February 21-24, 2012
New York, New York
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ABOUT THE SPEAKERS

Mark Acierno, DVM, DACVIM (SAIM)
Dr. Acierno is an Associate Professor of Veterinary Medicine at Louisiana State University Department of Veterinary Clinical Sciences. After obtaining his DVM from Mississippi State University, he completed an internship at Red Bank Veterinary Hospital and Referral Service and a medicine residency at Tufts University School of Veterinary Medicine. At Tufts, he learned to perform intermittent hemodialysis, and upon moving to LSU, he started a CRRT and IHD unit. His clinical interests are Nephrology and Urology, and his Research interests are renal replacement technologies and hypertension.

Linda Barton, DVM, DACVECC
Dr. Barton received her DVM from the University of Florida. She completed an internship at the University of Pennsylvania. After spending time in private practice, she completed a residency in emergency and critical care in Milwaukee, Wisconsin. She developed and directed the emergency and critical care medicine service at The Animal Medical Center in NYC for 10 years. She moved to the Veterinary Specialty Center of Seattle and developed the renal dialysis team there, which she currently heads.

Allyson Berent DVM, DACVIM (SAIM)
Dr. Berent graduated from Cornell University College of Veterinary Medicine in 2002. She completed a one year rotating small animal internship at the University of Minnesota. She then completed her internal medicine residency at the Matthew J. Ryan School Veterinary Hospital of the University of Pennsylvania. Following her residency she did a fellowship in interventional radiology and interventional endoscopy. Her research interests are in minimally invasive diagnostics and therapeutics including: endourology, laser lithotripsy, hepatic and biliary interventions, and intrahepatic portosystemic shunting. She is the Director of Interventional Endoscopy Services at The Animal Medical Center. She is a leading world expert in ureteral and urethral stent, as well as many other innovate urinary tract procedures.
Larry Cowgill, DVM, PhD, DACVIM (SAIM)
Dr. Cowgill received his DVM degree from the University of California at Davis and completed his internship and residency training at the University of Pennsylvania. He was a National Institutes of Health Special Research Fellow at the Renal and Electrolyte Section of the University Of Pennsylvania School Of Medicine and earned a PhD in Comparative Medical Sciences. He is Board Certified in Small Animal Internal Medicine and is Associate Dean for Southern California Clinical Programs, Co-Director of the UC Veterinary Medical Center-San Diego (UCVMC-SD), and Professor in the Department of Medicine and Epidemiology. He oversees the Clinical Nephrology programs and the Companion Animal Hemodialysis Units at the Veterinary Medical Teaching Hospital at Davis and the UCVMC-SD. Dr. Cowgill has more than 35 years of experience in veterinary internal medicine, nephrology, and teaching and has trained many of the leading veterinary nephrologists throughout the world. He is a pioneer in the application of hemodialysis in companion and remains a leading authority in the development of blood purification therapies for renal diseases in animals and people.

Adam Eatroff DVM, DACVIM (SAIM)
Dr. Eatroff graduated from the College of Veterinary Medicine at Cornell University in 2006. He completed an internship in small animal medicine and surgery at Oradell Animal Hospital in Paramus, NJ in 2007, and a residency in small animal internal medicine at Cornell University in 2010. He is currently in the second year of his training as the Renal Medicine/Hemodialysis Fellow at the Animal Medical Center in New York City. Dr. Eatroff’s professional interests include nephrology and renal replacement therapy, specifically hemodialysis. His research interests include body fluid homeostasis and novel treatments for acute kidney injury.

Robert J. Harman, DVM, MPVM
Dr. Harman founded and is the CEO of Vet-Stem, the first US-based commercial veterinary stem cell company. For 15 years prior to that, he was the CEO of HTI-Bio-Services, a preclinical research company for veterinary and human pharmaceutical development. He has authored more than 500 contract study reports for animal health companies throughout the world and for submission to the FDA and USDA in support of the development of new animal and human health products. In his current position, he is the CEO and principal clinical development director of the programs at Vet-Stem to bring stem cell therapy to veterinary medicine. He has been a frequent speaker at stem cell conferences in North America, Central America, Europe and the Middle East. He has authored seven peer-reviewed publications on stem cell therapy.
Cathy Langston, DVM, DACVIM (SAIM)
Dr. Langston graduated from Louisiana State University, completed an internship and residency in internal medicine at the Animal Medical Center, and a fellowship in Renal Medicine and Hemodialysis at the University of California, Davis. She has been the head of the AMC Renal Medicine Service and Hemodialysis Unit at AMC since 1999. Her current clinical interests include treatment of chronic kidney disease, complications of hemodialysis, and treatment of anemia of chronic kidney disease. She is one of the conference organizers for the only veterinary hemodialysis seminar, Advanced Renal Therapies Symposium.

George Lees, DVM, DACVIM (SAIM)
Dr. Lees obtained his DVM degree from Colorado State University, followed by an internship at the University of California at Davis and a medicine residency at the University of Minnesota. He is currently a professor of Small Animal Medicine & Surgery at Texas A&M University, College Station, TX, and he is the Director of Texas Veterinary Renal Pathology Service. His scholarly interests include urinary tract diseases and renal pathology in companion animals. He is a recipient of The Robert W. Kirk Award for Professional Excellence from the American College of Veterinary Internal Medicine (ACVIM). He has a strong interest in canine hereditary nephritis and has discovered a canine model of Alport’s syndrome.

Matt Mellema, DVM, PhD, DACVECC
Dr. Matt Mellema is a native of northern California and did his undergraduate training at UC Berkeley. He received his DVM degree from UC Davis in 1994. Following graduation, he completed a focused internship in Small Animal Emergency Medicine at Tufts University and remained at Tufts as an instructor for an additional year. He then went to work for Cardiopet, Inc. (now part of IDEXX), as a consultant in cardiothoracic medicine. He completed a residency in emergency and critical care medicine at UC Davis in 2000. Following his residency, Dr. Mellema went back to Boston yet again to get his PhD in respiratory physiology at Harvard University. He joined the faculty at UC Davis in 2007 as an assistant professor of Small Animal Emergency and Critical Care. At present his laboratory in focused heavily on vascular pathobiology and the exploration of endothelial microparticles as diagnostic and therapeutic tools in veterinary medicine. Dr. Mellema’s other research interests include nitric oxide biology and non-endothelial cellular microparticles in health and disease. Once upon a time he was a respiratory physiologist, but he says that now seems like a lifetime ago. At present, his clinical practice is limited to small animal intensive care. He is co-director of the SA-ICU and the SA-E/CC residency program at UC Davis.
Jan A. Nolta, Ph.D.
Dr. Nolta, stem cell program director at UC Davis, is one of the nation’s leading stem cell researchers. She received a Bachelor of Science degree from California State University Sacramento, took master’s classes at UC Davis and earned a Ph.D. in molecular microbiology from the University of Southern California. She was a post-doctoral fellow at Children’s Hospital of Los Angeles and an assistant professor at the USC School of Medicine before being appointed as an associate professor at Washington University School of Medicine. Dr. Nolta joined UC Davis in 2006 after directing an R01-funded stem-cell research lab and overseeing the work of 14 other scientists at Washington University School of Medicine in St. Louis. She also served as the Scientific Director for the university’s Good Manufacturing Practice (GMP) Facility for cell and gene therapy, where she helped investigators move promising bench research into clinical cellular therapy trials. Her laboratory used human hematopoietic, mesenchymal, and endothelial stem and progenitor populations to examine the recruitment of adult stem cells to areas of tissue damage in immune deficient mice. A scientist with more than 20 years’ experience with human stem cells, Dr. Nolta has served on more than 34 National Institutes of Health review panels and is a full-time member of the Hematopoiesis Study Section at the NIH. She was recently invited to participate in the strategic planning meetings in the area of cellular therapeutics at the National Heart, Lung, and Blood Institute. She has served as editor and editorial board member on six scientific journals and belongs to a number of national and international science committees.

Carrie Palm DVM, DACVIM (SAIM)
Dr. Palm graduated from the University of California, Davis and completed an internship and residency at the University of Pennsylvania. She is Board Certified in Small Animal Internal Medicine. Following a year in a specialty practice at Veterinary Medical and Surgical Group in Ventura, CA, completed a fellowship in hemodialysis and joined the faculty in Clinical Small Animal Internal Medicine.

Karen Poeppel, LVT
Ms. Poeppel has been a part of the AMC Dialysis Unit since its inception, as the Head Hemodialysis Nurse. She has trained all of the dialysis technicians and fellows that have rotated through the service. Her skill in managing dialysis patients and incorporating innovative dialysis technology in unsurpassed.
**Yann Quéau, DVM, Dipl. ACVN**

Yann Quéau graduated from the National Veterinary School of Toulouse (France) in 2007 after completing a veterinary thesis on the effect of aging on glomerular filtration rate in dogs. Following graduation, he completed an internship in Renal Medicine and Hemodialysis, and a residency in Small Animal Clinical Nutrition at the University of California, Davis. He became a Diplomate of the American College of Veterinary Nutrition in 2011, and now works at the Royal Canin Research Center in France.

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**Jessica Quimby, DVM, DACVIM (SAIM)**

Following graduation from the University of Wisconsin-Madison School of Veterinary Medicine, Dr Quimby completed a small animal rotating internship in Sacramento, CA, and subsequently spent two years in private practice in Grand Rapids, Michigan. Dr. Quimby completed a small animal internal medicine residency at Colorado State University during which she performed several clinical studies concentrating on feline renal disease and respiratory disease. She is now finishing a PhD, which explores several aspects of feline renal disease including telomere senescence and palliative stem cell therapy.

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**Roberta Relford, DVM, PhD, DACVIM (SAIM), DAVCP**

Dr. Relford graduated from Auburn University in 1982 and was a small animal practitioner for 4 years. She obtained an MS in pathology at Mississippi State University and a PhD in pathology from Texas A&M University, where she also pursued a residency in small animal internal medicine. Dr. Relford is board-certified in Internal Medicine and also in Clinical Pathology. Her areas of interest are internal medicine, coagulation, cytology, and kidney disease. She is the Division Vice President of Pathology, Internal Medicine and Strategic Operations at IDEXX Reference Laboratories and is instrumental in development of new biomarkers of kidney disease.
Sheri Ross, DVM, PhD, DACVIM (SAIM)
Dr. Sheri Ross is a faculty member of the renal medicine and hemodialysis service at the University of California Veterinary Medical Center, San Diego. Her specific research interests include the influence of dietary modifications on the progression of chronic kidney disease, and urolithiasis, with particular interest in feline ureteral stones and acute ureteral obstruction.

Gilad Segev, DVM, Dipl ECVIM-CA
Dr. Segev is Lecturer of Veterinary Medicine and Head, Department of Small Animal Internal Medicine; Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel. Dr. Segev has actively focused his clinical interests and research in nephrology and has recently established a hemodialysis program at the Koret School of Veterinary Medicine in Israel. He established a novel scoring system which effectively predicts the probability for survival in dogs treated with renal replacement therapy for the management of acute kidney failure. He has also described some of the challenges and complications faced with the long-term management of dogs with advanced chronic kidney disease including the hyperkalemia associated with the use of therapeutic renal diets and aluminum toxicity in the management of the hyperphosphatemia of CKD.

Carrie White, DVM, DACVIM
Dr. White is a graduate of Tufts University School of Veterinary Medicine. She completed an internship at Veterinary Referral & Emergency Center and then joined AMC as a Resident in Internal Medicine. She has been a staff doctor at AMC since the completion of her residency, and she has active research interests in hematology and hematologic diseases.
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Overview of biomarkers in acute kidney injury

Gilad Segev, DVM. Dip ECVIM-CA
Koren School of Veterinary Medicine
Hebrew University Jerusalem, Israel

Severe acute renal failure (AKI) is characterized by an abrupt and sustained decrease in the glomerular filtration rate (GFR). It is a common disorder in companion animals and humans, and is associated with high treatment costs as well as high morbidity and mortality. Four phases are currently recognized in AKI: initiation, progression, maintenance, and recovery. Using the common clinicopathologic markers (e.g., serum creatinine), the disease is recognized only in the maintenance phase, and when clinical signs are overt. Typically, at this point more than 80-90% of the kidney function is already lost.

Despite advances in the management of AKI patients, including renal replacement therapies, the mortality rate among human and animal patients remains unacceptably high. Over the past 50 years, mortality rates of human patients with AKI in the intensive care units have remained as high as 70%. One of the speculated reasons for the high mortality is the late recognition of the disease and consequently the narrow window of opportunity for therapy. The term AKI has been adopted in human medicine, and more recently in veterinary medicine, also to emphasis the need to recognize the disease early, before overt renal failure is evident, and when therapeutic interventions may be more effective. The late identification of the disease provides only a narrow window of opportunity for therapy before patients die from uremia. Therefore, there is a need for early identification of AKI in both human and veterinary medicine. In veterinary medicine, this need is further emphasized because renal replacement therapies are not readily available.

Despite the improvement that had occurred in other fields (e.g., use of biomarkers in cardiology), serum creatinine concentration, despite its multiple shortcoming, remained in use as a marker for AKI during the last few decades. Substantial changes in GFR may be associated with relatively small changes in serum creatinine concentration in the first 24–48 hours following initiation of AKI. This delay inhibits the ability to accurately estimate timing of injury and to assess the severity of dysfunction following injury. Increases in the serum creatinine concentration as little as 0.5 mg/dL have been associated with increased mortality. Moreover, even a transient rise of serum creatinine concentration (for 1–3 days) resulted in an increased odds ratio for in-hospital mortality, and with the need for chronic dialysis over the ensuing 3 years.

In recent years there is a growing research in nephrology with the attempt to identify sensitive and specific biomarkers of AKI, mostly in human patients. The data in veterinary medicine is still scarce, and further work is warranted. Each biomarker has its individual strengths and weaknesses in diagnosing AKI, thus, most likely, an array of biomarkers will be used in the future, not only to identify the disease early, but also to aid in the determination of the etiology and the prognosis.

For biomarkers to be useful, clinicians need to test patients with risk for kidney injury and before overt renal failure is evident. There are many biomarkers that have been assessed, mostly in human medicine, however some were anecdotally evaluated in veterinary medicine as well.
**Gamma-glutamyl transpeptidase**

Gamma-glutamyl transpeptidase (GGT) is located at the proximal renal epithelial cells and can be detected in the urine. Its instability requires samples to be analyzed quickly after collection, thus limiting its clinical utility. In one study, 24 hour urine GGT activity was measured in dogs with experimentally induced AKI using gentamicin. Increased GGT activity was documented as early as day 1, whereas serum creatinine concentration increased only 7 days after the study initiation, suggesting that GGT is highly sensitive marker.

**N-acetyl-β-glucosaminidase**

N-acetyl-β-glucosaminidase (NAG) is a proximal tubule lysosomal enzyme. During kidney injury uNAG/creatinine ratio increases and thus can be used as a marker for kidney injury. Increased NAG levels have been reported in human patients with nephrotoxicity, in delayed renal allograft function, and following cardiopulmonary bypass procedures. It has also been shown to precede the increases in serum creatinine concentration by 12 h to 4 days, thus may facilitate treatment. It has also been shown that NAG is associated with the severity of the injury and its increase was correlated with the need for renal replacement therapy as well as death. The fact that NAG may be increased in variety of other non renal disorders (e.g., rheumatoid arthritis hyperthyroidism) renders its specificity lower, thus false positive predictions may occur.

**α1 and β2-microglobulins**

β2-microglobulin is an 11.8-kDa protein expressed on the cell surface of all nucleated cells. β2-microglobulin is typically filtered by the glomerulus, reabsorbed and catabolized almost entirely by the proximal tubular cells. It has been shown to be an early marker of tubular injury in a number of settings, including nephrotoxicant exposure, cardiac surgery, and renal transplantation. β2-microglobulin was found to precede increases in serum creatinine concentration by 4–5 days. Its major downside is its instability in urine.

α1-microglobulin is a ~30-kDa protein which is synthesized by the liver and its free form is readily filtered by the glomerulus. It is then reabsorbed by proximal tubule cells. Unlike β2-microglobulin, α1-microglobulin is stable, and thus is considered more practical as a marker for proximal tubule dysfunction. Unfortunately, number of conditions have been identified to alter its plasma and serum levels (e.g., liver disease), therefore decreasing its specificity.

**Retinol Binding Protein**

Retinol binding protein (RBP) is a 21-kDa protein, synthesized by the liver, freely filtered by the glomerulus and subsequently reabsorbed and catabolized by the proximal tubule. Retinol binding proteins were found to be highly sensitive indicators of renal tubule dysfunction, preceding urinary NAG elevation. For example, in infants following birth asphyxia, increased RBP concentration were predictive of AKI. As other biomarkers, serum RBP levels are influenced by other factors, thus false negative predictions may occur.
Retinol binding protein was assessed in dogs with pyometra as markers of proximal tubular function and found to be significantly increased compared to healthy controls. In this study 68% of the 17 non-azotemic dogs had increased concentrations of urinary biomarkers indicating that dogs with pyometra sustain AKI that is often not detected using the routine markers.\textsuperscript{17}

**Cystatin-C**

Cystatin-C, a 13-kDa protein, is a cysteine protease inhibitor that has been studied relatively extensively as a marker for AKI in human patients. Unlike serum creatinine concentrations it is not affected by sex, age, and muscle mass, thus is considered a specific marker of kidney function. Due to its low molecular weight Cystatin-C is freely filtered by the glomerulus. It is subsequently reabsorbed and catabolized, but not secreted, by the tubules. Urinary and serum Cystatin-C were found to be sensitive markers of AKI, superior predictors of the disease compared to serum creatinine,\textsuperscript{18,19} and associated with the prognosis.\textsuperscript{20}

**Kidney Injury Molecule-1**

Kidney injury molecule-1 (KIM-1) is a type I cell membrane glycoprotein. It is substantially up regulated in AKI and was found to be a sensitive marker of the disorder.\textsuperscript{21,22} Urinary KIM-1 was found to be elevated within 12 hours after an ischemic renal insult, prior to the appearance of casts in the urine or increase in serum creatinine concentration.\textsuperscript{22} KIM-1 was also found to be an outcome predictor in 202 patients with established AKI, and demonstrated that elevated levels of urinary KIM-1 were significantly associated with death or dialysis requirement.\textsuperscript{19}

**Neutrophil Gelatinase-Associated Lipocalin**

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein initially identified bound to gelatinase in specific granules of the neutrophil. NGAL was found to be up-regulated more than tenfold within the first few hours after ischemic renal injury in a mouse model. It was also found as an early urinary biomarker for ischemic renal injury.\textsuperscript{23} NGAL was detected in mice urine as early as three hours after cisplatin administration.\textsuperscript{24} In pediatric patients undergoing cardiopulmonary bypass NGAL preceded the increase in serum creatinine by 1–3 days.\textsuperscript{25} It has been shown to increase in number of inflammatory conditions in which it is filtered by the glomerulus and may be found in the urine

**Interleukin-18**

Interleukin-18 (IL-18) is a cytokine and as such is elevated in a variety of inflammatory conditions. Nonetheless, it is constitutively expressed in the distal tubular epithelium and its levels are elevated in patients with AKI. It has been shown to increase in patients with delayed graft function compared with normal patients that had other urinary system disorders as prerenal azotemia, urinary tract infection, chronic kidney disease, and nephrotic syndrome.\textsuperscript{26} In a study of critically ill adult patients with acute respiratory distress syndrome, increased urinary IL-18 was found to be an early marker of AKI, preceding changes in serum creatinine by 1–2 days, and was associated with the prognosis.\textsuperscript{27}
Fatty Acid–Binding Protein

Fatty acid-binding proteins (FABPs) are 15 kDa proteins that are expressed in tissues with active fatty acid metabolism. There are 2 types of FABP, of which the liver-type is found in the proximal tubule and the heart-type in the distal tubule. Urinary liver FABPs were found as markers of number of urinary tract disorders such as chronic kidney disease, diabetic nephropathy, IgA nephropathy, and contrast nephropathy. In patients undergoing cardiopulmonary bypass it has been shown to be an early predictor of AKI.

References

SDMA – A Potential Surrogate For GFR

Roberta Relford, DVM, PhD, DACVIM, DACP
Idexx Reference Labs

Investigators

- Maha Yerramilli MS, Ph.D.
- Edward Obare, BS
- Murthy Yerramilli, Ph.D.
- Melissa Beall, DVM, PhD
- Jane Robertson, DVM, MS, DACVIM

Biomarker….what is it?

