithiasis) compared to healthy dogs.[30] As NGAL was identified originally as a component of neutrophil granules and is upregulated in response to inflammatory signals, it was tenable neutrophil associated inflammation could have been the source

of urinary NGAL in these dogs.[39] However, it is difficult to exclude the presence of subclinical kidney involvement in this lower urinary tract cohort. The increased urinary NGAL expression may have forecasted kidney involvement that was otherwise subclinical and undetectable.

To gain preliminary insight into this question, in collaboration with Drs. Shelly Vaden and Murthy Yerramilli, the one of the authors[c] recently has screened urine from 36 dogs with documented urinary tract infection for the presence of urinary cystatin C, urinary NGAL, and urinary clusterin as predictors of concurrent active kidney injury. No dog had clinical evidence of AKI although 6 dogs (17%) had historical CKD. The urine was considered positive for active kidney injury if at least 2 of 3 biomarkers were above the normal threshold or if urinary clusterin (of renal tubular origin) alone was above the reference threshold. For the 30 dogs with no documented CKD, 43% demonstrated biomarker predicted active kidney injury, 37% had no biomarker predicted active kidney injury, and 20% demonstrated equivocal biomarker activity. (Table 1, excludes the equivocal group) Although these data are preliminary and require additional prospective evaluation and validation of the long-term outcomes of these cases, they might suggest a large proportion of dogs with active urinary tract infection may simultaneously have upper tract kidney involvement promoting subclinical and otherwise undetectable active injury. Identification of active kidney injury concurrent with lower urinary tract disease may suggest the kidneys are concurrently infected and potentially a source for recurrent infection of the lower

urinary tract. If not infected, the active injury may involve alternative mechanisms associated with lower tract infection and pose a risk for erosion of renal reserve and progressive CKD. If these findings are confirmed by further validation studies, it suggests a new paradigm and significance to urinary tract infection and portends new diagnostic and therapeutic responsibilities.

Active kidney injury in acute and chronic cardiorenal syndrome: Acute and chronic cardiorenal disorders are another clinical arena in which the kidney may be subjected to subclinical and potentially sustained active injury secondarily to disease, failure, or management of primary cardiovascular disease. Severe and persistent heart failure is commonly associated with progressive CKD that may be punctuated by episodes of acute kidney decompensation concurrent with decompensation of cardiac function or escalation of drug therapy. (Figures 2) Active kidney injury biomarkers may facilitate recognition of the incipient kidney damage. This would permit more conscientious management of the cardiac disease and proactive preservation of kidney function and protection from kidney injury with its management. (Figure 4)

Figure 2 illustrates the influence of progressive venous congestion on the kidneys (congestive nephropathy) subsequent to right heart failure and the development of ascites and increased intraabdominal pressure. With abdominocentesis the intraabdominal pressure and congestion are relieved temporarily with an associated dramatic improvement of the active injury markers (urine clusterin and serum inosine)

with the initial abdominocentesis. Although improved, the markers further illustrate the ongoing kidney injury associated with ongoing cardiac disease and progressive congestion between abdominocentesis procedures and their prediction of the progressive CKD.

Is Progressive CKD a Slow Moving AKI: A New Look at CKD

As discussed and illustrated above, novel kidney biomarkers can expose subtle and subclinical kidney disease that may otherwise remain undiscovered with conventional diagnostic assessment. More importantly, "active kidney injury" biomarkers have potential to establish a new understanding of our traditional views of CKD including its early identification and possible mediators of its progression. In a recent evaluation of urinary NGAL in dogs with kidney disease, there was significantly greater NGAL excretion in dogs with CKD than noted for healthy control dogs or those with lower urinary tract disease.[30] The urinary NGAL excretion was clearly less than in dogs with AKI, but this observation suggested some degree of active injury may be ongoing in these dogs absent the typical presentation of acute-on-chronic disease. Similar findings were reported for serum NGAL in dogs with CKD, in which serum NGAL was significantly higher than control dogs and increased with IRIS CKD Stage. However, changes in serum NGAL are less specific than urine NGAL for predicting active kidney injury.[32]