Substance than can indicate a biological state in association to:

- disease
- Progression of disease
- Response to therapy or intervention

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Insert B
IDEXX Reference Labs
2012
SDMA – a potential surrogate for GFR (validation and stability data)

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SDMA – A Potential Surrogate For GFR
Roberta Relford, DVM, PhD, DACVIM, DACP
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Biomarker….what is it?

Substance than can indicate a biological state in association to:

- disease
- Progression of disease
- Response to therapy or intervention

___________________________________

___________________________________

___________________________________

___________________________________

___________________________________
Current Status

- **Creatinine**
  - Pro:
    - Reliable and easy to measure
    - Inexpensive
    - Good sensitivity and specificity
  - Con:
    - Estimates of GFR from creatinine are relatively insensitive
    - Interindividual variability due to muscle mass, protein intake, age, sex

- **GFR**
  - Measurement of GFR with inulin, iohexol, gadolinium or creatinine are cumbersome
    - Multiple injections
    - 24 hr urine collections
    - Analytical availability is limited

SDMA as a renal biomarker

- Literature review published in 2006
  - Kielstein, 2006 Nephrol Dial Transplant
  - 18 studies, N=2136
  - SDMA correlated with inulin clearance
    - R=0.85 (CI 0.76-0.91)
  - SDMA correlated with creatinine
    - R=0.75 (0.46-0.88)

- Cats with CKD and Hypertension
  - Jepson, 2008 JVIM
  - N=69
  - SDMA correlated with creatinine
    - R=0.74 (P< .001)

- Breed, Gender, exercise, white-coat
  - N=10 (2-4 yr, lg breed)
  - N=38 (3-10 yr med-lg breed)
  - No white-coat effect on SDMA

Methylated Arginines: ADMA and SDMA

**What are they and why do they matter...??**

- Arginine
  - Conditional essential amino acid
  - Most animals make their own
  - Synthesis of arginine is via GI-Renal collaboration.
  - Citrulline is produced by the epithelial cells of the small intestine
  - Citrulline is in turn extracted by the proximal renal tubular cells and converted to arginine and released into circulation.
  - Small intestinal disease or renal disease can reduce endogenous synthesis of arginine whereby the body would need to rely on dietary sources.
  - Most dietary sources that contain protein are adequate
  - Arginine methylation occurs in every nucleated cell
  - Two isomers are created:
    - Asymmetric dimethylarginine (ADMA)
    - Symmetrical dimethylarginine (SDMA)
Methylated Arginines: ADMA and SDMA

Why is arginine (and ADMA & SDMA) important…?
- Used in RNA translation, protein shuttling, and signal transduction
- One key pathway is availability of nitrous oxide for endothelial cell function
  - Vasodilation
  - Blood pressure
  - Blood flow
- ADMA - interferes with NO activity by inhibition of nitrous oxide synthase
- SDMA - interferes with the production of nitrous oxide

As biomarkers...
- ADMA
  - Correlates with cardiovascular function
  - A strong predictor of cardiovascular events and death.
- SDMA
  - Correlated with glomerular filtration rate (GFR)

Can SDMA help to detect CKD earlier?

- Biomarkers?? i.e. ↑SDMA?
  - ↑Creatinine
  - ↑BUN
  - ↑Microalbumin
  - ↑UPC

Why? Early detection improves outcome
SDMA as Diagnostic Assay

- SDMA - Work to date
  - LCMS method is developed (for research)
  - ELISA is in development (for commercial assay)

Validation of SDMA

Method Validation - SDMA

Validation characteristics – CL/MS (liquid chromatography – mass spectrometry)
- Sensitivity
- Crossreactivity and Interferences
- Matrix Effect and Recovery
- Linearity
- Accuracy
- Precision
- Ruggedness
- Stability
- Interferences
Sensitivity and LLOQ

- **LLOQ** (Lower Limit of Quantitation): Lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy.
- LLOQ is much lower than the 1.56 µg/dl.

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Conc (µg/dL)</th>
<th>Accuracy (%)</th>
<th>Signal (mm)</th>
<th>Baseline noise (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>1.56</td>
<td>100.23</td>
<td>208.00</td>
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</tr>
<tr>
<td>2.00</td>
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<td>103.16</td>
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<td></td>
</tr>
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<td>101.24</td>
<td>207.00</td>
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</table>

**Mean**

<table>
<thead>
<tr>
<th>Conc (µg/dL)</th>
<th>Accuracy (%)</th>
<th>Signal (mm)</th>
<th>Baseline noise (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.58</td>
<td>101.10</td>
<td>208.00</td>
<td></td>
</tr>
</tbody>
</table>

**SD**

<table>
<thead>
<tr>
<th>Conc (µg/dL)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>1.81</td>
</tr>
</tbody>
</table>

**Signal/Noise ratio**

- 104.00

**Acceptance criteria:**

- Signal/noise > 10
- 4 out of 5 LLOQ must be 80-100%
- %CV < 20%

Carryover and Interferences

- Serum/plasma blanks were analyzed for SDMA carryover after injection of STD 9 (ULOQ).
- The carryover was calculated as a percentage of STD 1 (LLOQ).

Linearity

- Ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to concentration of analyte. (1.56 µg/dl to 100 µg/dl)
## Intra-day (Within) Precision and Accuracy

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration (µg/dl)</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
<th>% Accuracy</th>
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</thead>
<tbody>
<tr>
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<td>5</td>
<td>1.56</td>
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<td>1.61</td>
<td>1.54</td>
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<tr>
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<td>3.08</td>
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<tr>
<td>3</td>
<td>6.25</td>
<td>5</td>
<td>6.33</td>
<td>0.1309</td>
<td>1.78</td>
<td>98.67</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>18.75</td>
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<tr>
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<td>5</td>
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<td>8</td>
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<td>9</td>
<td>100</td>
<td>5</td>
<td>103.07</td>
<td>1.4923</td>
<td>1.48</td>
<td>100.62</td>
</tr>
</tbody>
</table>

Acceptance criteria: %CV <15% except LLOQ <20%
% accuracy 85-115% except LLOQ 80-120%

## Inter-day (Between) Precision and Accuracy

Acceptance criteria:
%CV <15% except LLOQ <20%
% accuracy 85-115% except LLOQ 80-120%
N = 5 runs
9 conc levels

## Ruggedness

<table>
<thead>
<tr>
<th>Operator</th>
<th>Run Number</th>
<th>Y-Intercept</th>
<th>Slope</th>
<th>Correlation Coefficient ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.00113</td>
<td>0.0514</td>
<td>0.9997</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.000678</td>
<td>0.0504</td>
<td>0.9998</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.00784</td>
<td>0.0496</td>
<td>0.9996</td>
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<tr>
<td>2</td>
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<td>0.00453</td>
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<td>0.9998</td>
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<tr>
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<td>5</td>
<td>0.00547</td>
<td>0.0522</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

Total: $R^2 >0.99$
### Ruggedness

<table>
<thead>
<tr>
<th>Levels</th>
<th>Mean</th>
<th>Mean</th>
<th>% difference of mean</th>
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</thead>
<tbody>
<tr>
<td>STD1</td>
<td>1.36</td>
<td>1.73</td>
<td>1.55</td>
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<tr>
<td>STD2</td>
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<td>STD3</td>
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<td>STD4</td>
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<td>STD5</td>
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</tr>
<tr>
<td>STD6</td>
<td>24.63</td>
<td>24.83</td>
<td>24.73</td>
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<td>STD7</td>
<td>51.14</td>
<td>48.64</td>
<td>49.89</td>
</tr>
<tr>
<td>STD8</td>
<td>74.74</td>
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<tr>
<td>STD9</td>
<td>100.43</td>
<td>99.81</td>
<td>100.12</td>
</tr>
</tbody>
</table>

Acceptance criteria:
- Slope %CV < 20%
- % difference of concentration levels < 15%

Y = 0.0502x + 0.00921 (r=0.9978)

### Sample Stability

**Purpose:**
Long-term sample stability for SDMA at the following conditions.

**Conditions tested:**
- Room Temperature
- Freeze thaw 3X (Cycled between -80°C and RT)

**Sample population groups:**
- ENDO = Sample from Healthy population (low end of range)
- MID = Sample from Healthy population (Mid - upper end of range)
- HIGH = Sample from abnormal population (above range)

**Testing plan:**
1. Freeze thawed 3X
2. Room temperature tested at day 1, 3, 7, 10, and 14
3. Room temperature tested at day 1, 3, and 7

**Sample Type - Serum and plasma acceptable**

**Comparison of tubes at RT**

**Comparison of tubes at FT**

**Comparison of tubes at FT-High**

---

**Sample Stability Protocol**

- Long-term sample stability for SDMA at the following conditions.
- Conditions tested: Room Temperature, Freeze thaw 3X (Cycled between -80°C and RT).
- Sample population groups: ENDO = Sample from Healthy population (low end of range), MID = Sample from Healthy population (Mid - upper end of range), HIGH = Sample from abnormal population (above range).
- Testing plan: 1) Freeze thawed 3X, 2) Room temperature tested at day 1, 3, 7, 10, and 14, 3) Room temperature tested at day 1, 3, and 7.

---

**Sample Type - Serum and plasma acceptable**

- Serum and plasma acceptable.
Sample stability

Affects of Hemolysis

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Serum Conc. (µg/dL)</th>
<th>Actual Conc. (µg/dL)</th>
<th>% change</th>
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<tbody>
<tr>
<td>Serum control</td>
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<td></td>
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</tr>
<tr>
<td>Hemolysis level 1</td>
<td>5.17</td>
<td>5.41</td>
<td>4.47</td>
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<tr>
<td>Hemolysis level 2</td>
<td>6.25</td>
<td>6.20</td>
<td>-</td>
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<tr>
<td>Hemolysis level 3</td>
<td>7.32</td>
<td>6.90</td>
<td>-6.14</td>
</tr>
<tr>
<td>Hemolysis level 4</td>
<td>8.40</td>
<td>7.72</td>
<td>-8.83</td>
</tr>
<tr>
<td>Hemolysis level 5</td>
<td>9.48</td>
<td>8.96</td>
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</tr>
<tr>
<td>Lysate</td>
<td>10.78</td>
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<td></td>
</tr>
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</table>

Affects of Lipemia

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Conc. (µg/dL)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum control</td>
<td>7.63</td>
<td></td>
</tr>
<tr>
<td>Lipemia level 1</td>
<td>7.40</td>
<td>3.01</td>
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<tr>
<td>Lipemia level 2</td>
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<td>Lipemia level 4</td>
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<tr>
<td>Mean</td>
<td>7.39</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td>2.37</td>
<td></td>
</tr>
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</table>
### Affects of Bilirubin

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Conc. (µg/dL)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum control</td>
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<td></td>
</tr>
<tr>
<td>Icterus level 1</td>
<td>7.99</td>
<td>-6.82</td>
</tr>
<tr>
<td>Icterus level 2</td>
<td>7.48</td>
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<td>Icterus level 3</td>
<td>7.46</td>
<td>0.27</td>
</tr>
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<td>Icterus level 4</td>
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<td>1.20</td>
</tr>
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<td>Icterus level 5</td>
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<tr>
<td>mean</td>
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<tr>
<td>SD</td>
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<td></td>
</tr>
<tr>
<td>%CV</td>
<td>2.88</td>
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</tbody>
</table>

### Conclusions of SDMA Validation

- The sensitivity of the assay is adequate for analysis of SDMA in serum and plasma samples, if used under conditions specified.
- The assay is linear from 1.56µg/dl to 100µg/dl with an R²=0.99 over multiple replicates.
- The %CV of between-run and within-run accuracy and precision is well below the 15% acceptance criteria.
- The assay is robust in that there were no major differences between users or different columns or different instruments.
- Stability studies indicate that the SDMA is stable in several collection devices as well as in acid and basic conditions.

### Now we have an assay for SDMA…now what??

- Will SDMA help with early detection of CKD?
- Is SDMA influenced by breed, age, weight?
- Is SDMA influenced by hydration status?
- Is SDMA influenced by diet?
- Will SDMA be a more sensitive indicator of progression?
- Can SDMA have value as a prognostic indicator? Trending?
- Is production of SDMA stable?
- Is production of SDMA affected by presence of other diseases?
- ... ????
Application of Novel Biomarkers in Dogs with Experimental Acute Kidney Injury

Carrie A. Palm, DVM, DACVIM
Davis, CA

Acute kidney injury (AKI) represents a spectrum of disease severity, ranging from injury that is clinically non-detectable to severe damage that can progress to acute renal failure (ARF). In both humans and animals, ARF is the most severe stage of AKI and is associated with mortality rates that often exceed 60%.1 This in large part because of the inability to recognize renal injury soon after an initial insult. Creatinine is the most commonly utilized marker of renal function, but is insensitive for early detection of renal injury as elevations in creatinine outside of the reference range do not occur until there is loss of approximately 75% of renal function. In addition, factors such as breed, age, body condition, fluid volume status and laboratory variation contribute to the insensitivity of creatinine for early AKI diagnosis. A staging system for AKI has recently been adopted from human medicine for use in veterinary medicine.2 This staging system allows for categorization of patients into 5 stages of AKI, ranging from non-azotemic AKI to severe AKI and ARF. These categories are based on absolute levels of azotemia, as well as changes in azotemia over a specified period of time. The concept of staging patients with AKI stresses the need to identify renal injury early in its course and highlights the limitations of utilizing serum creatinine as a maker for early renal injury.2 Diagnosis of renal injury earlier than is currently possible with traditional evaluation of creatinine, may allow for therapeutic interventions that could lead to better prognoses. In addition, ability to recognize injury that is not significant enough to cause obvious creatinine elevations, may give a better understanding of variables causing injury that are not currently obvious.

Because serum creatinine is not ideal for early diagnosis of AKI, there is an interest in evaluating renal biomarkers to assess for evidence of early kidney damage. Biomarkers are natural substances that can be detected in urine or blood, which may allow for early diagnosis of AKI, either individually or in combination. An ideal marker of AKI should be easily detectable, sensitive and specific, have rapid laboratory or point-of-care turnaround time, and of course, should have significant and earlier elevations as compared to creatinine. Several candidate markers are being evaluated in human medicine, including Neutrophil-Gelatinase Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), N-acetyl-b-d-glucosaminidase (NAG) and cystatin-C. None of these have been widely investigated in veterinary medicine although studies are published evaluating their utility in animal models of AKI, and in human medicine.4 These markers, and many others, hold promise for allowing early diagnosis of AKI and may provide an opportunity for veterinarians to treat AKI in its earliest stages before high mortality occurs.

NGAL is a 25kDa protein that can be measured in both blood and urine. It has been shown to be one of the earliest and most robustly elevated markers in AKI.5 In human medicine, cardiac surgery is commonly associated with AKI. In one study, a large number of children under going elective cardiac surgery developed AKI (as defined by an increase in serum creatinine by 50%) within 2-3 days post-procedure. In that same study, significant elevations in NGAL occurred within 2 hours of surgery, several days prior to the changes in creatinine.5 This supports that NGAL may be of benefit to detect early declines in renal function. A canine specific ELISA NGAL kit is currently available for evaluation in the research setting (Abbott Laboratories, Abbott Park, IL).

KIM-1 is a transmembrane protein that is highly over-expressed in proximal tubule cells after ischemic or nephrotoxic renal injury in animal models.6 It has been reported that urinary KIM-1 can be used to distinguish ischemic AKI from prerenal azotemia and from chronic kidney disease.6 Both a human and rat ELISA, as well as a point-of-care (Rena-stripe) KIM-1 tests (BioAssay Works®, LLC, Ijamsville, MD,) are available, but these tests have not been validated in the dog.6,8 Both the KIM-1 ELISA...
and Rena-strip have been shown to be sensitive and specific markers of ARF in humans and rats. In addition, the KIM-1 Rena-strip also has the advantage of being a point-of-care test, which allows for a rapid turnaround time.

Because these two urinary markers seem promising in human and animal models, we have started investigations to evaluate their utility in dogs. The first part of the study evaluated if these specific markers could be detected in the urine of dogs. The second part determined if these markers differentiated AKI from other types of urinary tract diseases. Finally, biomarkers were evaluated in 5 dogs with experimentally induced AKI in the laboratory setting, to determine if these markers had significant increases sooner than serum creatinine. Daily blood and urine samples were collected from dogs before and during experimental induction of kidney injury (as defined by a 50% increase in creatinine values from baseline). In addition, blood and urine samples were obtained during renal recovery, to allow for comparison of biomarker concentrations through all stages of AKI.

Evaluating urinary biomarkers may lead to a better understanding of the progression to ARF and will determine if there is a more sensitive and specific test to diagnose AKI before it develops into a life-threatening condition. Recognition of AKI in the initial stages, may allow for early intervention and a reduction in the high mortality occurs with this disease. In addition, the availability of biomarkers that allow for early recognition of AKI may give a better understanding of processes that are causing significant renal injury.

REFERENCES:

Severe acute kidney injury (AKI) is characterized by an abrupt and sustained decrease in the glomerular filtration rate (GFR). It is a common disorder in companion animals and humans, and is associated with high treatment costs as well as high morbidity and mortality. Four phases are currently recognized in AKI (i.e., initiation, progression, maintenance, and recovery), however using the common clinicopathologic markers (e.g., serum creatinine), the disease is recognized only in the maintenance phase, and when clinical signs are overt.

Despite advances in the management of AKI patients, including renal replacement therapies, the mortality rate among human and animal patients remains unacceptably high. Over the last few decades, mortality rates of human patients with AKI in the intensive care units have remained as high as 70%\(^1\). One of the potential reasons for the high mortality is the late recognition the injury using the common clinicopathologic markers. This late recognition narrows the window of opportunity for therapy. Therefore, markers that can identify the disease early in its course are needed, both human and veterinary medicine. In veterinary medicine, this need is further emphasized because renal replacement therapies are not readily available, and the window of opportunity for recovery is even narrower.

Despite the improvement that had occurred in other fields (e.g., use of biomarkers in cardiology), serum creatinine concentration, despite its multiple shortcomings, remained in use as a marker for AKI during the last few decades. Increases in serum creatinine concentration are not sensitive and once documented represent kidney failure. Moreover, substantial changes in GFR may be associated with relatively small changes in serum creatinine during the first 24–48 hours following initiation of AKI. This delay inhibits the ability to accurately estimate timing of injury and to assess the severity of dysfunction following injury. Increases in the serum creatinine concentration as little as 0.5 mg/dL have been associated with increased mortality.\(^2\) Even a transient rise of serum creatinine resulted in an increased odds ratio for in-hospital mortality,\(^3\) and with the need for chronic dialysis over the ensuing 3 years.\(^4\) It is therefore clear that markers that can identify injury (and not only failure) are in need.

Routine and novel biomarkers of kidney injury have been recently studied, mostly in human patients. The use of readily available markers such as fractional excretion of electrolytes has multiple advantages, since such markers are readily available and cost effective. Fractional excretion of sodium has been evaluated in human medicine both as a prognostic indicator and as a marker for differentiating between pre-renal from intrinsic renal failure in azotemic patients. A high sodium fractional excretion was more common in patients with severe morphological tubular injury whereas lower sodium fractional excretion was documented in transient AKI and non oliguric AKI. Sensitivity and specificity of sodium fractional excretion were 78% and 75%, respectively for diagnosing patients with severe AKI,\(^5\) but the use of diuretics substantially decreases the performance of sodium fractional excretion.\(^5\) These findings indicate that sodium fractional excretion can be used as a surrogate for the severity of the injury.\(^5\)–\(^8\) There are data to suggest that fractional excretion of sodium and chloride can be used as markers of kidney injury severity in dogs, and their normalization during the course of the disease represent recovery.
Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein initially identified bound to gelatinase in specific granules of the neutrophil. NGAL was found to be up-regulated more than tenfold within the first few hours after ischemic renal injury in a mouse model and is considered one of the most promising biomarkers. It was also found to be an early urinary biomarker for ischemic renal injury.\(^9\) It was detected in mice urine as early as three hours after cisplatin administration.\(^10\) In pediatric patients undergoing cardiopulmonary bypass NGAL preceded the increase in serum creatinine by 1–3 days.\(^11\) Unfortunately NGAL has also been shown to increase in number of inflammatory and infective conditions in which it is filtered by the glomerulus and appears in the urine, thus decreasing its specificity.

This lecture will present unpublished data regarding the use of routine biomarkers and NGAL in dogs with AKI.