Progressive CKD generally develops in dogs with heart disease as the heart disease transitions from compensated to incipient or overt failure or with escalation in cardiac therapy. Concurrent with this transition in cardiac function, there is expression of active kidney injury markers (an IRIS AKI Grade I? or IRIS CKD Stage 1?) which precedes the temporal progression to more advanced CKD Stages. (Figure 2) Absent active injury, kidney function typically remains stable. (Figure 5) Similarly, as suggested in Table 1, animals with urinary tract infection and identifiable "active kidney injury" biomarkers might be classified as IRIS AKI Grade I or CKD Stage 1 (depending on the duration of infection and initial creatinine concentration) on the basis of biomarker expression but by most other criteria would otherwise have unrecognized kidney disease. With persistent expression of "active injury kidney" biomarkers, there would ultimately be the expectation for transition to higher grades or stages of kidney disease and the detection of progressive CKD over time.

With this overview, a hypothesis has been proposed that progressive CKD may result from clinically occult or overt acute insults to the kidneys which are episodic or sustained in character (a slow-moving or sustained AKI) subsequent to exposure to diverse processes including metabolic and/or physiologic stresses. Documentation of active and especially persistently active kidney injury may be predictive of patients who are at risk for progression of CKD. Patients where evidence of active kidney injury is absent are more likely to maintain stable kidney function. To support this hypothesis, prospective studies involving canine and feline patients with well characterized CKD

are need. The advent of "active kidney injury" biomarkers is likely to provide a renewed perspective and clinical significance to both IRIS CKD Stage 1 and IRIS AKI Grade I classifications. Although patients with these classifications are non azotemic and generally asymptomatic, detectible "active kidney injury" markers may provide justifiable criteria for them to be classified with early kidney disease thus distinguishing them from animals with normal kidneys. IRIS CKD Stage 1 and IRIS AKI Grade I classifications identified on the basis of biomarker criteria warrant heightened clinical significance as a recognizable gateway to more advanced kidney disease and progressively worsening kidney function. It is worth noting that the transition from Stage 1 to Stage 2 CKD is associated with proportionally greater loss of functional renal mass than occurs with the transition between more advanced CKD Stages. When followed over time or, ideally, coupled with markers of kidney repair, Stage 1 or Grade I classification might be further defined as "continued active", "resolving", or "inactive" to guide clinical decision making and therapeutic monitoring.

Sensitive and specific biomarkers are likely to lead to new insights into clinical kidney disease and facilitate new diagnostic and therapeutic approaches. Detection of active kidney injury (whether classified as CKD or AKI) may require a new designation depending on whether the patient's condition resolves, fails to develop progressive clinical manifestations over time, or progresses to overt clinical disease. Given the common pathophysiologic foundations and mediators working in the same cellular milieu, the distinctions between CKD and AKI might be viewed more interactively as a

singular process in both early manifestations and advancing stages. It is easy to foresee new opportunities and applications for biomarker diagnostics and their applications for early recognition of kidney injury associated with:

1. Critical care
2. Systemic diseases and their management including (e.g., heart failure, proteinuria, infectious disease, immune-mediated disease)
3. Anesthesia and invasive procedures.
4. Animals presenting for vague illness
5. Acute-on-chronic kidney disease and progression of CKD

If validated for specificity, sensitivity, and clinical utility, to be useful diagnostically, clinicians will need to be proactive in testing patients at risk for active kidney injury before the disease is identified by conventional diagnostic parameters or overt clinical signs. The established use of "active kidney injury" biomarkers will require changes in practice patterns related to CKD and its potential progression. Clinicians by necessity will need to recognize and anticipate the potential risks and clinical circumstances that might predispose to progressive CKD including comorbid diseases (e.g., hypertension, pre-existing kidney disease, heart failure, pancreatitis, heat stroke, vomiting), medications (e.g., ACEI, diuretics, antimicrobials, NSAIDs), and diagnostic and therapeutic procedures (e.g., anesthesia, surgery, dentistry, contrast administration).

This potential new diagnostic paradigm also will require an updated consensus for diagnostic and therapeutic responses to Stage 1 or Grade I kidney injury. It promises answers to the questions, what triggers or processes promote progression of CKD; and how can progression be recognized and potentially mitigated at its earliest stage to preserve kidney function. Similarly, therapeutic approaches may be monitored to biomarker endpoints and logically adjusted or extended until the biomarker activity is nullified.

Footnotes:

[a]**Napa Scientific Panel**: Larry D. Cowgill (Co-Chair), David Polzin (Co-Chair), Gilad Segev, Greg Grauer, Astrid van Dongen, Jonathan Elliott, Cathy Langston, Mary Nabity, Scott Brown. **IDEXX Sponsorship Team:** Roberta Relford, Jane Robertson, Murthy Yerramilli, Jason Lee, Troy Goddu, Leif Lorentzen, Donald McCarann, Lori Jackowitz.

[b]Abbott Laboratories, Abbott Park, IL

[c]Larry D. Cowgill

Figure Legends:

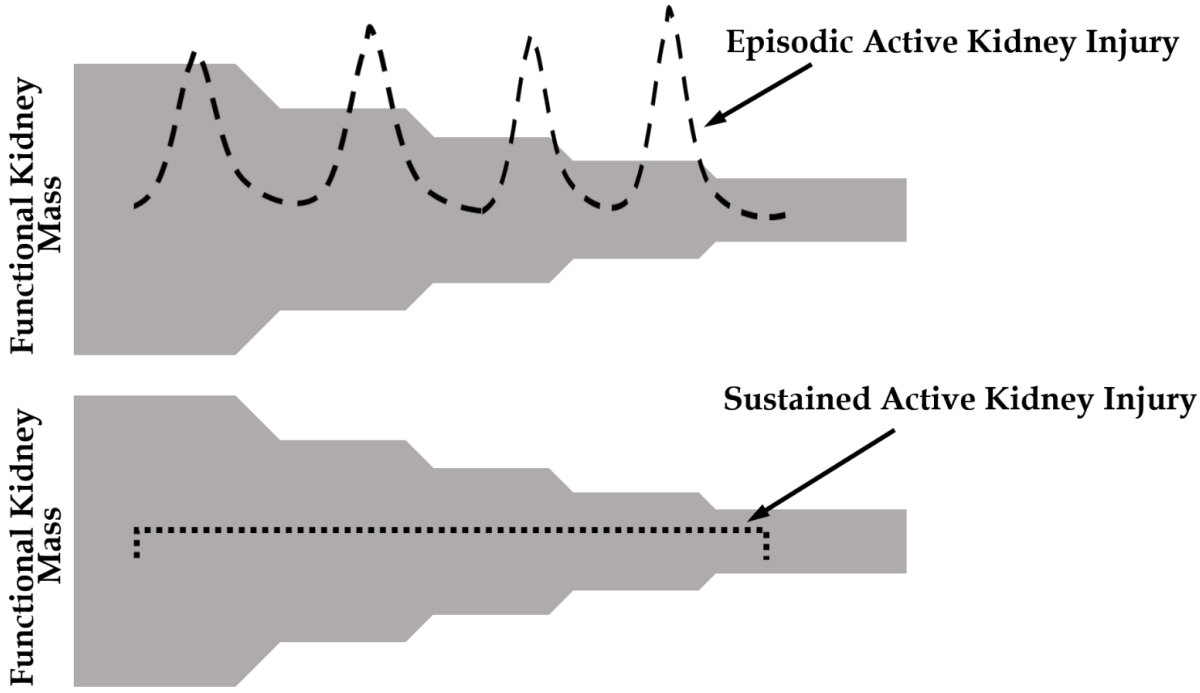

Figure 1. Schematic illustration of progressive CKD causing a decrease or worsening in functional kidney mass (or GFR) over time in response to step-wise or episodic insults to the kidney (upper panel) or subsequent to sustained active kidney injury resulting from intrinsic stress or disordered metabolism associated with the established CKD (lower panel).