References

Biomarkers in Dogs with Proteinuric Nephropathies

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Texas A&M University, College Station, TX

INTRODUCTION

The search for better non-invasive biomarkers of kidney disease is an active area of research, and several promising urine and serum biomarkers have been investigated in acute and chronic renal diseases. Although many urinary proteins indicating renal damage and/or dysfunction have been variably studied in dogs with renal disease, only a few have been studied sufficiently to begin to glean a sense of how they might be useful in detecting and monitoring chronic kidney disease (CKD) due to proteinuric nephropathies. This session provides an update on some of these biomarkers with regard to their clinical utility based on several recent studies.

URINARY BIOMARKERS OF TUBULAR DAMAGE/DYSFUNCTION

Proteins of tubular origin can be present in the urine due to release by damaged tubular cells or by impaired absorption of proteins normally present in the glomerular filtrate. These proteins have great potential as early biomarkers of tubulointerstitial (TI) damage. While their sensitivity in acute renal disease has been documented, their ability to identify TI damage earlier than conventional tests (e.g., serum creatinine (sCr)) in dogs with CKD needs further evaluation. In particular, their specificity for TI damage must be verified. While associated structural changes have been observed histologically in dogs with elevated urinary biomarkers, some studies call into question the influence of proteinuria due to glomerular damage on these tubular proteins. For instance, in dogs with progressive CKD due to X-linked hereditary nephropathy (XLHN), several urinary biomarkers were increased as compared with normal dogs well before an increase in sCr, but typically only after development of proteinuria. Factors such as in vitro hematuria, pyuria, and bacteriuria did not appear to have a significant effect on urinary N-acetyl B-D-glucosaminidase (NAG) and retinol binding protein (RBP); however, the influence of in vivo inflammation and hemorrhage have not been adequately assessed for any of the urine biomarkers. Two tubular proteins evaluated in multiple recent studies are discussed in more detail below.

N-acetyl B-D-glucosaminidase (NAG)

NAG is a lysosomal enzyme present in proximal renal tubular cells, and it is released from the cells secondary to tubular damage. Therefore, it is generally accepted as a biomarker of tubular injury, which has been supported by the increase seen secondary to administration of nephrotoxic drugs. Analytically it is an attractive protein because it can be measured using an enzymatic assay and is therefore easily adapted to different species, unlike many of the immunoassays that require a species-specific antibody. Because of this, it is one of the most studied of the non-albumin urinary proteins in renal disease. In normal dogs, activity of this enzyme is generally low, although leukocyte contamination and semen contamination can result in increased activity. While there is some overlap with that seen in normal dogs, activity is typically higher in dogs with renal disease. Activity was increased in dogs with pyometra-associated renal disease, and it significantly decreased, typically to a level comparable with normal dogs, after ovariohysterectomy. While there was moderate correlation with severity of glomerular and tubular damage in dogs with progressive CKD due to XLHN, correlation with proteinuria was higher. In addition, urinary NAG activity was compared with severity of glomerular and tubular damage based on renal biopsy in ~100 dogs with various renal diseases, typically manifested as protein-losing nephropathy (PLN). NAG activity demonstrated moderate correlation with severity of glomerular damage but poor correlation with severity of TI damage (Nabity, unpublished observations).
Therefore, whether urinary NAG is more likely present due to glomerular or tubular damage in dogs with PLN is questionable, and further investigation of its urinary origin needs to be performed. Even if present due to ongoing tubular damage, preliminary results suggest that NAG activity does not appear to be a useful indicator of the severity of tubular damage in dogs.

Retinol binding protein (RBP)

RBP is a low molecular weight (LMW) protein that, when circulating freely, will readily pass through the glomerular filtration barrier but is essentially completely reabsorbed by the proximal tubules in health. As tubular function decreases, reabsorption of RBP also decreases, resulting in increased urinary excretion. While it is therefore considered a marker of tubular dysfunction, glomerular proteinuria can interfere with absorption of LMW proteins. RBP is increased in dogs with proteinuric renal disease as compared with normal dogs, and it is also typically increased in dogs with pyometra-associated proteinuria, with resolution after treatment of the pyometra. A study of dogs with XLHN showed that RBP correlated well with sCr and GFR, and it correlated more strongly with histologic indicators of renal injury severity than did sCr and GFR. In addition, RBP was found to be markedly increased in the urine of XLHN dogs with proteinuria and early azotemia as compared with when those dogs were proteinuric but not azotemic. These studies show promise in the utility of RBP for the early diagnosis and monitoring of TI dysfunction. However, Raila et al evaluated several subsets of dogs with various combinations of proteinuria, azotemia, and reduced creatinine clearance. This study found that urinary RBP was of no diagnostic value for the early detection of reduced GFR in nonazotemic dogs, and it demonstrated a moderate correlation with UPC, which could suggest a greater influence of proteinuria than tubular damage on its urinary presence. Therefore, further evaluation of RBP is required before its clinical utility in dogs can be adequately assessed.

URINARY BIOMARKERS OF GLOMERULAR DAMAGE/DYSFUNCTION

The predominant urinary protein present with glomerular damage is albumin. However, large proteins such as intact immunoglobulins may also be present, and it is thought that the greater the compromise to the filtration barrier, the larger the proteins that can pass through. Because so little protein is present in the urine of normal animals, even mild glomerular damage can often easily be detected. The most studied non-albumin glomerular protein is immunoglobulin G (IgG). IgG is a high molecular weight protein (~160 kDa) that is normally present in very small amounts in normal canine urine. Like NAG and RBP, it is increased in the urine of dogs with CKD and pyometra-associated proteinuria, and it typically decreases to normal concentrations with treatment of pyometra. In a study of dogs with progressive CKD due to XLHN, IgG did not correlate any better with the severity of glomerular damage than UPC. However, in ~90 dogs with various protein-losing nephropathies it did correlate much better than UPC with severity of glomerular damage (Nabity, unpublished observations). It may therefore be useful as a non-invasive indicator of the extent of glomerular injury. Interestingly, despite its much larger size (~900 kDa), urinary IgM did not correlate as well as IgG with severity of glomerular damage in these dogs with PLN (Nabity, unpublished observations).

ENDOGENOUS MARKERS OF GFR

In addition to urinary markers of renal damage, the search for endogenous serum/plasma markers of GFR that can perform better than sCr and BUN continues. One promising molecule is symmetric dimethylarginine (SDMA), which originates from hydrolysis of methylated proteins. This molecule is increased in people with CKD, and preliminary studies in dogs show that it correlates well with sCr but that it might have a tighter reference interval in normal dogs as compared with sCr (IDEXX, unpublished observations). Therefore, it might be of more use than conventional tests in indicating a
decreased GFR based on a single blood sample. However, additional studies are in progress to further evaluate this molecule.

REFERENCES:
Over fifty years ago the first stem cell transplantations were performed in human patients, after being studied for nearly a decade in beagle dogs. Thus began the field of bone marrow transplantation. The field of bone marrow, cord blood, and mobilized stem cell transplantation has now saved countless lives, has donor registries that can be searched world-wide, and is becoming routine for patients who need replacement of their hematopoietic, or blood-forming, systems. Over the next 50 years we are poised to see remarkable advances in the use of stem cells to treat disease and heal tissue injuries outside of the blood-forming system. Stem cell treatments for athletic injuries in horses and companion animals are currently ongoing and in many cases will lead the way to human clinical trials and future cellular therapies. In particular the field of adipose-derived mesenchymal stem cell therapy, or “stromal vascular fraction” therapy to repair canine and equine injuries and degenerative conditions is becoming increasingly available to the public, and can be administered by qualified veterinarians through the company Vet-Stem and others. Vet-Stem, for instance, has recently announced that over 8,000 animals have now been treated with their therapy, with “greater than 75% of horses treated with Vet-Stem cell therapy for tendon and ligament injuries able to return to their previous level of performance, and dog owners reporting that greater than 80% of dogs treated with Vet-Stem cell therapy having an improved quality of life.” This high degree of regenerative medicine success rates in treating spontaneously-occurring disorders in large animals with an intact immune system is a highly applicable precedent for similar treatments in humans, as well as being great news for dog and horse owners.

Mesenchymal stem cells/marrow stromal cells (MSC) are the cell type most commonly investigated for tissue repair therapies. These interesting cells, derived most often from the bone marrow or fat, can directly contribute to the repair of bone, tendon and cartilage, and can serve as “paramedics” to help heal tissue through local and systemic secretion of proteins. In addition to bone marrow and adipose tissue, MSC-like populations can be isolated from different tissues such as muscle, tendon, dental pulp, periodontal ligament, umbilical cord blood, placenta, liver, cartilage, synovium, synovial fluid, spleen, and thymus, using criteria established to describe bone marrow derived MSC (reviewed in Arthur et al.). These different MSC-like populations share similar phenotypes and capabilities suggesting a similar ontogeny. The perivascular niche is a common stem cell microenvironment for resident MSCs within the different tissues (reviewed in Caplan). An interesting tissue compartment is the “stromal vascular fraction” from adipose tissue, which is a rich source of perivascular MSC in both humans and animals.

To date, MSC from bone marrow and adipose tissue have been extensively tested and proven effective in pre-clinical animal models of many injuries and disorders. The most common method for administration of the cells is through intravenous infusion. Following infusion, MSC are capable of systemic migration, in particular to areas of hypoxia, inflammation or other tissue injury, are not prone to tumor formation, and appear to tolerate the immune response across donor mismatch. After homing to or lodging in the region of tissue injury, MSCs release trophic factors that hasten endogenous repair. These secreted bioactive products can suppress the local immune system, enhance angiogenesis, inhibit fibrosis and apoptosis, and stimulate recruitment, retention, proliferation and...
differentiation of tissue-residing stem cells. These paracrine effects exerted by MSC are distinct from the classical model of direct differentiation of stem cells into the tissue to be regenerated. Some current studies aim to enhance these paracrine effects through forced over-production of various growth factors and other proteins, to further hasten the endogenous repair processes.5, 10, 19-21

MSCs can be infused without tissue matching, since they shield themselves from the immune system18. The ability to be transplanted without tissue matching has allowed large multi-center trials to be conducted with direct comparison of the same batches of MSC without adverse events or rejection reactions.22, 23 Human MSCs are currently being tested in clinical trials for numerous applications including, but not limited to: myocardial infarction, stroke, knee injury, Crohn’s disease, critical limb ischemia, neurodegenerative disorders, kidney disease, graft-vs.-host disease and autoimmune disorders.3, 21, 24-26 Companies such as Osiris, Mesoblast, and Athersys are in Phase I – III clinical trials using large lots of MSCs expanded from a single or several donors. The cells are infused without tissue matching, due to their ability to shelter or “hide” from the immune system. MSCs have well-documented immunosuppressive activities (reviewed in 18, 27, 28) and MSCs are now being used to successfully treat steroid-refractory Graft vs. Host disease.29-31

In veterinary medicine, the animal’s own stem cells are used as an “autologous” product, rather than using a banked allogeneic product expanded from a donor animal. However the clinical trials currently underway in humans suggest that safely expanded and well-qualified allogeneic MSC products might be considered in future veterinary applications, with more careful study. The benefit would be the availability of “off the shelf” products able to be infused almost immediately in instances of acute injury. However, a key factor in the processing of MSC batches for human clinical trials is the complete elimination of hematopoietic stem cells and macrophage components, since those will invoke immune attack and inflammation in the recipient and could even lead to graft vs. host disease. If allogeneic products are to be used in veterinary medicine in the future, we must understand more of the basic science and develop better markers to qualify batches of cells for therapeutic application, following guidelines and minimal safety standards currently used in the manufacturing of expanded human mesenchymal stem cell products.

For human clinical trials, the path to the clinic is long and arduous in comparison to veterinary trials. For human cellular therapy trials involving more than minimal manipulation of the cells, an Investigational New Drug (IND) application must be prepared with all supporting documentation and submitted to the FDA for eventual clearance to proceed with the Phase I clinical trial, a process that can take several years and a high budget. In contrast the path to veterinary trials, using the patient’s own cells, is far more streamlined. This advantage allows clinical trials of novel stem cell therapies in horses and companion animals to lead the way in many cases. For mesenchymal stem cells, since the expansion methods, biology, and in vivo trafficking of the cells are highly conserved from large animals to man, lessons learned from the veterinary trials can be directly translated to human cellular therapies, and vice versa. Therefore, through the concept of “one health”, in addition to helping our animal companions through novel stem cell-based approaches, veterinarians who conduct clinical trials are also helping to advance the field of human regenerative medicine.

References


Feline Chronic Kidney Disease: Cell Therapy Perspectives

Robert Harmon, DV, MPVM
Vet-Stem

What are Stem Cells?
Stem Cells are:
– Primitive cells present in almost every tissue
– Able to become different types of tissue: Tendon, Ligament, Bone
– Self-renewing
– Trophic Factors

Overview of “Trophic Effects”
Vet-Stem Adipose Processing Lab

1. Immediate use
2. Frozen for future
3. Lifetime supply by culture

Stem Cell Processing Lab Quality

CRITICAL ELEMENTS:
- Quality Control Systems
- Sterility
- Validated procedures
- Certified trained technicians
- Calibrated equipment
- Quality Assurance Inspections
- Dose Control – cell count/viability

Potency Testing

- Adipogenic Differentiation
  - Oil Red O: Stains triglycerides, lipids and fat deposits.
- Chondrogenic Differentiation
  - Alcian blue: Stains glycosaminoglycans, esp. sulfated like chondroitin sulfates
- Osteogenic Differentiation
  - Alizarin Red S: Binds calcium deposits in matrix mineralization

Control Media

Induction Media
CD Marker Analysis

SVF Cells
Expression dependent upon donor:
CD 34: hematopoietic progenitors and endothelial cells
CD 45: pan-leukocyte marker

Expression dependent upon donor:
CD 44: ASC marker
CD 90: ASC marker

Y-axis CD45 – 14.7%
X-axis CD34 – 75.7%
Y-axis CD90 – 94.7%
X-axis CD44 – 80.8%

CD Marker Analysis

Cultured Cells
< 10% of the cells will express
CD 34: hematopoietic progenitors and endothelial cells
CD 45: pan-leukocyte marker

CD 44: ASC marker
CD 90: ASC marker

Y-axis CD45 – 2.5%
X-axis CD34 – 0.0%
Y-axis CD90 – 99.9%
X-axis CD44 – 99.1%

What is the Stem Cell Content of the SVF from Adipose Tissue

Reprinted by Collas et al and Yoshimura et al.
Why Adipose as Stem Cell Source?

- High healing cell count – No culturing required
- 1000X stem cell concentration as bone marrow
- Family of healing cells - heterogeneous
- Rapid, Easy to access
- Over 1700 peer reviewed papers published

In-vitro Comparison Adipose to BM

Current Supported Indications Canine/Feline

- Osteoarthritis
- Polyarthritis
- Tendonitis
- Ligament injury
- IBD Pilot
- Compassionate Use
Feline CKD Autologous Cases
Pilot Compassionate Use

Protocol:
1. Requested Data
   - Age, weight, sex, breed
   - Complete physical, CBC, Chem, UA, Urine cult
   - Renal Specific: UPC, Blood pressure, ultrasound
2. Inclusions
   - Full work up for complete diagnosis
   - Agreement to provide follow up data
3. Exclusions
   - Active cancer
   - Other disease that would make surgery too risky
   - Unreasonable expectations

Feline CKD Autologous Cases
Pilot Compassionate Use

Protocol:
1. Submission of compassionate use request
2. Review of clinical data – approve or deny
3. Collection of adipose tissue - FedEx
4. Processing in lab – create immediate IV dose, freeze balance
5. Cells returned fresh for intravenous slow administration
6. Repeated doses per clinical condition and available frozen cells
7. Attempted minimum dose of \(0.55 \times 10^6\) MNC/kg

Feline CKD Autologous Cases
Overview

<table>
<thead>
<tr>
<th># Cases Treated</th>
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<tr>
<td>Average time*</td>
<td>776 days</td>
</tr>
<tr>
<td>Longest</td>
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<tr>
<td>Average Dose</td>
<td>(5.4 \times 10^6) MNC</td>
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<tr>
<td>Average Dose/kg</td>
<td>(1.47 \times 10^6) per kg</td>
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<tr>
<td>Average # Doses</td>
<td>1.7</td>
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<tr>
<td>Percent Still Alive</td>
<td>84%</td>
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* Excludes cases less than 6 months since treatment
### Feline CKD Autologous Cases
#### Veterinary – Response to Treatment

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<tr>
<td>None</td>
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</tr>
<tr>
<td>Mild</td>
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<tr>
<td>Good</td>
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<tr>
<td>Excellent</td>
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<td>TOTAL</td>
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<tr>
<td>No outcome yet</td>
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### Feline CKD Autologous Cases
#### Veterinary – Quality of Life

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<tr>
<td>Worse</td>
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<td>7.7%</td>
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<tr>
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<tr>
<td>Mild</td>
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<tr>
<td>Significant Improve</td>
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<td>53.8%</td>
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<tr>
<td>TOTAL</td>
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<tr>
<td>Waiting for outcome</td>
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### Feline CKD Autologous Cases
#### Change in Clin Path Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>#</th>
<th>%</th>
<th># Cases with Clin Path follow-up</th>
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</thead>
<tbody>
<tr>
<td>BUN</td>
<td>19.6%</td>
<td></td>
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</tr>
<tr>
<td>Creatinine</td>
<td>3.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>15.6%</td>
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<tr>
<td>Average time for changes</td>
<td>450 days</td>
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<tr>
<td>IRIS Stage Change pre/post</td>
<td>3.0 / 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPC not consistently reported</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Feline CKD Autologous Cases
Summary of Experience

1. There was a trend to improvement in BUN, Creatinine, RBC, and IRIS staging.
2. Poor compliance with UPC and blood pressure measures, so no conclusions possible.
3. No obvious correlation between starting IRIS stage and ability to respond.
4. No reported adverse events related to this therapy.

Positive outcomes and good safety with fairly low dose intravenous administration has led to clinical trial design and collaboration to evaluate intra-renal arterial administration compared to intravenous and placebo at AMC.
Assessment of Allogeneic Mesenchymal Stem Cell Therapy for Feline Kidney Disease

Jessica Quimby, DVM, DACVIM
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INTRODUCTION
Stem cell therapy is an innovative new field of scientific investigation and clinical application that holds promise for a variety of diseases in veterinary medicine. Recent years have brought increased interest in the potential for adult stem cells to help in the treatment of many diseases through both their regenerative properties as well as their apparent ability to alter the environment in injured and diseased tissues. In particular, mesenchymal stem cells can migrate to affected areas and may be able to support the growth of other stem cells as well as moderate the response of the immune system.

STEM CELLS
A stem cell is a generic term referring to any unspecialized cell that is capable of long-term self-renewal through cell division but that can be induced to differentiate into a specialized, functional cell. Stem cells are generally divided into two groups, embryonic stem cells and adult stem cells. Adult stem cells can be obtained from many differentiated tissues including but not limited to bone marrow, bone, fat, and muscle. Obtaining adult stem cells also does not raise ethical concerns. For most studies, the adult stem cell in question is actually a mesenchymal stem cell or mesenchymal stromal cell. Mesenchymal stem cells are multipotent but not pluripotent, which means they can differentiate into some, or “multiple,” but not all tissue types.\(^1\)

Characterization
Mesenchymal stem cells or mesenchymal stromal cells (MSC) are plastic adherent and assume a fibroblast-like morphology during culture. They proliferate easily in culture and can be cryopreserved without loss of phenotype or differentiation potential. Additionally cell surface marker characterization via flow cytometry differentiates them from hematopoietic cells, though no truly unique MSC molecule has been identified. In part, the lack of definitive markers probably reflects the diverse lineage of MSC and the fact that each MSC population reflects to some degree the characteristics of tissues from which they were derived. Most importantly stem cells possess the ability to differentiate into cell types of multiple lineages including adipocytes, chondrocytes, and osteocytes.\(^1\)

Immunologic properties
Mesenchymal stem cells clearly modulate immune responses, as demonstrated by both in vitro and in vivo studies. For example, MSC are poor antigen presenting cells and do not express MHC class II or co-stimulatory molecules and only low levels of MHC class I molecules.\(^1\) Thus, MSC are very non-immunogenic and can be transferred to fully allogeneic recipients and still mediate their immunologic effects. Among their other immunological properties, MSC inhibit lymphocyte proliferation and cytokine production, suppress dendritic cell function and alter DC cytokine production, and decrease IFN-g production by NK cells.\(^1\) These properties of MSC can be harnessed therapeutically (as discussed below).
STEM CELLS AND KIDNEY DISEASE

There are numerous studies of MSC therapy in rodent models of renal failure, though most studies have focused on models of short-term protection from acute renal disease.\(^2\)\(^-\)\(^5\) The majority of these studies provide evidence that systemic administration of bone marrow-derived or adipose-tissue derived MSC can help preserve renal function in the face of acute insults and can also help reduce tubular injury and fibrosis.\(^2\)\(^-\)\(^5\) Several studies have also demonstrated incorporation of small numbers of MSC into the renal parenchyma.\(^4\)\(^,\)\(^6\)\(^,\)\(^7\) It has been proposed that some of these MSC may actually differentiate into functional renal tubular epithelial cells, though this theory remains controversial. Other investigators propose that paracrine effects from the injected MSC are more important than the effects of direct cellular incorporation into the kidney.\(^8\)\(^,\)\(^9\) Thus, the available data indicate that systemically administered MSC can help improve or stabilize renal function in acute renal disease by a variety of mechanisms.

Fewer studies have investigated the effects of MSC therapy in chronic renal failure models in rodents.\(^10\)\(^-\)\(^15\) In the CKD rodent models that have been investigated, administration of MSC has been beneficial, especially with respect to reducing intra-renal inflammation and suppressing fibrosis and glomerulosclerosis.\(^10\)\(^,\)\(^11\)\(^,\)\(^13\)\(^,\)\(^14\)\(^,\)\(^16\) Results from a recent study, which utilized a rat remnant kidney model of CKD, are particularly noteworthy.\(^10\) In this study, the authors found that 3 i.v. injections of relatively low numbers of MSC led to significant and sustained improvement in renal function (eg, reduced serum creatinine concentrations and proteinuria) and markedly suppressed intra-renal fibrosis and inflammation. Repeated injections of MSC were found to be more effective than a single injection, and MSC additionally MSC were identified in the kidney after systemic injection. Thus, there is compelling evidence from rodent models of CKD that MSC injections can lead to significant improvement in renal function. MSC secrete a variety of biologically active factors and release of these factors, either systemically or locally into the kidney parenchyma, could affect renal function both directly and indirectly. In vitro studies have demonstrated that MSC can produce growth factors, cytokines, and anti-inflammatory mediators, all of which could help maintain or improve renal function and suppress intra-renal inflammation.\(^8\)\(^,\)\(^17\)\(^,\)\(^18\) The ability of MSC to suppress inflammation appears to be mediated both by secreted factors and by direct contact with inflammatory cells.\(^17\)\(^,\)\(^18\) Thus, MSC have the potential to strongly suppress intra-renal inflammation.

FELINE CKD

CKD remains a leading cause of illness and death in cats in the United States. This is a progressive disease, and currently no treatment short of renal transplantation has been shown to reverse or halt declining renal function for any significant period of time. This condition is characterized by tubulointerstitial damage, fibrosis and progressive loss of renal function, and is commonly described as the final common pathway after any one of multiple types of renal insults. Regardless of the initial insult, once a threshold of renal damage has been reached, progression is irreversible and appears consistent in character.\(^19\) The process of inflammation appears to be integral to the progressive, irreversible nature of CKD. It is based on this premise that mesenchymal stem cells have potential to ameliorate this disease process.

CURRENT CLINICAL STEM CELL RESEARCH FOR FELINE CHRONIC KIDNEY DISEASE.

At present, there is little published work regarding the use of MSC for treatment of naturally-occurring CKD. At the Center for Immune and Regenerative Medicine at Colorado State University, we are currently conducting research into the immunological properties of feline MSC, as well as the potential use of MSC for treatment of CKD in cats. We previously completed a pilot study investigating the safety and potential effectiveness of unilateral intra-renal MSC injections for cats with CKD.\(^20\) In that
study, we found that the MSC injections were well-tolerated and may have improved renal function in some animals. However, the number of sedations required for the procedure limited its effectiveness, and also made management of CKD in those patients more difficult. In a recently completed pilot study, we investigated the effectiveness of intravenously delivered MSC for treatment of feline CKD, using allogeneic cryopreserved MSC derived from healthy young donor animals. Preliminary results from this study are mixed, and additional pilot studies are needed to further explore efficacy.

REFERENCES

Chronic Kidney Disease (CKD) is characterized by progressive loss of renal function, and culminates with total organ failure. CKD is among the most common clinical diagnosis in middle-aged to older feline patients in which there is a loss of renal function. In our older feline patients CKD is one of the leading causes of death, affecting anywhere from 1.6-20% of all cats. In aged populations of cats, CKD was found to affect up to 35% of cats, resulting in clinical azotemia and ultimate death. There is little data on the long-term survival in cats with CKD, and many suggest the mean survival is approximately 1-3 years depending on the stage at which they are diagnosed. Knowing how common this condition is amongst feline patients, therapeutic options to prolong survival times are needed. Currently there are no known therapies to aid in the reversal of this condition and the focus, to this point, has been in supportive care including dietary changes, improving hydration, management of proteinuria and hypertension, chelation of excessive phosphorus and controlling renal secondary hyperparathyroidism. Renal transplantation has been the only available treatment option to improve renal function for a patient, which is expensive, associated with a high morbidity and mortality rate, and met with many inherent immunologic difficulties. Most cats are diagnosed early in the stage of their disease and progressive decline in renal function is witnessed.

Protein losing nephropathy (PLN) is the leading cause of renal disease and ultimate renal failure in dogs. In a randomly selected group of dogs the incidence of glomerular lesions was documented to be as high has 43-90%. This is most commonly associated with one of 2 forms: membranoproliferative glomerulonephritis (MPGN) and membranous nephropathy (MN). Immune complexes that are deposited in the glomerulus can initiate glomerular damage. Cell-mediated immune mechanisms take part in the pathogenesis and once the damage has been initiated activation of complement, influx of inflammatory cells, release of proteolytic enzymes, synthesis of cytokines and growth factors, and generation of proinflammatory mediators occurs resulting in a highly inflammatory environment and severe cell damage. There are some immunosuppressive trials looking at the effect on PLN in dogs with immune-complex deposition and inflammatory infiltrative causes of PLN, but overall outcomes in dogs with PLN is considered extremely poor. Traditionally, dogs with GN that are azotemic were all expected to survive less than 3 months. In one study the median survival of 53 dogs was 28 days. A recently reported study in 2011 compared 234 dogs with PLN and found that the median survival times (MST) for dogs with a creatinine level over 1.5 mg/dL was only 13 days versus a normal creatinine with PLN was 407 days. For dogs with PLN and nephrotic syndrome (hypoalbuminemia, hypercholesterolemia, proteinuria and 3rd space effusion) the MST was only 12.5 days versus 104.5 days in dogs without nephrotic syndrome. The investigation of alternative approaches, such as mesenchymal stem cell (MSC) therapy, in which the potential for slowing disease progression exists, is of great clinical importance.

One of the largest challenges facing veterinary nephrology is to reduce the incidence of end-stage kidney disease and progression of PLN. This can be done by blocking, or slowing down, the progression of the disease. The therapeutic strategies currently available should aim at preventing disease progression and treating the underlying condition, but this is not currently possible in most cases. In veterinary medicine, we often treat the symptoms while progression ensues.

Mesenchymal stem cells (MSC) are multipotent cells that reside in various parts of the body like fat and bone marrow. These cells are capable of differentiating in vitro and in vivo into different cell types. They are isolated from tissue based on their ability to adhere to plastic surfaces and to adopt a fibroblast-like morphology, termed colony-forming unit-fibroblasts. They are attractive candidates for renal repair because nephrons are of mesenchymal origin and stromal cells are of
crucial importance for signaling, which can lead to the differentiation of both nephrons and collecting ducts. MSC express cell surface markers such as CD29, 44, 73, 90, and 105. Previous studies in animal models have shown the MSC have the potential to enhance recovery from acute renal tubular injury and repair in both interstitial and glomerular diseases. The ultimate goal is to create an environment to impede glomerular and tubular inflammation and the progression of glomerular and interstitial fibrosis. In 2006 Kunter et al reported on the use of intra-arterial MSC injection into a rat model with mesangioproliferative glomerulonephritis. In this study 20-50% of glomeruli had fluorescently-labelled MSC detected within them. This study showed a 50% reduction of mesangiolysis, a 3-4 fold higher intraglomerular cell proliferation, reduction in proteinuria by 28%, increased glomerular cell proliferation, and more rapid mesangial reconstitutions. In this rat model acute renal failure was ameliorated by MSC injection after disease induction. This study also documented MSC secreted high amounts of vascular endothelial growth factor and TGF-B1, which have anti-inflammatory properties. Overall, MSC were shown to markedly accelerate glomerular recovery most likely related to the paracrine growth factor release.

The ultimate goal of this study is to investigate the use of regenerative medicine through adipose derived mesenchymal stem cell (MSC) therapy to aid in nephron repair, improved nephron function, decrease renal fibrosis, and ultimately slow the progression of CKD in cats and PLN in dogs. Stem cell therapy has been shown to have many potential benefits in tissue recovery, regeneration and differentiation of damaged cells by various mechanisms including the release of chemical mediators for the trophic effects (paracrine effects of growth factors and cytokines) for signal transduction, angiogenic effects, anti-apoptosis, antifibrosis and the anti-inflammatory effects. Stem cells are thought to work in 5 main ways: 1) by trophic support in response to a diseased environment through growth factors and cytokines/chemokines; 2) through anti-inflammatory effects by decreased proinflammatory T cell function and increase the anti-inflammatory natural killer cell function (through IFN-gamma); 3) by the ability to differentiate into desired tissues; 4) innate homing abilities to a site of disease through signaling and diepedesis at the site of injury; and 5) through immune system modulation by various cytokine effects on surrounding tissues.

In CKD the spontaneous healing processes are ameliorated once the phase of chronic kidney disease ensues and the hypertrophic mechanisms are exhausted. Because of this, stem cell therapy has great potential to accelerate regeneration of the damaged nephrons and microenvironment for improved renal function. Since renal failure is so common in cats, and PLN is so highly inflammatory and devastating in dogs, with renal cell death being the ultimate result, improving the health and environment of the viable cells that remain could improve the overall function of the kidneys, glomerular filtration rate, survival times and quality of life of these patients.

Because of the homing capacity of MSC in the body, once injected, these cells will ultimately be trapped peripherally in any area of diseased tissue. Older animals often have a relatively high incidence of other concurrent maladies like inflammatory bowel disease, chronic liver disease, endocrine disease (hyperthyroidism, hypothyroidism), heart disease and osteoarthritis. With intravenous (IV) systemic stem cell delivery the MSC’s will not be delivered directly to the renal tissue, but instead will home to all areas of the body with concurrent inflammation. Stem cells that have been delivered intravenously have first pass delivery to the pulmonary capillary beds before being distributed to the rest of the body. If the cells are directly delivered into the renal artery, then the first capillary bed to uptake the stem cells with the highest concentration of exposure would be the glomeruli and vast blood supply of the diseased nephrons. This could potentially result in more dramatic regenerative results. It has been shown for both myocardial disease and acute cerebral injury using rodent models that intra-arterial (IA) stem cell infusion resulted in significantly higher colonization of stem cells into the diseased area. In one study where sheep had IA infusion of MSC directly into the renal artery there was no evidence of renal damage that occurred during or after the
injection, confirming the safety and reliability of the IA procedure. These patients were also shown to have higher engraftment of MSC’s in the glomeruli, which is rarely reported in studies after intravenous systemic delivery. The speculation was that MSC were stopped in the glomeruli by a trapping phenomenon within the microcapillary vessels at the end of the glomerular tuft, which would not occur after first-pass elimination in the pulmonary capillary beds.

Intravenous (IV) injection is the least invasive method for delivery but may lead to low numbers of engrafted cells inside the diseased kidneys, and major trapping in the lung or other peripheral sites of concurrent injury (osteoarthritis, inflammatory bowel disease, inflammatory liver disease, hyperthyroidism, etc). Intraarterial (IA) injection, by the use of interventional radiologic (IR) techniques, may be used to bypass the initial uptake of the stem cells by the non-renal systemic organs and deliver larger number of cells directly to the diseased renal tissue. Anecdotally, both canine and feline patients have been injected with autologous MSC intravenously for various conditions without any known ill effects (osteoarthritis, inflammatory bowel disease, inflammatory liver disease, and chronic renal disease) but evidence of long-term benefit is not currently available.

The authors’ have delivered MSC intra-arterially into the renal artery in 11 dogs and 6 cats to date, with various forms and stages of AKI, PLN and CKD. The details of the procedure and some preliminary data will be presented in this lecture.

Since stem cells are thought to improve, repair, and aid in the growth of damaged tissue through various mechanisms and renal cell death is the ultimate result of chronic renal disease, improving the health and environment of the cells that remain could improve the overall function of the kidneys and ultimately improve the survival times and quality of life in these patients. For dogs with PLN, this can be more of an acute process which progresses quickly to chronic inflammation of fibrosis. With the association of immune complex deposition at the level of the glomerular basement membrane, interstitial nephritis and fibrosis, the benefits of stem cell therapy may also be promising.

The technique of stem cell delivery for kidney disease is potentially transferable to humans. A majority of the studies using stem cell therapy for chronic kidney disease and glomerulonephritis were performed in normal animal models (rats, sheep, pigs, etc) where various forms of kidney disease was artificially created. Using a natural model of disease and long-term follow-up data can be a beneficial model for humans.

At the Animal Medical Center we are currently conducting 2 separate randomized, placebo controlled studies. The aims of our feline study are two-fold: 1) to initially perform a pilot study on 6 cats with IRIS stage III CKD using intra-arterial autologous MSC delivery into the renal artery to assess for short term safety, and 2) if phase 1 proves safe and effective over a 3 month period then the aim is to investigate and compare the effect of autologous MSC injected via either the renal artery or cephalic (or saphenous) vein on CKD progression in a randomized placebo controlled study. The aims of our canine PLN study are 1) investigate if the use of MSC in azotemic canine patients with PLN-CKD and show they will improve renal function and protein loss over a 1 year period; 2) investigate if the delivery of the MSC selectively (IA) vs. non-selectively (IV) vs. IV placebo, will improve clinical outcome in these canine patients over a 1 year period.

The authors hypothesize that MSC therapy coupled with conventional therapy will 1) improve renal function based on biochemical data (GFR studies, creatinine levels, proteinuria, serum phosphorus concentration, etc), 2) reduce the mortality from renal failure, 3) reduce all cause mortality, 4) is safe and 5) will improve QOL compared with animals treated only by conventional therapy. In addition, MSC therapy delivered via renal arterial injection prolongs survival compared with animals receiving MSC through peripheral venous injection.

This lecture will focus on the procedure of IA stem cell injection and expand upon some of the preliminary data seen in our 17 patients to date. The study is currently on-going.
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Fluid Status and Fluid Overload in Acute Kidney Injury

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In veterinary medicine, misconceptions exist regarding the appropriate use of intravenous fluid therapy. These misconceptions frequently lead to administration of excessive fluid volumes and fluid overload. While administration of intravenous fluids is essential for volume resuscitation in hypovolemic states, there is no evidence that administration of fluids beyond the point of restoration of normal perfusion can augment renal function. Furthermore, when vasomotor dysfunction is present (e.g. the systemic inflammatory response syndrome) intravascular fluid losses may be restored to a volume sufficient to generate adequate cardiac preload, but vasodilation results in vascular pooling, inadequate cardiac preload, and poor cardiac output. In this situation, additional fluid administration rarely results in sustained normalization of cardiac output due to equilibration of fluid between the intravascular and extravascular space. Because the volume of the extravascular space is four-fold that of the intravascular space, the majority of fluid rapidly redistributes, thus minimizing the effect of the administered fluid load. Administration of large volumes of replacement fluids ultimately leads to fluid accumulation in all body water compartments, but most notably the extravascular space.

Fluid overload is defined as a 10% increase in body weight from hospital admission, due to administration of enteral and parenteral fluids. Fluid overload has been shown to be associated with poor outcomes in both adults and children with acute kidney injury. In one study of adult humans with acute kidney injury, the odds ratio for death associated with fluid overload was 2.07 in dialyzed patients and 3.14 in non-dialyzed patients. In another study of children treated with continuous renal replacement therapy for acute kidney injury, the severity of fluid overload was associated with mortality (odds ratio 1.03, suggesting a 3% increase in mortality for each 1% increase in fluid overload above 10%).

Whether fluid overload is a marker or mediator of disease severity has not been definitely determined. However, there are several mechanisms by which fluid overload may affect outcome. Fluid overload can adversely affect almost all body systems, including the cardiovascular system (myocardial edema can result in impaired contractile function) and the gastrointestinal tract (edema can prevent nutrient absorption and cause ileus). Additionally, fluid overload can affect renal perfusion and renal function by means of renal venous congestion and an increase in renal interstitial pressure (the kidney is housed in a non-distensible capsule, so edema may compromise renal perfusion and tubular flow by increasing intracapsular pressure). Lastly, fluid overload can result in pulmonary interstitial and alveolar edema, which can lead to hypoxia and a pseudo-acute respiratory distress syndrome. These patients are usually administered mechanical ventilation which increases the risk of mortality.

Assessment of optimal fluid balance is essential for preventing fluid overload. Unfortunately, there are few reliable tools for this purpose available to veterinarians in clinical practice. Serial quantifications of fluid input and output and serial measurements of body weight are two of the most objective, repeatable, and reliable tools for assessment of trends in fluid accumulation. Assessment of these parameters is both inexpensive and easy, and should be employed in all cases of acute kidney injury.
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Managing Volume Overload

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Volume overload is a relatively common complication of treatment of patients with acute kidney injury (AKI). While volume excess has been correlated to worse outcomes, recent literature suggests that volume excess contributes to worsening outcome, and is not merely a marker of more severe disease. Patients with over 10% volume overload are 2-3 times more likely to die than those with less overhydration (Bouchard 2009). Swelling and edema in various organ systems, including the kidney, gut, and brain to mention only a few, impairs function. In the case of the kidneys, this further impairs the ability to excrete a water load.

In general, anuric and oliguric renal failure have a worse outcome than polyuric renal failure in population studies, although this rubric cannot be applied to an individual patient (Bagshaw 2008). It is important to keep in mind that patients with non-oliguric renal failure who have a fixed urine output, even if it is a larger volume than considered oliguric, do not have the ability to increase urine output in response to a fluid challenge. While many clinicians are meticulous about restricting fluid load in an anuric or oliguric patient, these non-oliguric patients frequently do not have precise urine volume measurement, and are at risk for developing volume overload with therapy.

Fluid therapy is the mainstay of treating AKI (Prowle 2010). Many drugs have been evaluated, and the only intervention that improves outcome is careful fluid therapy. Fluids are given to patients with AKI to restore and maintain hydration, which is an important goal. About 30-75% of AKI arises from renal perfusion problems, including hemodynamic (pre-renal) disorders and renal ischemia. Despite common perception, fluid administration does not improve renal perfusion once AKI has become established. Fluids are also used to maintain electrolyte and acid-base status. It is commonly held that fluid administration dilutes nephrotoxins and enhances elimination, but there is no data to support this claim. It is also commonly held that a forced diuresis will enhance urine output and uremic solute excretion, but this is also not supported by the literature (Smith 2008). The kidneys are better adapted to conserving sodium and water than excreting an excess of either. Hypotension, pain, and renal injury activate the sympathetic nervous system, renin-angiotensin-aldosterone system, and antidiuretic hormone release, all of which trigger salt and water retention, not excretion (Prowle 2010).

Avoiding volume overload from the outset logically seems prudent. Multiple studies of early goal directed therapy have shown that aggressive fluid resuscitation at emergency room admission followed by a more restrictive fluid administration plan improves outcome and may decrease the incidence of AKI (Schrier 2010). In randomized studies comparing a liberal fluid administration plan (standard of care currently) with a more restrictive fluid plan, the restrictive plans improved number of days in ICU, days on the ventilator, oxygen status, number of organ failures, post-operative complications, and mortality. None of the restrictive plans worsened renal function or increased mortality, and some studies showed improvement in these parameters (Prowle 2010, Patalak 2011).