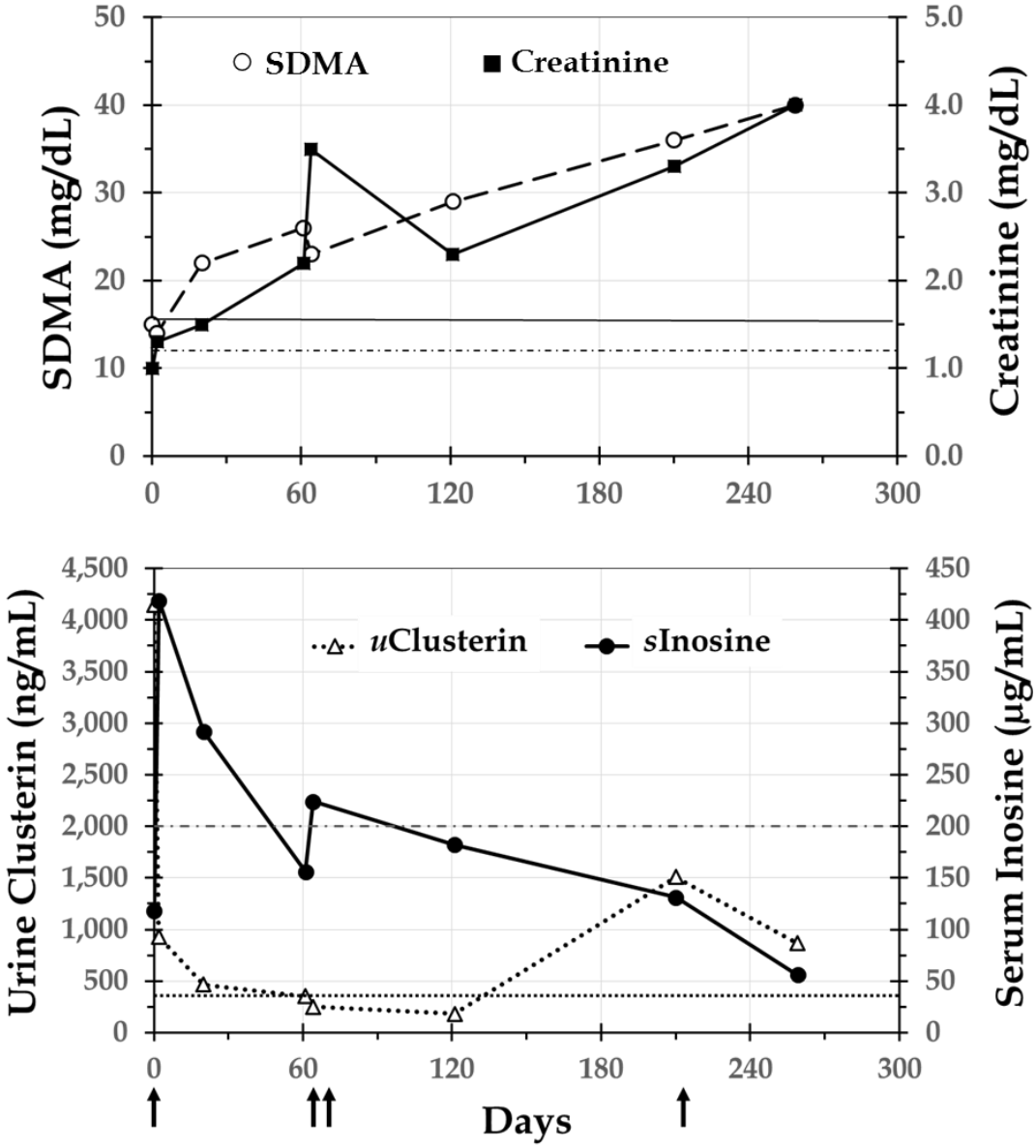

Figure 2. Progressive chronic kidney disease (CKD) in a dog with mitral and tricuspid valvular insufficiency and regurgitation and right-sided congestive heart failure, ascites, and increased intraabdominal pressure. Both NTproBNP and cTroponin I (not shown) were markedly elevated consistent with heart failure. (Upper Panel) Changes in serum creatinine (solid squares) and IDEXX SDMA™ (open circles) over time illustrating progressive worsening of kidney function. The solid and dashed horizontal lines represent the upper reference range for creatinine and IDEXX SDMA™, respectively. (Lower Panel) Associative changes in the "active kidney injury" biomarkers, urine clusterin (open triangles) and serum inosine (closed circles), to periodic abdominocentesis (arrows) and decreased intraabdominal pressure. The mixed dashed horizontal line represents the lower reference threshold for serum inosine and the dotted horizontal lines represents the upper reference range for urine clusterin. Note the marked improvement in both urinary clusterin and serum inosine subsequent to

institution of medical therapy and abdominocentesis (arrows) on day 0. Although improved, both markers demonstrate only transient resolution of the ongoing kidney injury which progresses over time and between abdominocentesis procedures as the congestive heart failure progresses. The biomarker-predicted active injury is further associated with the progressive CKD.

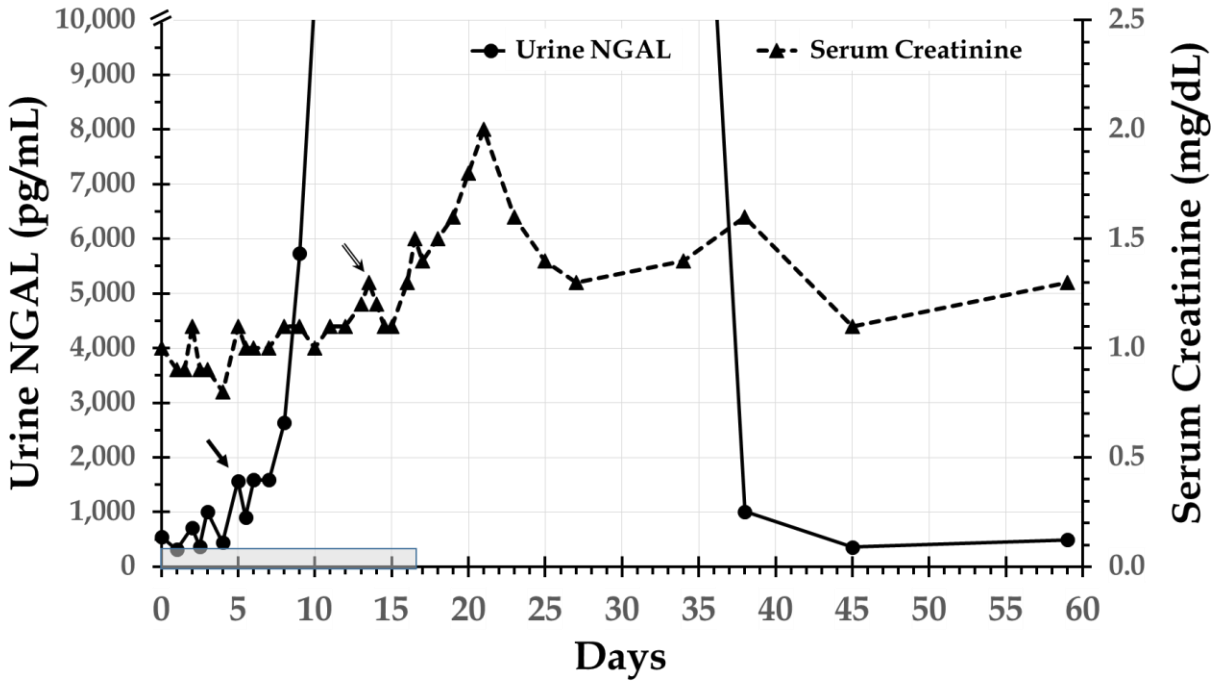

Figure 3. Changes in urine NGAL (solid triangles and curve) and serum creatinine (solid circles and dashed curve) in an experimental model of gentamicin nephrotoxicity in a dog. Gentamicin was injected subcutaneously twice daily for 16.5 days (shaded area) until the serum creatinine increased by 50%. Urinary NGAL predicts the "active kidney injury" by day 5 (solid arrow) whereas the AKI was not recognized until day 14 by IRIS AKI guidelines (open arrow). Note the discontinued left axis as urinary NGAL increased by more than 480 times the baseline concentration to a peak value to 264,000 pg/mL at day 19 before decreasing to the baseline over the subsequent 26 days. (Data from Palm, et. al., 2016: reference 31)