Although the data for the benefits of restricting fluid is becoming clear, from a practical perspective, there are a number of hurdles. Patients with AKI typically receive numerous medications to treat the underlying condition (i.e., antibiotics for infection-induced AKI), uremic complications (i.e., gastroprotectants for uremic gastritis), or secondary complications (i.e., aspiration pneumonia from vomiting). The volume of medications adds to the fluid burden. Additionally, malnutrition worsens outcome in critically ill humans; one would expect the same to be true in veterinary patients. However, enteral nutrition via a feeding tube has an obligate volume load for practical reasons (keeping the food consistency thin enough to not clog the tube), whereas parenteral nutrition also has an obligate volume
load to control osmolality issues. Other sources of fluid administration, such as saline used to flush catheters, are frequently not included in the fluid calculations, but can add a significant amount to the daily fluid load.

**Diuretics**

Diuretics are commonly used in an attempt to convert anuric/oliguric patients to a non-oliguric state. They are also used to effect volume removal via urine, particularly in patients with congestive heart failure. Furosemide is one of the most commonly used diuretics. Furosemide is secreted by the proximal tubule into the ultrafiltrate, where it travels to the ascending thick limb of the loop of Henle. It binds to the potassium binding site of the sodium-potassium-2 chloride pump on the luminal side, impairing sodium reabsorption. The increase in sodium in the ultrafiltrate impairs water reabsorption, increasing urine volume.

Despite the well-recognized effect of furosemide to increase urine output, it does not improve renal function or outcome (Bagshaw 2008). In one meta-analysis evaluating 962 human patients, there was no improvement in mortality, and there was no decrease in the number of patients needing renal replacement therapy, although the study was underpowered to find an effect on need for RRT (Ho 2010). Addition of furosemide may be part of a restrictive fluid plan despite the lack of improvement in GFR. There may be improvement in lung function. In a study of healthy cats, a combination of furosemide and dopamine did not increase GFR or renal blood flow despite an increase in urine output (McClellan 2006). Total urine output is higher when furosemide is given as a constant infusion compared to intermittent bolus administration (Adin 2003).

Osmotic diuretics are an alternative to furosemide and other chemical diuretics. Osmotic diuretics are freely filtered at the glomerulus, and an ideal osmotic diuretic would not be reabsorbed or metabolized. Mannitol is a classic osmotic diuretic. In addition to increasing urine volume, mannitol may expand the extracellular fluid volume, decrease blood viscosity and inhibit renin release. It can increase renal blood flow, decrease tubular swelling and help flush out obstructing tubular casts. It also acts as a free radical scavenger. Despite all of these potentially beneficial effects, mannitol, compared to fluids alone, shows no benefit in prevention or treatment of AKI.

Other diuretics have been evaluated as treatment for AKI. Diltiazem, a calcium channel antagonist, is one such drug. In a pilot study of 18 dogs with AKI from leptospirosis, dogs receiving diltiazem had a non-significant increase (p = 0.06) in urine volume compared to dogs not receiving diltiazem (Mathews 2007). Fenoldopam, a selective dopamine-1 receptor agonist, did not increase GFR in dogs and only transiently increased GFR in cats after a short infusion (Simmons 2006, Zimmerman 2003). It did increase urine output in cats. The effects of fenoldopam in dogs and cats with acute kidney injury remain to be reported.

Natriuretic peptides are synthesized in the heart. Atrial natriuretic peptide (ANP) is synthesized in the atria, whereas brain natriuretic peptide (BNP) is synthesized in the ventricles. BNP was originally identified in the brain, hence the name. These peptides are released in response to various stimuli, including myocardial stretch, ischemia, hypoxia, and neurohormonal upregulation. The release of ANP in response to atrial stretch caused by volume excess inhibits renal reabsorption of sodium and water, mainly in the collecting ducts. Natriuretic peptides also have vasodilatory effects. BNP and its metabolites are perhaps known best in the context of diagnostic testing for heart disease. However, the clinical effect of BNP led to the development of a BNP analog for therapeutic use. Nesiritide is a synthetic B-natriuretic peptide that has been approved for use in treating congestive heart failure in people. Unfortunately, a recent large study failed to find any improvement in parameters relating to CHF in people (O’Connor NEJM). Studies of this drug to prevent AKI after cardiac surgery found some renoprotective effects, but did not find an improvement in mortality.
Ultrafiltration

Ultrafiltration is a method of removing water from the patient. With extracorporeal therapies, including intermittent hemodialysis and CRRT, the fluid removal is based on a hydrostatic pressure gradient. Blood is pumped through the dialyzer by a mechanical pump. The blood is contained within multiple hollow fibers in the dialyzer. With dialysis, dialysate is pumped through the dialyzer by a mechanical pump. The dialysate is outside the hollow fibers, so it is effectively in a different compartment, but the pores in the fiber membrane allow fluid and other substances to diffuse across. By applying a mild vacuum to the outgoing side of the dialysate compartment, more water is removed from the dialysate compartment than is put in. The only place for that water to come from is the blood compartment. With hemofiltration, the same sort of vacuum is used, but the fluid that is removed is called effluent. Comparing this to peritoneal dialysis, with PD, a hypertonic dextrose solution is instilled into the abdomen. The increased osmolarity of the peritoneal dialysate compared to the blood osmolality draws water into the abdomen, and a larger volume of dialysate will be drained out than was instilled. Despite the differences in mechanism, both methods of ultrafiltration can be used to improve volume overload.

The total target volume loss may not be able to be achieved in one dialysis treatment. Rapid rates of fluid removal may cause intravascular volume depletion if the removal rate exceeds the rate of vascular space refilling from the interstitial and intracellular compartments, leading to symptomatic hypotension.

Aquaretics

Antidiuretic hormone, also known as vasopressin, is synthesized in the hypothalamus and released from the posterior pituitary in response to hypotonicity or hypovolemia. In the collecting ducts, vasopressin stimulates vasopressin receptors, which triggers translocation of aquaporin 2 channels from intracellular vesicles to the luminal surface. These pores allow free water reabsorption from the urine back into the body, concentrating the urine and conserving water. A V2 receptor antagonist prevents the expression of aquaporin-2, thus forcing free water loss through the urine, without simultaneous causing a natriuresis. An oral V2 receptor antagonist, tolvaptan, has been marketed for treatment of hyponatremia. The main indications are end stage congestive heart failure, cirrhosis, and SIADH. Whether there is a role for this drug in treating volume excess resulting from kidney disease remains to be seen.

References


Monitoring Fluid Volume in Critical Care Nephrology

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Monitoring the Status of Body Fluid Volume
Alterations of fluid balance are common and significant therapeutic targets for all stages and presentation of critical care nephrology. Both the state of hydration (the overall loss or gain of fluid) and volemia (the relative volume of the vasculature) must be estimated at presentation and reassessed at regular intervals to direct the therapeutic plan. Hypovolemia and dehydration are typical at the primary presentation, but in the critical care setting, overhydration is the prevailing consequence and must be avoided proactively.

Under most circumstances, fluid balance is judged on the basis of clinical assessments. Most patients with acute or chronic uremia have associated reductions of fluid intake and/or excessive fluid losses through vomiting, diarrhea, hemorrhage, or polyuria leading to dehydration, hypovolemia, and variable hypotension at presentation to the emergency or critical care unit. Dehydration and hypovolemia exacerbate the azotemia by superimposing hemodynamic contributions to the underlying uremia and predisposing the kidneys to additional ischemic injury and decreased urine output. In contrast, overhydration and hypervolemia are common complications of hospitalization and aggressive fluid management during the initial stages of management. Hypervolemia imposes a risk of pulmonary and peripheral edema, pleural effusion, systemic hypertension, congestive heart failure, and worsened outcomes.

Many clinical parameters are at the clinician's disposal to assess the initial and ongoing fluid status of uremic animals including: body weight, heart rate, pulse character, capillary refill time, mucous membrane wetness, chest auscultation, skin turgor, tear production, serous nasal discharge, blood pressure, central venous pressure, plasma proteins and hematocrit. Technologies including multifrequency bioimpedance spectroscopy provide additional options to quantitate noninvasively and sequentially static or real-time changes in extracellular fluid volume, intracellular fluid volume, and total body water.

Clinical Assessment and Monitoring of Fluid Balance
Despite attraction to sophisticated technology and monitoring equipment in the critical care setting, there is little substitution for thorough and comprehensive clinical assessment of the patient. Progressive increases in body weight, tachypnea, increased breath sounds, increased skin turgor, chemosis, serous nasal discharge, peripheral or pulmonary edema, circulatory congestion, ascites, and systemic hypertension are predictive of overhydration in animals receiving fluids. Oral mucous membrane wetness does not reflect hydration status in uremic animals due to decreased salivary secretion (xerostomia). Reliance on this sign often causes an erroneous underestimation of the true hydration status and inappropriate administration of additional fluid to already overhydrated animals. Fluid overload may be impossible to correct in animals with oliguric stages of AKI. Consequently, and contrary to a nearly universal clinical temptation, all fluid delivery should be curtailed or discontinued at the first evidence of overhydration.

Foremost and usually the most predictable monitor of ongoing fluid balance is serial measurement of body weight. In the critical care setting, increases or decreases in body weight reliably predict both the direction and magnitude of fluid balance. The history should include documentation of the patient’s historical weight which can be used as an estimate of the appropriate weight when the patient was normally hydrated. A 2 kg increase in a patient's body weight between observations predicts a 2 kg (2L) increase in fluid balance. This represents a typical response when patients are subjected aggressive fluid therapy.
Conversely, a 2 kg decrease predicts a 2 kg (2 L) decrease in fluid balance. This represents a typical response when patients become acutely polyuric during the course of acute uremia.

The clinical goal is to manage fluid balance by providing adequate volumes of fluid of appropriate composition to relieve deficits and ongoing losses without promoting overt excesses or continued deficiencies. Ideal dry body weight is a useful concept barrowed from the dialysis arena which is a progressively derived value designated as the body weight at which additional fluid removal would produce hypotension or signs of Hypovolemia. Alternatively, ideal dry weight is the body weight at which extracellular fluid volume is physiologic. By extension, dry weight is a useful benchmark that can be used to prevent inadvertent overhydration. Ideal dry weight usually is predicted from recent historical weight measurements before the onset of illness, or it is estimated from the weight when blood pressure was controlled (neither hypertension or hypotension) or there was no demonstrated fluid accumulation. If the patient is undergoing renal replacement therapy, the dry weight can be predicted more precisely by the response to ultrafiltration at the end of the treatment. Ideal dry weight should not be considered a static parameter but should be redefined regularly to compensate for on-going changes in the animal’s lean body mass and fat mass. Lean mass and fat mass typically decrease over time (Figure 2), and consequently, body weight will decrease independently of fluid balance. Failure to update the targeted ideal dry weight can trigger inappropriate fluid administration or ultrafiltration prescription during hemodiaysis sessions that could lead to hypovolemia or progressive overhydration, respectively, as the patient gains or loses non fluid mass. Progressive deviation from dry weight also can be recognized by routine clinical assessment of body condition. The determination of dry weight can be elusive on the basis of clinical parameters alone and often is facilitated by more objective techniques including blood volume assessment and bioimpedance spectroscopy.

A variety of additional clinical parameters are used in concert to facilitate the assessment of hydration status. Skin turgor should be assessed regularly and semi-quantitatively. It requires some interpretation as age of the patient and body condition can induce artifactual changes in skin turgor, but generally it is a reliable parameter which can be used to direct clinical decisions. To be of value, the clinician must “calibrate” their assessment of skin turgor to patients who are at ideal dry weight. Many critical care clinicians seem to have an “offset” in their assessment favoring overhydration. Many patients also have regional differences in skin turgor that require global assessment of the patient. For example, the skin turgor may appear reduced over the dorsum of the patient suggesting dehydration while it is very elastic on the ventral half of the patient associated with overhydration. Similarly, skin turgor in the cranial half of the patient may be inelastic while the caudal half is very fluid.

Tear production will often correlate with overall hydration status. A patient with a moist, glistening cornea and evident fluid in the commissures of the eyes is usually amply hydrated and typically overhydrated. Similarly, the presence of chemosis is a predictor of overhydration. Oppositely, a dull non glistening cornea and sunken eyes reflect negative fluid balance. While examining the head and face, evidence of a small, continuous serous discharge from the nares is a very predictable and useful sign of overhydration.

Cardiovascular parameters including heart rate, pulse quality, blood pressure, and central venous pressure contribute importantly to the assessment, but must be interpreted in context with other clinical features rather than as absolutes. Heart rate can increase with both dehydration/hypovolemia and overhydration in addition to pain, anxiety, and a host of non-volume or cardiovascular disorders; so it requires careful evaluation. When managing overhydration with ultrafiltration, increases in heart rate may predict impending hypovolemia and hypotension. In this setting, it usually will precede decreases in arterial
blood pressure and should not be ignored. A more sensitive harbinger of hypovolemia is a progressive
decrease in venous oxygen saturation or a visible darkening of the blood in the extracorporeal circuit.
(Figure 1)

Systemic blood pressure is influenced directly by volemia and hydration but may not reflect
pathologic alterations in these parameters due to concomitant changes in vascular resistance, presence of
antihypertensive medications, and cardiovascular disease. Predictably, over hydration in animals with renal
failure (in the absence of overt cardiovascular failure) will be associated with increases in arterial blood
pressure, and improvement or correction of the positive fluid balance will improve blood pressure
alterations correspondingly. Increased systemic blood pressure, therefore, is useful to help substantiate
clinical suspicions of over hydration. We have noted when dogs are hospitalized for management of renal
failure, there is a dramatic increase in the prevalence and severity of systemic hypertension that is caused
invariably by iatrogenic excessive fluid overload and hypervolemia.

Adjunct Assessment and Monitoring of Fluid Balance

During the extracorporeal management of critical patients, continuous in-line blood volume
monitoring has become nearly indispensable for the real-time assessment of changes in intravascular
volume, responses to resuscitative fluids, and to monitor the safe removal of excessive fluid burdens. The
monitoring of blood volume is especially critical in patients weighing less than 10 kg in which rapid fluid
removal or subtle and unrecognized fluid shifts could promote profound hypovolemia. The most commonly
used in-line blood volume monitor utilizes a noninvasively in-line hematocrit sensor which optically monitors
the hematocrit in a disposable volumetric chamber placed in the extracorporeal path. Time, hematocrit,
oxigen saturation, and percent change in blood volume can be determined continuously at 20-second
intervals for the duration of the extracorporeal procedure. The percent change from initial blood volume is
derived from the sequential changes in hematocrit according to the formula: \[ \frac{Hct_o}{Hct_e} - 1 \times 100 \], where
Hct_o is the initial or starting hematocrit and Hct_e is the hematocrit at subsequent time intervals. (Figure 1)

Venous oxygen saturation can be measured continuously and simultaneously with the in-line
haematocrit measurements or observed visibly as darkening (desaturation) of blood in the
extracorporeal circuit and also is a sensitive indicator of hemodynamic stability. Sudden or progressive
decreases in venous oxygen saturation usually reflect directional decreases in cardiac output secondary
to hypovolemia and can foreshadow impeding hypotensive events. Any decrease in venous oxygen
saturation should prompt immediate assessment of the patient and possible adjustment to the fluid
delivery or removal goals.
Figure 1 Changes in on-line hematocrit (top panel), blood volume (middle panel) and venous oxygen saturation (bottom panel) over time in response to fluid removal by ultrafiltration during hemodialysis. The excessive rate of ultrafiltration is reflected by the approximately 20+% decrease in blood volume and decline in venous oxygen saturation approaching 20% (normally venous O₂ saturation is 50-75% during hemodialysis). The venous oxygen saturation is noted to increase when the prescribed ultrafiltration was stopped at hour 4. The lack of vascular refilling suggests the patient was at “dry weight” and could not tolerate additional fluid removal.

Multifrequency bioelectric impedance spectroscopy (BIS) is a useful adjunct to supplement and quantitate the clinical assessment of static fluid balance, the nutritional status of the patient, and sequential or real-time changes in body fluid volumes. Bioelectric impedance spectroscopy uses the resistive and reactive properties of the body’s fluid compartments to non-invasively estimate their respective volumes in response to the passage of a series of imposed but imperceptible electrical currents. The volumes are derived by complicated algorithms which must be verified and validated in the specie of interest. Multifrequency BIS provides reproducible estimates of ECF, ICF, and total body water as static assessments (i.e., daily) or in real-time in response to ongoing clinical trends or therapeutic interventions. If carefully performed and validated, BIS provides the static or real-time change of the absolute volume of each compartment or can be used with less rigor to predict relative changes and trends over time. We routinely
assess the following parameters sequentially or continuously to evaluate and monitor the volume and nutritional status of patients receiving renal replacement therapy:

- Extracellular fluid volume (ECF) (L) to predict hydration status. (Figure 2)
- Intracellular fluid volume (ICF) (L) to predict changes in hydration and nutritional status. (Figure 2)
- Total body water (TBW) (L) to predict hydration status and to validate the quality of the measurements.
- ICF/kg to estimate hydration status (dehydration, <55%; overhydration, >63%)
- ECF/kg to estimate hydration status (dehydration, <20%; overhydration, >24%)
- Real-time change in ECF to assess the efficacy of ultrafiltration and intercompartmental fluid shifts.
- Real-time changes in ICF to assess perturbations of the ICF and intercompartmental fluid shifts.

![Figure 2](image.png)

**Figure 2** Sequential changes in the BIS measured ECF and ICF volume in a dog undergoing chronic intermittent hemodialysis. The measurements depict the relative stability of the ECF fluid volume over time but document the increase in lean body mass (ICF volume) with the onset of enteral feeding (days 15-80) and the subsequent malnutrition occurring from day 100 through day 270.
Figure 3  Example of a real-time BIS plot of ECF volume (black circles) and ICF volume (red circles) in an 8kg cat (predialysis serum sodium, 135 mmol/L) undergoing hemodialysis with sodium profiling from 135 mmol/L to 155 mmol/L over the course of the treatment to prevent cerebral edema and dialysis disequilibrium syndrome. The cat was also receiving 100 ml/hr of ultrafiltration. The plot demonstrates the effects of the sodium profile to reduce intracellular volume by 290 ml or by 22.3% of the initial 1.3 L of ICF volume. Presumably, this promotes similar effects on the brain volume to prevent brain swelling during the treatment. Despite the large ultrafiltration rate of 12.5 ml/kg/hr, sodium profiling also blunted the contraction of the ECF volume.
Intradialytic hypotension (IH) is defined by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines as a decrease in systolic blood pressure by $\geq 20$ mm Hg or a decrease in MAP by 10 mm Hg associated with symptoms including abdominal discomfort, yawning, nausea, vomiting, muscle cramps, restlessness, dizziness and anxiety. IH is a common adverse event reported to occur in 15-25% of all human dialysis treatments. It is problematic not only due to patient morbidity; its occurrence often results in delivery of an inadequate dose of dialysis and sub-optimal fluid removal. The problem of IH becomes more significant in critically ill patients receiving dialysis as patient-related factors leading to hypotension compound the dialysis-related causes. Additionally, acutely injured kidneys often have impaired autoregulation of renal blood flow making them particularly sensitive to additional injury from decreased perfusion.

Arterial blood pressure is determined by the plasma volume, cardiac contractility, afterload, heart rate and rhythm and the peripheral vascular resistance. Hypotension should not be thought of a problem to be corrected by any means, but rather as a symptom reflecting a problem in one or more of the factors influencing blood pressure. The treatment of hypotension should focus on correction of the underlying abnormality.

The etiology of IH is multifactorial, however reduced circulating plasma volume caused by decreased extracellular osmolality and ultrafiltration in excess of plasma refilling and in small patients the volume of the extracorporeal circuit appears to be the most important factor. Priming the extracorporeal circuit with a synthetic colloid or blood helps to prevent hypotension in small patients. If hypotension develops, ultrafiltration should be discontinued. If the blood pressure does not correct, a fluid bolus (Hetastarch 5 ml/kg, balanced crystalloid 20 ml/kg) is the appropriate treatment. Additionally, there are several dialysis interventions that can be employed to prevent and/or treat intradialytic hypotension. The rate-limiting step for fluid removal via ultrafiltration is the movement of water from the extravascular to the intravascular compartment, or plasma refilling. The use of a dialysate sodium concentration lower than the serum sodium concentration results in fluid shifts from the extracellular to the intracellular compartment as diffusion lowers the serum sodium and plasma osmolality. In contrast, high dialysate sodium promotes vascular filling by delivering osmolar solutes to the plasma water. The side effects of high sodium dialysis include increased thirst and polydipsia leading to increased interdialytic weight gain and hypertension. This side effect is less relevant in the acute hospitalized patient than in the outpatient setting, but can be minimized with sodium modeling. Most modern dialysis machines offer the option to alter or profile the dialysate sodium concentration during the course of the dialysis treatment. The profile recommended to minimize IH delivers a higher dialysate sodium concentration at the beginning of the treatment during the period of maximal solute and fluid removal, with a progressive reduction in the sodium concentration to near normal levels before the end of dialysis. Sodium modeling allows modification of the plasma osmolality during a dialysis treatment to minimize side effects while preventing sodium overload. Isolated ultrafiltration followed by isovolemic dialysis, a strategy to encourage plasma refilling by maintaining a steady plasma osmolality has also been suggested.