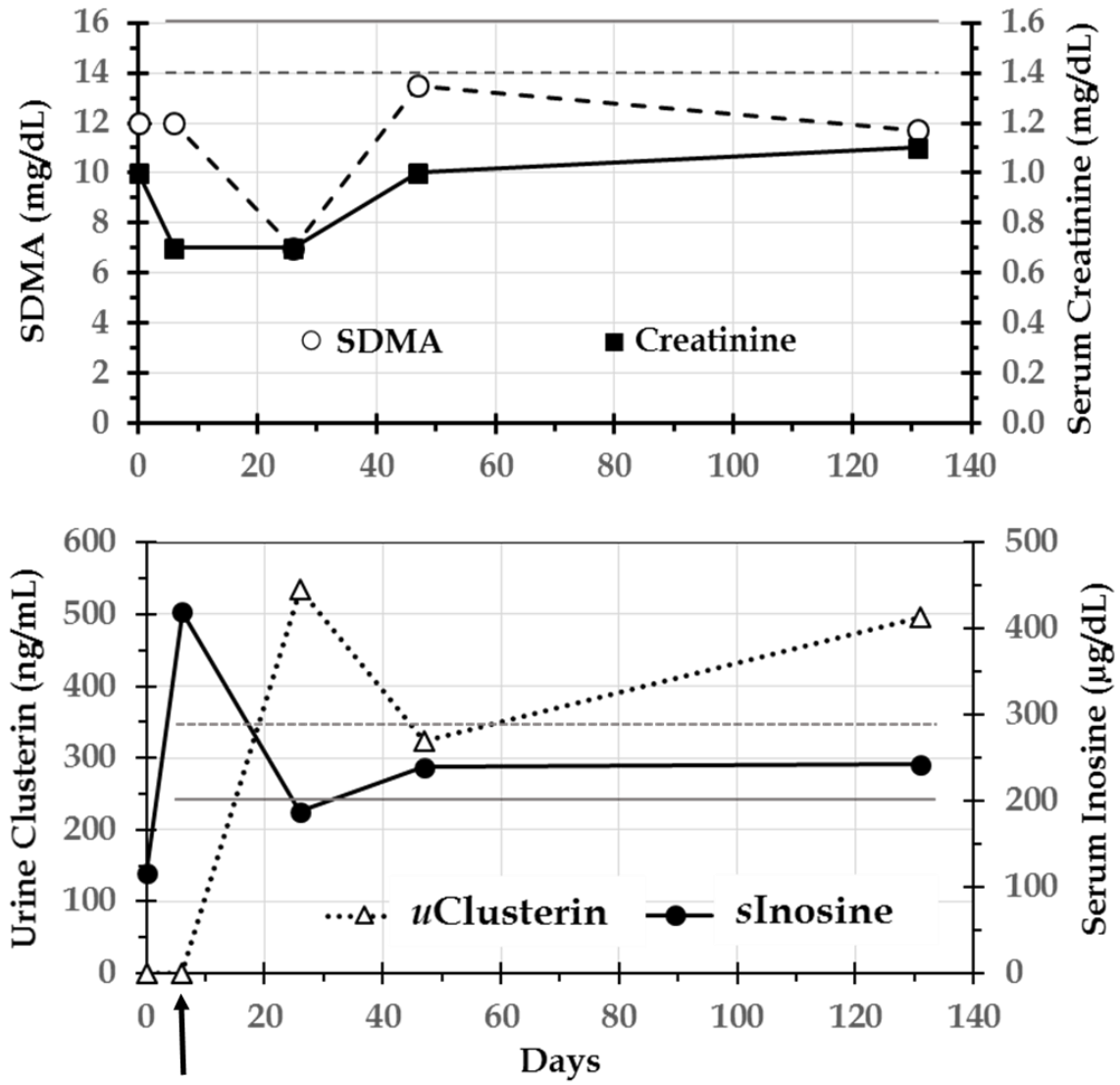

Figure 4. Changes in conventional function test and novel "active kidney injury" markers in a dog with progressive valvular heart disease and impending cardiac failure. Cardiac therapy including Lasix and ACE inhibition was started on Day 0.. By Day 6, the dog's cardiac signs had improved. However, twelve hours following discharge on day 6, the dog was represented to the emergency service for acute respiratory signs and heart failure secondary to a ruptured chordae tendineae requiring a rapid escalated the cardiac medications (arrow). (Upper Panel) Changes in serum creatinine (solid squares) and IDEXX SDMA™ (open circles) over time. The solid and dotted horizontal lines represent the upper reference range for creatinine and IDEXX SDMA™, respectively. (Lower Panel) Associative changes in the "active kidney injury" biomarkers, urine clusterin (open triangles) and serum inosine (closed circles) over time. The solid line represents the lower reference threshold for serum inosine, and the dotted horizontal line represent the upper reference range for urine clusterin. The initial decrease in serum inosine suggest active kidney injury in response to the

impending cardiac failure. The initial medical management for incipient congestive heart failure resulted in a marked improvement (increase) in serum inosine as a measure of resolving active kidney injury/stress between day 0 and day 6. The subsequent acute decompensated heart failure and escalation of the medical management (arrow) promoted biomarker-predicted active kidney injury (decreased serum inosine and increased urinary clusterin) which persisted at a low level with compensation of the heart failure. The active kidney injury was not detected by serum creatinine or IDEXX SDMA™.

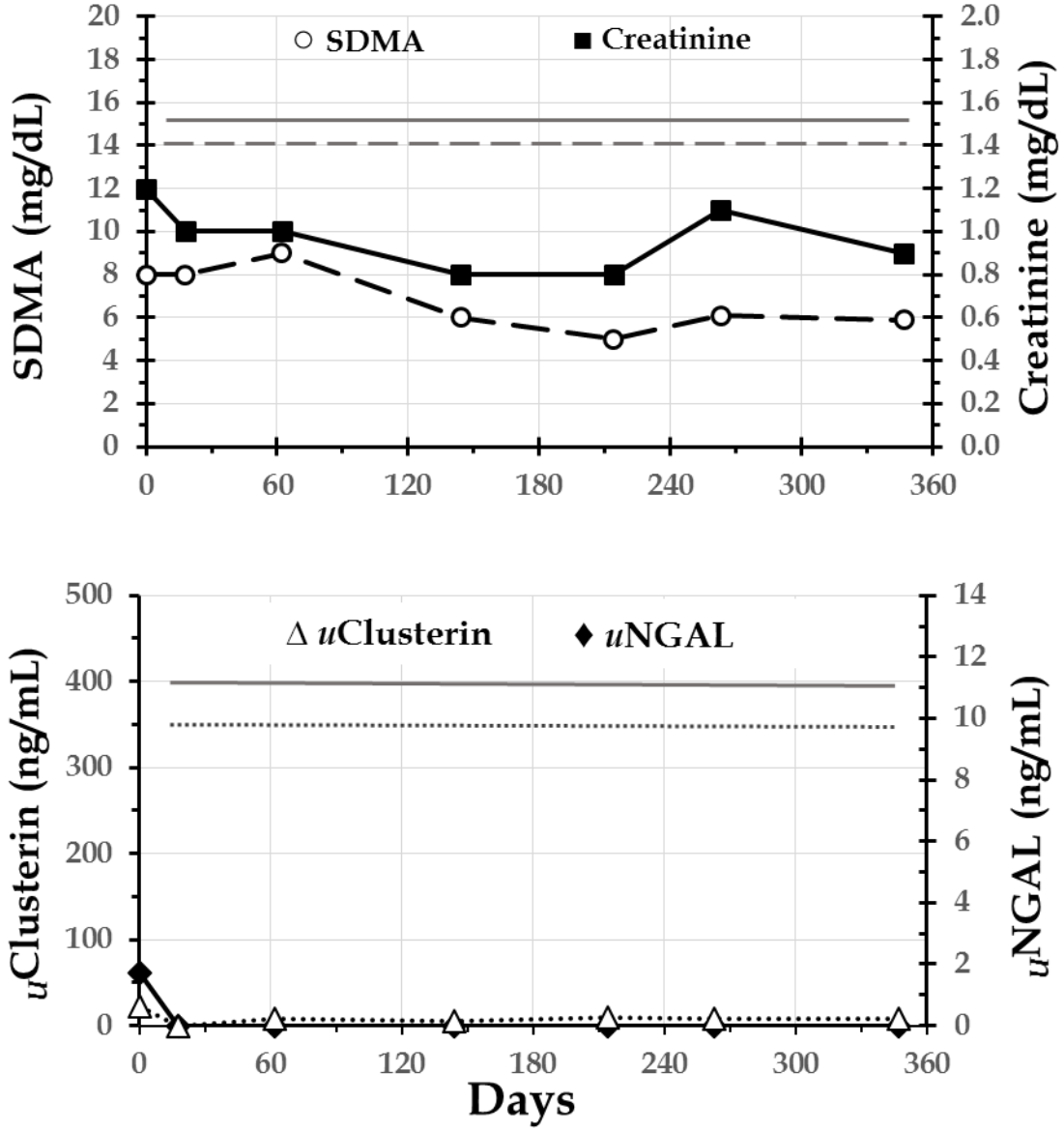

Figure 5. Changes in conventional markers of kidney function and novel "active kidney injury" markers in a dog with compensated mitral valvular heart disease. (Upper Panel) Changes in serum creatinine (solid squares) and IDEXX SDMA™ (open circles) over time. The solid and dotted horizontal lines represent the upper reference range for creatinine and IDEXX SDMA™, respectively. (Lower Panel) Associative changes in the "active kidney injury" biomarkers, urine clusterin (open triangles) and urine NGAL (closed diamonds) over time. The solid and dotted horizontal lines represent the upper reference ranges for urine NGAL and urine clusterin, respectively. Note with stable and compensated heart failure, there is remarkable stability of kidney function and no biomarker-predicted active kidney injury over an extended period of time.

Table 1. Blood and urine analytes from 30 dogs with documented urinary tract infection and no evident kidney involvement.