The normal compensatory responses to a reduction in circulating plasma volume (increased cardiac output, increased systemic vascular resistance) may not occur in the dialysis patient. Normal compensatory responses may be impaired by many factors including, concurrent medications, dialysate temperature, membrane biocompatibility, splanchnic fluid sequestration and tissue ischemia. The use
of cool dialysate has been recommended to manage IH. Cooling the dialysate by 1-2 degrees will induce catecholamine release leading to peripheral vasoconstriction and an increase in myocardial contractility.

Hypotension refractory to these interventions should be treated with vasopressors. Vasopressor agents used to increase blood pressure via vasoconstriction include dopamine and norepinephrine. Recommended dose ranges are listed in Table 1.

Increasing peripheral vascular resistance with vasopressors will increase the blood pressure at the expense of peripheral perfusion. In situations where SVR is thought to be normal to increased (pale mucous membranes, cold extremities), the addition of vasopressors should be considered only in the case of life-threatening hypotension. Temporary vasoconstriction, at the expense of peripheral perfusion may be necessary to support blood pressure and to keep the patient alive while the underlying disease process is being addressed. Hypotensive, volume-loaded patients with physical exam evidence of peripheral vasodilation (bright red membranes, rapid refill time, warm extremities) may benefit from vasoconstrictive therapy. Vasopressor drugs should be used judiciously with a goal-directed approach. Because raising blood pressure via vasoconstriction may result in decreased blood flow, a rise in blood pressure may not always be a surrogate of clinical benefit.

Cardiac output may be decreased due to diastolic dysfunction (hypertrophic cardiomyopathy, restrictive cardiomyopathy, pericardial tamponade), systolic dysfunction (dilated cardiomyopathy), severe bradycardia or tachycardia or arrhythmias. Arrhythmias should be treated if they are adversely affecting perfusion. Decreased contractility is the most significant factor responsible for hypotension in cardiogenic shock and often requires treatment with inotropic agents. In addition, animals with sepsis and other systemic inflammatory diseases are susceptible to myocardial depression. Dobutamine is primarily a beta agonist with relatively weak alpha and beta 2 activity. Administration produces positive inotrophic effect with little effect on systemic vascular resistance. At standard doses it provides increased cardiac output, increased stroke volume, and improved coronary arterial perfusion. Tachycardia is seen less frequently than with dopamine administration. It is the drug of choice for patients with low output heart failure (ie. Dilated cardiomyopathy). It can also be used in noncardiogenic shock. Seizures are occasionally seen in cats receiving dobutamine, necessitating the discontinuation of the drug.

A small study comparing these commonly suggested techniques in a small group of chronic dialysis patients prone to IH, supported sodium modeling as the most effective method to address IH\textsuperscript{1}. Cool-temperature dialysate and high sodium dialysate were also efficacious in the study population, but isolated ultrafiltration followed by isovolemic dialysis was found to be much less effective.

### Table 1: Commonly Used Catecholamines

<table>
<thead>
<tr>
<th>DRUG</th>
<th>RECEPTOR</th>
<th>CLINICAL EFFECT</th>
<th>DOSE RANGE</th>
</tr>
</thead>
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<tr>
<td>Dobutamine</td>
<td>β₁: +++</td>
<td>Inotrophy</td>
<td>5-20 µ/kg/min</td>
</tr>
<tr>
<td></td>
<td>β₂: +</td>
<td>Mild chronotrophy, mild vasodilation</td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>Dᵃ⁺: +++</td>
<td>Diuresis (dogs)</td>
<td>Dₐ⁺: 1-5 µ/kg/min</td>
</tr>
<tr>
<td></td>
<td>β: +++</td>
<td>Inotrophy, chronotrophy, vasoconstriction</td>
<td>β: 5-10 µ/kg/min</td>
</tr>
<tr>
<td></td>
<td>α: ++</td>
<td></td>
<td>α: &gt;10 µ/kg/min</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>β: +</td>
<td>Mild inotrophy</td>
<td>.05-2 µ/kg/min</td>
</tr>
<tr>
<td></td>
<td>α: +++</td>
<td>Vasoconstriction</td>
<td></td>
</tr>
</tbody>
</table>
Uremic Lung

Matt Mellema DVM PhD DACVECC
University of California, Davis

Introduction

Pulmonary edema is considered the most common complication leading to respiratory failure in patients with acute kidney injury. Uremic lung and uremic pneumonitis are two widely used labels to describe this condition in the clinical setting. Pulmonary complications are known to carry serious consequences in uremic human patients. AKI is likely both a cause and amplifier of respiratory failure syndromes. Critically ill human patients with AKI are twice as likely to require mechanical ventilation (IPPV; intermittent positive pressure ventilation) as those without AKI. Further, the subset of uremic patients which require renal replacement therapy are several fold more likely to require IPPV than those in whom uremia can be managed medically. The need for IPPV is a consistent predictor of mortality in AKI patients. When AKI and respiratory failure are both present mortality is greater than 80% in human patients.

Despite the importance of pulmonary complications in uremic patients, the terms “uremic lung” or “uremic pneumonitis” both tend to provoke a great deal of confusion and debate among veterinary clinicians. This is largely due to the changes in what these terms have meant over the last century. In addition, uremia-related lung pathology seems to follow rules that are not yet clear, intuitive, or predictable. This presentation will focus on the history of the process that has come to be called “uremic lung” and will attempt to clarify the distinguishing features that separate it from other respiratory disorders in uremic patients. The terms “uremic lung” and “uremic pneumonitis” will be considered to be synonyms based on historical usage in the literature. The history of the condition, modern updates on the pathophysiology, and important differential diagnoses will be emphasized.

Historical Perspective

Full credit must be given to Dr. Sarah Faubel of the University of Colorado for her recently published work documenting the long and colorful history of uremic lung. Her written works provide a clear historical roadmap to the controversy surrounding this disorder. The first part of this presentation will outline the historical highlights of the uremic lung literature in humans and is based largely on her exhaustive study of the problem.

Overview and definitions

Before the historical perspectives can be explored a few working definitions need to be established. The formation of pulmonary edema is central to the pathology of uremic lung. Pulmonary edema represents the accumulation of excessive fluid in the interstitium of the pulmonary parenchyma with or without flooding of the terminal airspaces. The interstitial edema accumulates on the “service side” of the alveolar septa (i.e. away from the gas exchange interface) and hypoxemia is not expected to be present until alveolar flooding with resultant V/Q mismatching occurs. Interstitial edema accumulates when the rate of formation exceeds the rate of lymphatic drainage. The two main mechanisms by which pulmonary edema fluid accumulates at excessive rates are increased pulmonary capillary hydrostatic pressure (cardiogenic or fluid overload edema) and increased pulmonary capillary permeability (non-cardiogenic edema). This distinction is of central importance to understanding the controversial nature of uremic lung. While low colloid osmotic pressure (COP) is a major determinant of transcapillary fluid flux in systemic capillaries, it is of lesser importance in the formation of pulmonary edema due to the significantly different reflection coefficient (σ) of pulmonary capillaries relative to their systemic counterparts (i.e. hypoalbuminemia is far less likely to cause clinically significant pulmonary edema on its own). Pulmonary lymphatics end at the level of the bronchioles and are not
present in the alveolar septa. Interstitial edema in the alveolar septa reaches the lymphatics via coiled wick-like protein bundles. The uninjured alveolus is relatively impermeable to paracellular fluid flow and alveolar flooding occurs via fluid movement across the leakier epithelium of the bronchioles and then flows into the alveoli. Pulmonary lymphatic flow can increase by as much as 20-fold and this represents a significant “safety factor” in the prevention of pulmonary edema accumulation. Once fluid has reached the airspaces its removal can be via either sweeping or coughing it up to the pharynx or by active pumping of electrolytes (with water following) via the alveolar epithelium. As will be discussed below, uremia interferes with several steps in both the accumulation and removal of pulmonary edema fluid. The four main mechanisms by which AKI leads to pulmonary edema may be summarized as follows: (1) impaired fluid excretion and volume overload, (2) impaired cardiac output, (3) direct capillary endothelial injury with increased capillary “leakiness” due to hemodynamic forces, and (4) indirect endothelial injury with increased capillary “leakiness” due to uremic toxins and inflammatory mediators.

Acute lung injury (ALI) is a broad term for respiratory dysfunction characterized by three main findings: (1) radiographic evidence of bilateral pulmonary infiltrates, (2) the absence of clinical evidence of congestive heart failure or fluid overload, and (3) a PaO2/FIO2 ratio of 201-300. Acute respiratory distress syndrome (ARDS) is a more severe form with largely similar criteria, but a more severe reduction in PaO2/FIO2 ratio. Some patients with ALI may progress to ARDS, but not all. Similarly, some patients will develop fulminant ARDS without clinical recognition of ALI preceding it. ALI and ARDS may be due to direct or indirect injury to the lungs. Common direct causes include aspiration pneumonia and inhalational injury. The most common indirect cause is systemic inflammatory response syndrome (SIRS) due to septic and non-septic extrapulmonary disorders. Acute kidney injury is considered an uncommon cause of ALI at present, but may be an important co-morbidity in critically ill patients with multiple organ dysfunction syndrome (MODS).

Uremic lung in the early to mid 20th century

The first report of pulmonary edema caused by renal failure was in 1901, but it was not until 1934 that Roubier and Plauchu coined the term “uremic edema”. Those authors believed a specific term was needed because they felt that the radiographic findings were unique to patients in uremia. It was at this point in history that the debate began over whether uremic edema was of cardiogenic or non-cardiogenic origin. Some 34 cases had been reported in the literature by 1952. The terms “uremic edema”, “uremic lung”, and “uremic pneumonia” were all used to describe the characteristic butterfly pattern of infiltrates. Clinicians of this era were now on the alert for this complication and felt that the infiltrates would resolve with resolution of fluid overload, uremia, or both. Authors from this era noted that the radiographic findings were often unexpected and many patients among this group lacked physical exam signs of respiratory compromise. Importantly, among this group of reported cases the pulmonary changes seemed unrelated to either the severity or chronicity of the uremia and that heart failure and fluid overload were not always present at the time the infiltrates were detected. Allwall and colleagues reported a case series of 16 patients with uremic lung in 1953. This group of patients included both AKI and CKD sufferers and the clinical finding of shortness of breath was common among them. The therapeutic approach to these patients focused on achieving a negative fluid balance and in all cases this led to successful resolution of the infiltrates. These authors and others subsequently proposed that the term “uremic lung” be replaced with “fluid lung” because they felt this terminology better reflected both the pathophysiology and appropriate course of action. While this study and many that followed seemed to make it clear that fluid overload contributes to pulmonary edema in many uremic patients, it has long since been evident that other factors play a significant role.

Pathologic diagnosis of uremic lung

The term “uremic pneumonitis” makes its first appearance in the literature in 1954, but this term has subsequently fallen out of favor in human medicine for a variety of reasons. The name was coined by Hopps and Wissler in a paper published in the American Journal of Pathology in a report of
pathologic changes in the lungs of uremic patients which were felt to be distinct. This was not the first such report (some 40 cases including pathologic descriptions had been published in total to this point), but it was certainly the largest (107 uremic patients with >3 times as many non-uremics) and best characterized up to that point. The authors characterized uremic pneumonitis as having the following diffuse and homogenous features: heavy and rubbery lungs, rich proteinaceous edema, hyaline membranes, and cellular exudates consisting primarily of macrophages. No evidence of left heart failure was present, and once again the severity and chronicity of uremia seemed to correlate poorly (or not at all) with the degree of pulmonary pathology.

In the early 1980’s uremic pneumonitis had been co-opted by criticalists as just another cause of ALI/ARDS. At this stage in medical history, a wide assortment of clinical disorders were being folded into the SIRS/sepsis/MODS milieu with important distinguishing features often being overlooked in a move to consolidate complex medical syndromes into one “grand unification theory of critical illness”. The findings in one study published in 1981 by Bleyl and colleagues confirmed those of Hopps and Wissler. These authors made particular note that uremic pneumonitis had to be distinguished from “uremic pulmonary edema” adding yet another term to the mix. In this report, the authors reported that uremic pulmonary edema lacked many of the characteristic features of uremic pneumonitis (hyaline membranes, proteinaceous edema, and desquamation/necrosis of alveolar epithelial cells). In this study, uremic pneumonitis was felt to be present only in AKI patients, but no correlation was found with the severity of uremia. Many have interpreted this report and others from this period as indicative that uremic pneumonitis is just ALI in a specific setting. However, this author’s impression is that the nature of the inflammatory exudates is actually different which raised doubts. Uremic pneumonitis has been fairly consistently described as having an inflammatory exudate that is dominated by macrophages. However, other forms of ALI classically have a massive influx of neutrophils from the onset of injury (more on this later). This disparity caused this author to reject the idea that uremic lung is merely another form of classic inflammatory ALI for many, many years, but research published in 2009 seems to suggest a feasible explanation for the discrepancy. It is important to note that reports from this era contain many patients with uremic lung that had only modest uremia for a short duration. Uremic lung does not appear to be a phenomenon limited in scope to terminally uremic patients.

Pathophysiologic studies in the 1960s and 70s

The pathophysiology of uremic lung in patients with end-stage renal disease was more thoroughly investigated than in patients with AKI during the 1960s and 70s. Advances in our understanding of uremic lung in AKI patients were limited until quite recently. Between 1962 and 1978, three important case reports/series were published in which evidence that uremic lung represents non-cardiogenic edema was presented. These reports included information that was lacking in earlier reports including right heart catheterization, BAL protein content, and resolution in patients with persistent positive fluid balances.

Pathophysiologic studies in the 1980s to the present

Increased production and activity of proinflammatory cytokines is thought to be central to the pathogenesis of ALI. Data derived from both animal models and clinical patients with AKI indicate that the condition is associated with a proinflammatory state. Serum levels of many classic proinflammatory cytokines and chemokines (IL-1β, IL-6, IL-8, and TNFα) are increased in AKI patients and IL-6 and IL-8 levels are predictive of mortality in humans. Rodent models of AKI due to ischemia-reperfusion injury (IRI) have demonstrated that the kidney is an important source of inflammatory cytokines. Interestingly, the kidney is a major source of cytokine clearance as well. The evidence to date suggests that while filtration and excretion contribute, it is proximal tubular cell uptake and metabolism of cytokines that is the principal clearance mechanism. Thus in AKI the proinflammatory state may reflect both increased production and reduced clearance of inflammatory mediators which represents a somewhat unique situation.
Rodent and sheep models have been an important source of information regarding the pathophysiology of AKI-induced lung injury. Both ischemic and bilateral nephrectomy models have been utilized and are summarized in Table 1 below. These models have focused on four primary outcomes:

- Lung histology
  - Whether it becomes abnormal, and if so how quickly it resolves
- Lung vascular permeability
  - Determined by wet-dry ratio, Evans Blue Dye extravasation, or BAL fluid protein levels
- Lung inflammation
  - Determined by leukocyte counts or myeloperoxidase activity
- Gene transcription/translation or protein expression
  - Looking at genomic effects or alterations in concentration/activity of specific proteins of interest

The two types of models are complementary. The bilateral nephrectomy model is attractive in that it removes the confounding factor of ischemia-reperfusion of other organs and allows more substantive investigation of the effects of uremia on the lungs. The findings from these studies support the concept that inflammatory injury and non-cardiogenic edema contribute to uremic lung pathology and that fluid overload is unlikely to be the sole etiology in all cases as was suggested in early reports of human patients. The studies in which genomic techniques have been utilized have illustrated that the IL-10 and IL-6 pathways may be of particular importance. These findings compliment other studies which suggest that attenuating IL-6 signaling improve lung function following AKI.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Species</th>
<th>Model</th>
<th>Duration</th>
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<td>Rat</td>
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<td>30'</td>
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<td>Kim do</td>
<td>2006</td>
<td>Rat</td>
<td>Ischemia</td>
<td>60'</td>
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<td>Mouse</td>
<td>Ischemia</td>
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<td>Mouse</td>
<td>Ischemia</td>
<td>40'</td>
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<td>22'</td>
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<td>22'</td>
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<tr>
<td>Rabb</td>
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<td>Rat</td>
<td>Bilateral nephrectomy</td>
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<td>Rat</td>
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<td>Hoke</td>
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<td>Klein</td>
<td>2011</td>
<td>Mouse</td>
<td>Bilateral nephrectomy</td>
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Recent studies of the pathophysiology of uremic lung

In the last ten years, several important studies have come out that have helped to fill in the gaps in our knowledge of the pathophysiology of lung dysfunction in uremic patients. Klein and colleagues used rodent models similar to those in Table 1 (but also including IL-6 knockout mice) to demonstrate several key points regarding lung injury in acute kidney injury and uremia: (1) they confirmed that AKI leads to increased vascular permeability and pulmonary inflammation, (2) they demonstrated that IL-6 is a direct mediator of the pulmonary effects of AKI, and (3) that the kidneys are not a required source of the IL-6. Work by Rabb and others has also added new insights into our understanding of uremic lung. Using multiple rat models of AKI, these authors demonstrated that AKI leads to dysregulation of lung salt and water regulatory systems. Specifically, they showed that AKI leads to down regulation of apical sodium channels, basolateral Na/K-ATPase, and some but not all aquaporins. These data suggest a mechanism by which uremia may lead to impaired clearance of fluid from flooded alveoli. Finally, one recent article that the author would like to call the audience’s attention to is by Okusa and colleagues (see Awad 2009 in recommended readings). This study revealed that in a mouse model of ischemic AKI there was an increase in neutrophil content in the lung. That was not a novel finding. What was novel is that the neutrophils were merely marginated and the interstitial neutrophil count did not change. These data suggest several important possibilities: (1) transmigration of neutrophils to the alveolar space is not necessary to induce lung injury in AKI and (2) uremic lung may be a unique form of ALI in which neutrophils do not accumulate within the airspaces to the same degree. This latter point may explain why the pathologic descriptions of the exudate in uremic lung are filled with macrophages not neutrophils as is seen in other forms of ALI. The result does conflict with the findings of others, but few studies have evaluated neutrophil location with this level of scrutiny and the same finding may have been present but missed by others. The result is curious in that rodent models have frequently shown that AKI causes an increase in lung levels of a chemokine called KC. This is the rodent equivalent of IL-8 in other species by which this author means it is a central neutrophil chemotactic cytokine. One could speculate that perhaps uremia doesn’t interfere with KC signaling, but rather inhibits the ability of the neutrophil to transmigrate across pulmonary vascular beds even in the face of a KC gradient promoting exactly that behavior. The findings of Okusa and colleagues are also supported by work using different models and approaches. Zarbock and colleagues used a model in which pulmonary ALI (acid aspiration) is superimposed upon both inflammatory (IRI) and non-inflammatory (bilateral nephrectomy) models of AKI. Their findings are also strongly indicative of a reduced capacity to recruit neutrophils to the pulmonary parenchyma in uremia.

Important differential diagnoses

Clearly not every patient with AKI or uremia that has an abnormal respiratory pattern/effort or altered blood gases has uremic lung. Uremic patients frequently vomit and in uremic dogs aspiration pneumonia remains a significant cause of respiratory compromise. Likewise, volume overload is an additional rule-out that is all too common in hospitalized AKI and CKD patients. Patients exhibiting tachypnea and/or hyperpnea may be attempting to maximally compensate for a metabolic acidosis. Patients with painful kidneys may be hyperventilating in response to this noxious stimulus. There is some limited evidence that hyperventilation may help to alleviate nausea and this would be another potential explanation for an altered respiratory pattern in uremic patients. Renal replacement therapy, with its requisite large central catheter placement, represents an increased risk factor for pulmonary thromboembolism in humans and perhaps veterinary species as well. Lastly, leptospirosis is a particularly challenging situation in which the clinician is faced with a multitude of potential causes of respiratory compromise beyond uremic lung alone. Pulmonary complications of leptospirosis in humans often result in diagnostic findings atypical of uremic lung including hemoptysis and nodular/coalescing infiltrates on thoracic radiographs. These findings suggest that while AKI due to leptospirosis can lead to uremic lung, it can also lead to other types of pulmonary injury that are distinct. The take home
message would be that clearly not every uremic patient with signs of respiratory compromise has uremic lung as the underlying cause.

**Future therapies**

In closing, the author is regrettably required to state that no specific therapies for uremic lung can be recommended at present. Mild to moderate hypoxemia may be managed with supplemental oxygen while more severe respiratory dysfunction is likely to require mechanical ventilation. Early and appropriate institution of supportive care, renal replacement therapy, and antibiotics in the case of infectious AKI all offer the best means at present of avoiding uremia-related respiratory failure. The need to implement mechanical ventilation in a patient with AKI likely represents a worsening of the prognosis in veterinary species as it does in humans, but there is no published data to establish this firmly at present. The avoidance of fluid overload as a primary goal is supported by much of the early literature regarding uremic lung, but many patient are likely to develop this form of ALI in spite of all efforts to that effect.

**Recommended readings**

GASTROINTESTINAL COMPLICATIONS OF UREMIA

Yann Quéau (yann.queau@royal-canin.fr)
Royal Canin Research & Development Center, France.

Uremia, and more broadly, renal dysfunction, have numerous systemic consequences, including on the gastrointestinal (GI) tract. This presentation reviews the alterations of GI physiology in the uremic state, describes their clinical consequences and reports their prevalence in dogs and cats with chronic kidney disease (CKD). Therapeutic strategies aimed at limiting the GI manifestations of renal disease are also discussed.

1. PATHOPHYSIOLOGIC EFFECTS OF RENAL FAILURE ON GI FUNCTION
Most studies are from the human literature, and caution should be exercised when extrapolating data to dogs and cats, as GI physiology and treatment modalities (e.g. dialysis) differ among species.

1.1. Gastrointestinal motor functions in uremia
Decreased esophageal transit time has been reported to occur in uremic human patients (1), but it usually remains subclinical. Gastric emptying in humans with chronic kidney disease has been variably reported to be delayed or similar to healthy controls, and to be affected by peritoneal or hemodialysis (2). However, altered gastric motility correlated poorly with clinical signs (vomiting or nausea) or GI lesions in those studies.

Dogs with experimentally induced azotemia (corresponding to IRIS stage 2 CKD), had irregular electrical activity of the small intestine and decreased propagation velocity of the myoelectrical complex in the duodenum and jejunum (3). Gastric emptying rate remained unaffected, but colonic transit time was decreased by 38%. To the author’s knowledge, no such data are available in cats.

1.2. Mucosal alterations
Xerostomia, manifested by dry buccal mucous membranes, can occur in patients with CKD secondary to a lack of salivary secretions (4); it should not be misinterpreted as dehydration. Buccal and lingual ulcerations are also seen, possibly as a result of the conversion of accumulating urea into ammonia by urease producing bacteria present in the oral cavity. Necrosis and sloughing of the cranial part of the tongue can be seen in most severe cases. Gastric mucosal ulcerations are frequent in human uremic patients, and might result from increased acidity from elevated gastrin levels, decreased pancreatic and duodenal bicarbonate secretion, altered mucosal barrier, increased ammonia or infection by Helicobacter pylori. However, gastric ulcers do not seem to be frequent in uremic dogs (5) and despite elevated gastrin levels found in cats with CKD (6), gastric hyperacidity is not a consistent finding, maybe because of gastric cell damage. Ulcerations of the upper small intestine may be more common. Gastrointestinal bleeding has been attributed to ulcerations, decreased platelet adhesiveness due to uremia, angiodysplasia and anticoagulant therapy during dialysis (2).

1.3. Effects on secretion and absorption
Decreased intestinal absorption of certain nutrients, including zinc and some B vitamins has been reported in rodents (7), but is of unknown clinical significance. In dogs, oral bioavailability of drugs/markers absorbed in the proximal and distal parts of the GI tract appears to be minimally affected in experimental IRIS stage 2 CKD (3). The total amount of water excreted in feces increased and was correlated with the colonic transit time (3). Small intestinal bacterial overgrowth has also been reported
to occur in 36% human patients with CKD, maybe as a consequence of altered GI motility (8), but no data is available in dogs or cats.

2. GASTROINTESTINAL CLINICAL MANIFESTATIONS OF RENAL FAILURE
Up to 80% human patients with end-stage renal disease present with various GI symptoms. Likewise, many GI signs are reported in uremic dogs and cats, but their prevalence remains poorly documented and is possibly different in acute vs. chronic renal disease.

2.1. Anorexia, nausea, vomiting
Anorexia or decreased appetite are multifactorial in uremic patients. Accumulation of toxic metabolic waste products (among which unidentified anorexigenic compounds) and decreased clearance of hormones (leptin, ghrelin) involved in the appetite regulation center in the brain are believed to play a key role. Hyperserotonergic state from increased tryptophan transport to the brain has also been postulated (10). Altered sense of smell, ulcerative oral lesions, stomatitis, gastritis, dehydration, anemia, metabolic acidosis or hypokalaemia, also contribute to anorexia in uremia. Nausea and vomiting, frequent in animals with advanced renal disease, also find their etiology in uremic toxin retention and uremic gastropathy, but non-renal causes should not be overlooked. Some commonly used drugs can induce nausea or alter the sense of smell or taste (opioids, antibiotics, anti-inflammatory drugs etc.).

2.2. Uremic gastropathy and GI signs
A recent retrospective study reported that gastric edema, vasculopathy and mineralization were significantly more frequent in dogs euthanized for renal failure than in control dogs (5). All these dogs presented with GI signs including inappetence and vomiting. Gastric ulcerations were not found. The prevalence of gastric lesions increased with the severity of azotemia, and the severity of mineralization was directly related to serum Ca*P product. The prevalence of uremic gastropathy has not been reported in cats. Uremic enterocolitis and diarrhea appear more frequent in uremic dogs than cats, and can be hemorrhagic. In cats, constipation, as a result to dehydration, is more common.

2.3. Pancreatitis
Acute pancreatitis is reported to be more frequent in human patients with end-stage CKD than in the general population, possibly as a result of increased concentrations of GI hormones such as cholecystokinin leading to hypersecretion of pancreatic enzymes (2). No information has been published in small animals, but in the author’s experience, pancreatitis is seen in a significant proportion of dogs with acute kidney injury.

3. THERAPEUTIC STRATEGIES TO CONTROL ADVERSE GI EFFECTS INDUCED BY RENAL FAILURE
Initial medical management of the patient to correct fluid, acid-base and electrolyte disturbances is a prerequisite as it can have significant effects on the GI condition.

3.1. Antacids, antiemetics and GI protectants
H2-receptor antagonists and proton pump inhibitors are frequently used in acute uremia to decrease gastric acid secretions. It has recently been shown that omeprazole is superior to famotidine for that purpose in healthy dogs (11). Sucralfate can be administered if there is evidence of GI ulceration (hematemesis or melena, discordant BUN:creatinine ratio). Nausea and vomiting are treated with
dopamine antagonists (metoclopramide), serotonin 5-HT3 receptor antagonist (ondansetron, dolasetron) or neurokinin receptor antagonist (maropitant).

3.2. Management of hypo- and anorexia, food aversions

Fluid, acid-base and electrolyte imbalances should be corrected first. Physical and environmental barriers to eating should be eliminated (e.g. treatment of oral ulcerations with chlorhexidine and/or topical lidocaine gel for pain relief, improvement of cage comfort, etc.). Appetite stimulants (cyproheptadine, mirtazapine, diazepam, propofol) can be tried for a limited period of time in inappetent patients, but they usually fail to promote sustained consumption of the animal’s energy requirement.

Improving palatability of the diet is key in uremic animals that can have altered sense of smell. Increasing dietary protein above the level found in commercial renal diets should be avoided, as it leads to retention of nitrogen waste products, perpetuating the vicious circle of anorexia. The adage “any food is better than no food” can be counterproductive in the long term. Increasing dietary fat is a commonly used strategy in renal diets to enhance palatability and caloric density, but can be inadequate in animals with pancreatitis, or those with risk of aspiration pneumonia (fat delays gastric emptying). Switching from a dry to a canned diet can work in some dogs, but can be unrewarding in cats with a learned texture preference. Warming up the food to body temperature may also prove useful by releasing aromas. The “buffet” approach with multiple foods must be avoided because learned taste aversion could result and limit the diet options for this pet in the future.

If the strategies described above are not successful in getting the patient to eat the right amount (sufficient to maintain weight) of the right diet, assisted feeding should be discussed, earlier in animals with poor body condition and in obese cats. Parenteral nutrition with restricted amount of protein can be used, but enteral nutrition is preferred if vomiting is absent. Nasoesophageal tubes only allow the use of liquid diets (some are formulated for renal disease) in the short term. Esophagostomy and gastrostomy tubes allow greater amounts of calories from canned renal diets to be delivered with limited complications. In one study (12), gastrostomy tubes were used in CKD dogs for up to 14 months, and body weight was maintained or increased in half the patients.

CONFLICTS OF INTEREST

Yann Quéau is an employee of Royal Canin, France. None of the studies cited in the manuscript were funded by Royal Canin.

REFERENCES


Acute kidney injury (AKI) leading to severe uremia is associated with high morbidity and mortality.\textsuperscript{1,2} A tool that can accurately and objectively assess the severity of the disease and its prognosis is thus vital.

Since the early 1980’s a number of scoring systems have evolved in human medicine to assess the severity and to forecast outcomes, mostly for emergency and critical care patients,\textsuperscript{3-6} however only a few have been developed or tested for human patients with AKI.\textsuperscript{3,7} In veterinary medicine, scoring systems have been developed to assess trauma, critically ill, and surgery patients.\textsuperscript{8-10} Recently few scoring systems were developed or tested in animal patients with AKI.

In one study, 182 dogs with AKI that were managed with hemodialysis, were used to develop and to assess a novel scoring system.\textsuperscript{11} This scoring system was not based on a known human scoring system, but rather was based entirely on statistical analysis, in which risk factors for mortality were identified and weighted based on their relative contribution to mortality. Causes for AKI in this study included leptospirosis (56 dogs), ethylene glycol intoxication (50 dogs), hemodynamic (18 dogs), and toxicoses (e.g., NSAID) other than ethylene glycol (11 dogs). Seventy variables were screened initially for a possible relationship with survival and 13 were used for subsequent analyses. Ten of these were continuous variables, and 3 reflected extra-renal system involvement (respiratory, neurological, DIC). Four models were generated, and in all higher scores were associated significantly with decreased probability of survival. The area under the receiver operating characteristic curve, as a measurement for the models’ performance was 0.88-0.91, suggesting high prediction value. It is difficult to speculate if this scoring system, which has been tailored for dogs requiring hemodialysis, can be applied reliably to dogs with less severe AKI that do not require hemodialysis for their management.

Similarly, a scoring system for cats with acute uremia, which were managed with hemodialysis, was developed using 132 cats. This scoring system was also derived statistically as was done in the previous study. Ureteral obstruction was the most common cause for acute uremia. In this scoring system 10 continuous variables and 5 categorical variables (reflected extra-renal system involvement) were included. Models developed in this study had an area under the receiver operating characteristic curve between 0.81-0.86. The relatively lower prediction value of the current scoring systems is probably related to the fact that acute uremia in dogs probably represented acute intrinsic kidney injury, whereas many cats presented for hemodialysis with acute uremia had some degree of pre-existing chronic kidney disease prior to the development of uremia, thus the cats represented a less homogenous group compared to the dog study.

Another recent study had evaluated retrospectively dogs with potential AKI based on subtle increases in plasma creatinine concentration.\textsuperscript{12} In this study 164 dogs were included, and a veterinary AKI scoring system, which was based on two human staging system (i.e., AKIN, RIFLE) was applied based on the increase in serum creatinine concentration during hospitalization as follows: stage 0, <150% increase from in serum creatinine concentration from baseline during hospitalization, stage 1, 150–199% or ≥0.3 mg/dL increase in serum creatinine concentration from baseline, stage 2, 200–299% increase in serum creatinine concentration from baseline, and stage 3, ≥300% increase in serum creatinine concentration from baseline. This study has demonstrated that the mortality rate is significantly higher for Stages 1–3 (54.2% mortality rate) compared to Stage 0 (15.7% mortality rate).
Even when considering dogs in Stage 1 alone, the mortality rate was significantly higher compared to dogs in Stage 0, suggesting that only mild increases in serum creatinine concentration in hospitalized patients may be detrimental.

Another recent study of 853 dogs with AKI has assessed prognosis of the disease based on the RIFLE (Risk, Injury, Failure, Loss and End-stage renal failure) criteria. The 30-day mortality in this study was 23.8% for dogs classified at the Risk category, 41.0% for dogs classified at the Injury category, and 78.5% for dogs classified at the Failure category. Using the dogs in the Risk category as the reference, the mortality of dogs in either the Injury or Failure categories was significantly higher. A modified score was then developed based on the RIFLE criteria including additional risk factors for mortality (diarrhea status and serum phosphorus concentration) was associated with area under the operating curve analysis of 0.8 (compared to 0.7 based on the RIFLE score alone). This study provides an additional support that the RIFLE criteria may be applied (with some modifications) for dogs with AKI.

Despite the relatively high prediction of the aforementioned scoring systems, these should be applied judiciously and with caution in individual patients. Scoring systems should not replace proper clinical assessment nor should they serve as a sole prognostic tool. Some of the models permit flexibility of clinician preference to either maximize sensitivity or specificity through individualized selection of cutoff points. Specific cutoff points also can be used to establish a “gray zone” for scores in which predictions will not be made due to the risk of misclassification. Only scores outside the “gray zone” are accepted to maximize sensitivity or specificity.

Scoring systems also can be used to objectively compare or classify the severity of AKI among different patient populations. Severity classification would facilitate comparison of reported outcomes in clinical trials with multiple patient groups or between centers. For example, if one would compare mortality rate of dogs with AKI between two different centers when the severity of the disease is center A is milder compared to center B, a conclusion that the mortality rate in center A is lower due to a specific therapeutic intervention would not be valid. Thus scoring systems are widely used in human medicine to compare the severity of illness at baseline between different populations to assure valid conclusions.

References

Clinical Staging of Acute Kidney Injury

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Acute kidney disease represents a spectrum of disease associated with a sudden onset of renal parenchymal injury most typically characterized by generalized failure of the kidneys to meet the excretory, metabolic, and endocrine demands of the body, i.e. acute renal failure. Acute kidney disease typically is recognized clinically by its advanced and most overt manifestations, acute renal failure (ARF). This convention and practice has relegated this syndrome to one of reactive rather than proactive intervention. Acute renal failure is characterized by rapid hemodynamic, filtration, tubulointerstitial, or outflow injury to the kidneys and subsequent accumulation of metabolic toxins (uremia toxins) and dysregulation of fluid, electrolyte, and acid-base balance. However, acute renal failure reflects only a subset of patients with kidney injury who have the highest morbidity and mortality. The term “acute kidney injury” (AKI) has been adopted in human medicine to better reflect the broad spectrum of acute diseases of the kidney and to reinforce the concept that AKI encompasses a continuum of functional and parenchymal damage from its least severe to its most severe manifestations. Kidney injury may be imperceptible clinically at early stages and culminate with the requirement for renal replacement therapy.

The clinical presentation of AKI includes prerenal and postrenal conditions which may be independent or combined with intrinsic renal injury depending on the functional origin, extent, and duration of the conditions inciting the disease. Animal patients most often are recognized with an acute uremia which must be differentiated subsequently into its prerenal, intrinsic renal parenchymal, and/or postrenal components for proper diagnostic evaluation, management, and staging. Acute kidney injury conceptually is a disease affecting intrinsically normal kidneys, but events predisposing to AKI frequently are superimposed on preexisting chronic kidney disease (CKD) to produce a seemingly acute uremia with similar clinical features. Currently, there are no discrete markers to define or stage the conditions that represent AKI, although some urine biomarkers are showing promise. Precise definitions for AKI have not been established in veterinary medicine. There also is no formal categorization of the spectrum of the functional deficiencies to standardize its classification, severity, stage, clinical course, response to therapy, or prognosis for recovery.

To better emphasize the concept that AKI represents a continuum of renal injury, two staging schemes (RIFLE and AKIN) have been proposed for human patients to stratify the extent and duration of renal injury and to predict clinical outcomes. There is considerable overlap between both system, and criteria for each staging category are based ostensibly on insensitive markers of renal injury including abrupt changes in GFR, serum creatinine, urine output, and duration of signs. Unfortunately, the criteria which define these staging schemes in humans are not as consistently applicable in animal patients with naturally occurring disease. In humans, AKI is most commonly a condition that manifests within the hospital setting. In animals, by contrast, AKI most commonly develops outside of the hospital setting in which the abruptness of the disease and the magnitude of changes in GFR, azotemia, or urine production are rarely known or quantitated.

The IRIS staging system for CKD was developed as a consensus scheme to promote the more uniform characterization and recognition of CKD in animals with the goal to promote understand of its
IRIS AKI Staging for Acute Kidney Injury in Dogs and Cats

IRIS AKI Stage I defines animals with historical, clinical, laboratory (biomarker, glucosuria, cylinduria, inflammatory sediment, microalbuminuria, etc.), or imaging evidence of acute kidney injury that are non azotemic and/or whose clinical presentation is readily fluid volume-responsive. IRIS AKI Stage I also would include animals with progressive (hourly or daily) increases in serum creatinine of 0.3 mg/dl within the non azotemic range during a 48 hour interval.

IRIS AKI Stage II defines animals with documented acute kidney injury characterized by mild azotemia in addition to other historical, biochemical, or anatomic characteristics of AKI. This would include animals that have an increase from their baseline serum creatinine associated with pre-existing CKD.

IRIS AKI Stages III, IV, and V define animals with documented AKI and progressively greater degrees of parenchymal damage and functional failure (uremia). Each stage of AKI is further substaged on the basis of current urine production as oligoanuric (O) or non oliguric (NO) and on the requirement for renal replacement therapy (RRT). The inclusion of substaging by urine production is based on the importance of the interrelationship of urine production to the pathological or functional contributions to the renal injury and its influence on the clinical presentation, therapeutic options, and outcome of AKI. Substaging on the requirement for renal replacement therapy is established on the need to correct life-threatening iatrogenic or clinical consequences of AKI including severe azotemia, hyperkalemia, acid-base disorders, overhydration, oliguria or anuria, or the need to eliminate nephrotoxins. The requirement for renal replacement therapy could occur at any AKI stage. Substaging on the requirement for renal replacement therapy has similar clinical, therapeutic, and prognostic implications as for urine production to categorize the severity of the renal injury as well as its influence on outcome.

Just as IRIS staging for chronic kidney disease has facilitated consistency of recognition and categorization of the management and outcome predictions for CKD, IRIS staging for AKI portends an instrument for the earlier recognition, therapeutic stratification, and outcomes assessment of acute kidney injury in dogs and cats. Animals recognized and managed with IRIS AKI Stages I and II may regain adequate renal function within 2 to 5 days, forestalling life-threatening azotemia and electrolyte disorders and usually need only short-term support. Those with higher IRIS Stages of AKI at presentation or whose IRIS AKI stage progresses during hospitalization may require weeks of supportive care before the onset of renal repair. Animals with severe kidney failure, IRIS AKI Stage IV or V, may die within 5 to 10 days despite appropriate conventional management unless supported with renal replacement therapy for an indefinite time. This disparity between the window of survival with conventional supportive therapy and the extended time required to repair severe acute renal injury underlies, in part, the poor prognosis and outcomes associated with severe stages of AKI.
Table 1. IRIS AKI Staging Criteria

<table>
<thead>
<tr>
<th>AKI Stage*</th>
<th>Serum Creatinine (mg/dl)</th>
<th>Clinical Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>&lt; 1.6</td>
<td>^Non azotemic AKI or volume-responsive AKI (^b) Historical, clinical, laboratory, or imaging evidence of renal injury (^c) Progressive non azotemic increase in serum creatinine; ≥0.3 mg/dl within 48 hours</td>
</tr>
<tr>
<td>Stage II</td>
<td>1.7 – 2.5</td>
<td>Mild AKI: Historical, clinical, laboratory, or imaging evidence of AKI and mild static or progressive azotemia</td>
</tr>
<tr>
<td>Stage III</td>
<td>2.6 – 5.0</td>
<td>Moderate to Severe AKI: Documented AKI and increasing severities of azotemia and functional renal failure</td>
</tr>
<tr>
<td>Stage IV</td>
<td>5.1 -10.0</td>
<td></td>
</tr>
<tr>
<td>Stage V</td>
<td>&gt; 10.0</td>
<td></td>
</tr>
</tbody>
</table>

*Each stage of AKI is further sub-staged on the basis of current urine production as oligoanuric (O) or non oliguric (NO) and on the requirement for renal replacement therapy (RRT); (see text)

Figure 1, above, illustrates the use and concept of IRIS AKI staging in 5 patients at presentation and during 5 subsequent days of hospitalization:

**Patient 1** illustrates a patient who has been admitted to the hospital for an acute history of anorexia and vomiting. On Day 1 (the first day of presentation) the patient has no evidence of renal dysfunction. However, on Day 2, despite the serum creatinine remaining within the reference range, it is clear the patient has an AKI and a diagnosis of IRIS AKI Stage I is established which prompts heightened therapeutic attention and monitoring. The patients remains at IRIS AKI Stage I for the next 2 days, but on Day 5 the AKI stage is revised to IRIS AKI Stage II indicating worsening of the renal injury.