Test (units; reference range)	No Biomarker Evident Kidney Injury (n = 11)	Biomarker Predicted Kidney Injury (n = 13)
	Clinical Parameters Median (range)	
Creatinine (mg/dL; 0.5-1.5)	0.9 (0.4-1.2)	0.9 (0.4-1.2)
BUN (mg/dL; 9-31)	14.0 (3-21)	21 (8-75)
IDEXX SDMA™ (µg /dL <14)	8.0 (6-16)	12.0 (7-17)
Uprot/creat (< 0.5)	0.26 (0.04-0.89)	1.46 (0.02-8.85)
Urine Specific Gravity	1.017 (1.006-1.032)	1.025 (1.013-1.042)
uWBC (/hpf; 0-5)	10 (0->500)	0 (0-20)
uRBC (/hpf; 0-5)	15 (0->500)	0 (0-15)
	Urinary Biomarkers Median (range)	
uCystatin C (ng/mL,<300)	57 (1-115.7)	370 (29->2500)
uNGAL (ng/mL,<10)	2.6 (1.6-5.8)	40.4 (0.26-220.1)
uClusterin (ng/mL,<600)	97 (0-274)	2010 (119-2527)

BUN, blood urea nitrogen; IDEXX SDMA™, IDEXX Laboratories symmetrical dimethylated arginine; Uprot/creat, urine protein/creatinine ratio; uWBC, urine white blood cell count; uRBC, urine red blood cell count; uCystatin C, urine cystatin C; uNGAL, urine neutrophil gelatinase-associated lipocalin; uClusterin, urine clusterin.

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