**Patient 2** is an 8 year old cat with a history of renal pelvic and ureteral stones and has been diagnosed previously with chronic kidney disease at IRIS CKD Stage II. Now the cat presents with an acute illness characterized by depression, lethargy, and anorexia. At presentation the azotemia is at...
historical levels, but is noted to be increased by 0.2 mg/dl on Day 2. On Day 3, however, the creatinine has increased by 0.3 mg/dl from the baseline (within 48 hours) and now prompts a diagnosis of AKI (IRIS AKI Stage II) on pre-existing CKD. Despite therapy, on Day 4, the AKI has worsened and is re-staged at IRIS AKI Stage III. Sequential updating the AKI stage documents the clinical course and severity of the kidney injury in a systematic manner that can be universally interpreted by attending colleagues or consultants. On Day 5 the AKI staging is updated to IRIS AKI Stage II predicting the therapy has started to work and the kidney injury is improving.

**Patient 3** is a 9 year old cat with no previous history of illness, but presents to the hospital with an acute onset of depression, lethargy, anorexia, fever, and large painful kidneys on abdominal palpation. Based on the clinical and laboratory findings (azotemia, bacteriuria) and results of an abdominal ultrasound that revealed mild pelvic dilation, a diagnosis of AKI (IRIS AKI Stage IV) secondary to pyelonephritis is made and the cat is started on antimicrobial therapy and fluids. Daily re-staging of the this patient reveals a progressive lowering of the AKI Stage on Days 3 through 5 categorizing the progressive improvement in the kidney injury.

**Patient 4** is a 4 year old Cocker Spaniel who presents to the emergency service for a 3 day history of acute depression, anorexia, and vomiting. The history reveals he has been treated for 16 days with daily subcutaneous injections of Amikacin for the management of an antibiotic resistant pyoderma. Based on these clinical findings and the laboratory evaluation, a diagnosis of AKI (IRIS AKI Stage IV, O) is established and the dog is started on conventional medical therapy. Despite the therapy, the azotemia progresses during hospitalization, and on Day 4, the AKI stage is updated to IRIS AKI Stage V (O) suggesting progressive and worsening kidney injury and lack of responsiveness to conventional therapy. On Day 5 the staging is further adjusted to IRIS AKI Stage V (O, RRT) indicating the decision to institute hemodialysis.

**Patient 5** is a 3 year old DSH cat who presents with a 4 day history of anorexia, depression, vomiting, and no notable urine production. The owners of the cat celebrated an anniversary 5 days ago with the introduction of several bouquets of flowers (including lilies) which they insist the cat could not have had access. On the basis of this information and the laboratory and imaging (abdominal x-ray and ultrasound) findings, a diagnosis of AKI was established. The condition was staged as IRIS AKI Stage V (O). On Day 2 the cat was started on hemodialysis and the staging was updated to IRIS AKI Stage V (O, RRR) for the following 4 hospital days. Although this illustration did not follow the cat beyond the initial 5 days of hospitalization, he remained dialysis dependent for 3 weeks before the kidney injury repaired, and he was subsequently discharged with an uneventful recovery.

As has been demonstrated in human nephrology, AKI staging has the potential to better discriminate the pathophysiologic and therapeutic spectrum of AKI. It, very importantly, has the potential to sensitize clinical evaluation of patients to promote earlier recognition of AKI. Like IRIS staging of CKD, staging of AKI should promote better comparative assessment of clinical status and therapeutic strategies. Finally, AKI staging (in conjunction with scoring strategies for AKI) will hopefully promote more realistic predictions of outcomes in affected patients.
Monitoring Renal Function In Acute Kidney Injury

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Acute kidney disease represents a spectrum of disease associated with a sudden onset of renal parenchymal injury and proportionate immediate losses of renal excretory functions including: glomerular filtration, tubular reabsorption, tubular secretion, and urine concentration/dilution. Over time, additional metabolic and endocrine functions of the kidney may become dysregulated and manifested clinically. Depending on the underlying etiology and extent of the injury, the onset of renal repair and recovery of renal function may be delayed by days, weeks, or months. Alternatively, the injury may be so extensive that recovery is not possible, and the patient progresses to a state of chronic dysfunction or CKD. For animals with severe stages of AKI, the opportunity for recovery of renal function may be preceded by death from the consequences of the renal failure if the patient is not supported with renal replacement therapy to permit recovery to proceed. Consequently, it is necessary to monitor the progress of renal functional to provide appropriate perspective and guidance on the progress of the disease and prognosis for recovery.

Ideally clinicians could monitor specific biomarkers for the process of morphologic repair to the glomerulus or tubular-interstitium to signal the onset of recovery prior to the return of discrete renal functions. Although, the hunt for such biomarkers is ongoing, concurrent with predictors of renal injury, currently there are no validated candidates available for clinical use. The clinician must therefore rely on conventional predictors of renal function or dysfunction to forecast the patient’s future.

Urine production:

Oliguric stages of AKI generally are associated with less favorable outcomes than non-oliguric conditions. The conversion of anuric or oliguric stages of AKI to a non-oliguric state has been an important early goal for the medical management of AKI and perceived as a harbinger of improving renal function for decades. The desire to promote a diuresis has solidified the empirical use of diuretics (mannitol and furosemide) and renal vasodilators (dopamine and fenoldopam) as components of conventional therapy when fluid therapy alone is ineffective. Induction of an effective diuresis has potential to improve fluid and electrolyte imbalances, promote clearance of endogenous and exogenous toxins, and facilitate delivery of adjunctive therapies which may appear to represent improved renal function. Theoretically, increases in urine production could augment intratubular fluid flow and removal of luminal debris, necrotic epithelial remnants, and tubular obstructions promoting and extending the tubular injury to facilitating renal repair. Nevertheless, these two discrete events are not explicitly coupled, and increased urine production may just mean...increased urine production without prognostic or outcome significance.

The pharmacologic or natural induction of urine production may disclose patients with less severe renal injury, but the validity of changes in urine production to predict outcome or improvement in renal function in human and animal patients with severe stages of AKI is losing credibility. Despite the early observational and experimental benefits ascribed to diuretic agents that have fostered the use of urine production as a surrogate for improved outcome, there has been little documented correlation between urine production and attenuation of the renal injury, improve mortality, or requirement for
renal replacement therapy. Outcomes analysis has not been performed in veterinary patients with naturally acquired AKI to confirm or refute the influence of urine production on outcome. However, in a recent but yet unpublished study in dogs with IRIS AKI Stage IV or V (O) AKI, dogs with leptospirosis induced AKI demonstrated increased urine production over the first 7 days of hospitalization which correlated with survival compared to dogs with other etiologies and no increase in urine production who did not survive. This observation may be predictive for leptospirosis specifically and not generalizable to other causes of AKI.

**Serum Creatinine and Urea:**
Progressive (and often subtle) azotemia is the hallmark of AKI, but the progressive component is observed inconsistently in animals because of variations in the stage and chronicity of disease at presentation. Serum creatinine increases proportionally with the severity of the renal injury or completeness of urinary outflow obstruction. Oppositely, reductions in lean body mass, creatinine generation, and overhydration can lower the serum creatinine concentration and overestimate monitored renal function. There is a curvilinear and inverse relationship between serum creatinine and GFR. Incremental changes in monitored serum creatinine will be small in IRIS AKI Stages I and II despite significant changes (increases or decreases) in GFR. Notwithstanding the insensitivity and limitations of serum creatinine as a biomarker of kidney injury, it currently stands as the best, most time-tested, and most familiar clinical marker of AKI. Serum creatinine has been designated as the surrogate marker of renal function for the IRIS staging of AKI, and changes in serum creatinine are used to predict improving or worsening stages of the condition. With careful interpretation, sequential monitoring of serum creatinine will provide sufficient clinical information to direct re-staging of the AKI, adjust to ongoing therapy, modify pharmaceutical doses, score the severity of the disease, and facilitate prognosis and outcomes predictions.

Serum urea nitrogen (or BUN) concentration increases with decreases in renal function, but unlike creatinine, urea is influenced by numerous extra renal factors which make its concentration less specific as a marker of kidney damage. Urea appearance, the net rate of urea accumulation in the body is influenced directly by its generation, distribution and removal. These parameters are influenced by exogenous and endogenous protein metabolism, hepatic function, hydration status, urine production, and diuretic therapy in addition to residual renal clearance (Kr). These global influences on serum urea concentration make it a useful predictor of the overall clinical severity of the uremic state (renal function, catabolism, nutritional adequacy) but less predictable as a marker of renal function or renal recovery.

When serum creatinine or urea are perturbed by hemodialysis, the sequential changes over time from the end of dialysis to the next dialysis session can be used to predict the status of residual renal function. After dialysis, BUN increases in proportion to urea generation from dietary nitrogen and endogenous protein catabolism and inversely with residual renal function (Kr) (Fig. 1). Higher dietary protein intake, increased catabolism, and lower residual renal function will produce a steeper increase and higher steady-state concentration of urea after dialysis until interrupted by an intervening dialysis treatment before achieving steady state. Conversely, if dietary and catabolic nitrogen turnover are constant, decreases in the predialysis urea concentration and a decrease in the slope of the change in BUN or creatinine over the interdialysis interval and a “flattening” of the exponential shape of the BUN (or creatinine) vs time curve between dialysis treatments suggest improvement of residual (continuous) renal function. (Figure 1) Due to the exponential shape of the curve during the interdialysis interval, it is important to make sequential comparisons of the slope of the curve over similar time intervals. The utility of these observations is usually more useful if extended over a weekend so there is an extra day between dialysis sessions. (Fig. 1 and 2).
Figure 4 Changes in BUN during and after 5-hour hemodialysis treatments in a 33-kg dog presented for acute kidney injury at varying degrees of residual urea clearance during recovery. The BUN increases immediately following dialysis to its steady-state over 3 to 6 days unless interrupted by a subsequent dialysis session. As illustrated by the 3 curves, the rate (steepness) of increase, the steady-state BUN concentration, and the shape of the curve following dialysis are influenced by the patient’s improving residual urea clearance (Kr, increasing from 0.4 ml/min to 4.5 ml/min) and can be used to help predict changes (improvement or worsening) of renal function over time.

Figure 5 Changes in serum urea nitrogen (BUN) during and between hemodialysis sessions in a dog with AKI (IRIS Stage III, O,RRT) secondary to ethylene glycol intoxication. Serum urea nitrogen drops dramatically during the hemodialysis session then increases during the interdialysis interval before the next treatment. The decrease in SUN during the hemodialysis session reflects the increased
“intermittent” clearance of urea by the hemodialyzer. Recovery of residual renal function is identified by the progressive decrease in the predialysis SUN on subsequent days (dashed line), and the change in slope of the increase in SUN in the interdialysis interval (A and B, dotted lines). The change in slope represents a decrease in urea appearance associated with increasing “continuous” urea clearance by the injured kidneys.

Generally, renal function can be monitored effectively with routine clinical and laboratory parameters discussed above. However, estimation of glomerular filtration rate (GFR) is regarded the most important predictor of renal function. It can be measured from either the urinary clearance or the plasma clearance of appropriate endogenous or exogenous markers of filtration. Urinary clearance requires accurate collection of timed urine samples and appropriately timed blood collections. Ordinarily, measurement of GFR is beyond the scope and utility of routine clinical practice, but many patients in the ICU have indwelling urinary catheters to monitor urine production and are readily poised for GFR measurement. The urinary clearance of endogenous creatinine is the most convenient and provides clinically relevant estimates of GFR. Procedurally, a well-mixed aliquot from an accurate, timed (30 minutes to several hours) urine collection from the catheter or closed collection system and a serum sample collected at the midpoint of the urine collection are submitted for creatinine determinations. The clearance of creatinine (as an estimate of GFR) is calculated from the equation, \[ C_{creat} = \frac{(U_{creat} \cdot V)}{P_{creat}} \]

- \( C_{creat} \), clearance of creatinine (ml/min)
- \( U_{creat} \), Urine creatinine concentration (mg/dl)
- \( V \), urine flow (ml/min)
- \( P_{creat} \), plasma creatinine concentration (mg/dl)

If the laboratory analyses are extended to include electrolytes and minerals on the urine and plasma samples, the clearance and fractional excretion of sodium, potassium, chloride, calcium, and phosphate can be determined concurrently with the GFR estimates. The fractional excretion (FE) is calculated as the \( \left( \frac{C_x}{C_{creat}} \right) \times 100 \), where \( C_x \), the urinary clearance of solute, x. The FE Na has been shown to correlate with improvement of renal function in some etiologies of AKI in dogs.

Alternatively, GFR can be estimated from the plasma clearance of exogenously administrated markers like creatinine or iohexol. Plasma clearance requires collection of serial blood samples over a variable time course which is dictated by the degree of renal failure and the distribution characteristics of the marker solute following intravenous administration. The clearance is calculated using the formula Clearance = marker dose/AUC, where AUC is the area under the plasma disappearance curve of the marker solute. Plasma clearance techniques are being used as an alternative to urine clearance methods to eliminate the requirement for urine collection. However, the utility of plasma clearance methods are often limited in patients with severe renal failure due to the necessity for very long sampling intervals.
Hospital-acquired Acute Kidney Injury: The Human (and Canine?) Perspective

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In recent years, the term acute kidney injury (AKI) has supplanted “acute renal failure” as the preferred description of a complex clinical syndrome involving renal parenchymal dysfunction. The change in terminology was intended to reflect a broader spectrum of clinical dysfunction, rather than just fulminant excretory failure that commonly requires renal replacement therapy and frequently leads to death. Multiple attempts have been made to further define and classify AKI, the most notable of which are the Risk, Insult, Failure, Loss, End-stage kidney disease (RIFLE) and the Acute Kidney Injury Network (AKIN) criteria (Tables 1 and 2). These staging schemes have been designed with the intent of identifying early and subtle changes in glomerular filtration rates, serum or plasma creatinine concentrations, and/or urine output that may indicate milder forms of renal dysfunction than what has been previously recognized. Identification of milder forms of AKI may not only allow for more accurate prognostication, but also for earlier recognition of kidney injury and an opportunity for earlier intervention.

Both the RIFLE and AKIN classifications schemes have been utilized in human medicine to determine an association between AKI and mortality, and both schemes have done so in a multitude of both retrospective and prospective studies evaluating a variety of populations, most of which consist of hospitalized patients. The vast majority of these studies demonstrated an association between even the mildest forms of AKI and mortality. Furthermore, increasing severity of AKI has been associated with higher mortality rates.

Recently, the veterinary scientific community has begun to evaluate the utility of these staging schemes for acute kidney injury. Thoen and Kerl retrospectively demonstrated an incidence of 14.6% for hospital-acquired AKI, using a modified version of the AKIN scheme. Although there were too few patients classified in the higher stages of the scheme to determine if severity of AKI was associated with mortality, a clear association between the development of AKI (any classification) and death was demonstrated. Additionally, Lee, et al have utilized a modified version of the RIFLE scheme to demonstrate that a higher stage of AKI is associated with a shorter survival time. However, this study did not differentiate hospital-acquired from community-acquired cases of AKI. Cowgill has proposed unique staging criteria (Table 3) that rely on absolute creatinine concentrations, rather than changes relative to a baseline creatinine concentration. This alteration may make this scheme applicable to both hospital-acquired and community-acquired AKI, the latter of which frequently occurs without documentation of a baseline creatinine concentration. This staging system has yet to be evaluated in the peer reviewed literature.

While progress in early identification of kidney injury has been made in veterinary medicine, the inherent limitations of retrospective studies limit the conclusions that can be drawn. Future studies should be prospective in nature, should evaluate novel veterinary staging schemes, and should include the feline population.
### Table 1: RIFLE criteria.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Creatinine (Cr)/GFR Criteria</th>
<th>Urine Output (UO) Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk</td>
<td>Increased Cr x 1.5 or GFR decreases &gt; 25%</td>
<td>UO &lt; 0.5 ml/kg/hr x 6 hr</td>
</tr>
<tr>
<td>Injury</td>
<td>Increased Cr x 2 or GFR decreases &gt; 50%</td>
<td>UO &lt; 0.5 ml/kg/hr x 12 hr</td>
</tr>
<tr>
<td>Failure</td>
<td>Increased Cr x 3 or GFR decreases &gt; 75%</td>
<td>UO &lt; 0.3 ml/kg/hr x 24 hr or anuria x 12 hr</td>
</tr>
<tr>
<td>Loss</td>
<td>Persistent ARF = complete loss of renal function for &gt;4 weeks</td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: AKIN criteria.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Creatinine (Cr)/GFR Criteria</th>
<th>Urine Output (UO) Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Increased Cr x 1.5 or ≥ 0.3 mg/dL</td>
<td>UO &lt; 0.5 ml/kg/hr x 6 hr</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Increased Cr x 2</td>
<td>UO &lt; 0.5 ml/kg/hr x 12 hr</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Increased Cr x 3 or Cr ≥ 4 mg/dL (with acute rise of ≥ 0.5 mg/dL)</td>
<td>UO &lt; 0.3 ml/kg/hr x 24 hr or anuria x 12 hr</td>
</tr>
</tbody>
</table>

Any patient receiving renal replacement therapy meets criteria for Stage 3, regardless of the Cr or UO.

### Table 3: Cowgill’s criteria.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Creatinine (Cr)</th>
<th>Clinical description</th>
</tr>
</thead>
</table>
| Stage I | < 1.6           | -Non-azotemic AKI or volume-responsive AKI  
                  -Historical, clinical, laboratory or imaging evidence of renal injury  
                  -Increase in Cr ≥ 0.3 mg/dL within 48 hours |
| Stage II | 1.6 - 2.5       | -Mild AKI: historical, clinical, laboratory, or imaging evidence of acute kidney injury and mild static or progressive azotemia |
| Stage III | 2.6 - 5.0      | -Moderate to severe AKI: documented AKI and increasing severities of azotemia and functional renal failure |
| Stage IV | 5.0 - 10.0      |                       |
| Stage V  | > 10.0          |                       |

Each stage of acute kidney injury is further sub-staged on the basis of current urine production as oliguric (O) or non-oliguric (NO) and on the requirement for renal replacement therapy (RRT)
References

The Polyuric Phase of Acute Kidney Injury

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The recovery phase of acute kidney injury can vary in the rapidity in which urine flow and excretory function returns to normal and the degree of residual tubular dysfunction that impedes complete return to normal function. The polyuria that accompanies the recovery phase has not been well documented in human or veterinary medicine, and no consensus exists in either field regarding pathophysiologic mechanisms or proper management strategies. In our hospital, polyuria most commonly manifests in the recovery phase of canine leptospirosis and feline urinary obstruction (specifically ureteral obstruction, although post-obstructive diureses do occur following relief of urethral obstruction, as well). During this phase, urine output can be substantial enough to affect the clinical course by putting the patient at risk for volume depletion and electrolyte abnormalities. A proper understanding of some of the mechanisms responsible for recovery phase polyuria may help veterinarians recognize and manage this condition appropriately.

Direct, sublethal injury to the renal tubular cells may cause loss of structural integrity, resulting in disorder of the actin cytoskeleton, disruption of cell polarity, and loss of transporters. This dysfunction can lead to massive solute loss in the urine, which produces an osmotic diuresis. The resultant polyuria can be manifested at any point during renal injury, but also during renal tubular cell recovery from sublethal injury or repopulation of tubules in which cells have undergone necrosis or apoptosis. Disruption or dysfunction of transporters can occur in both obstructive disease and leptospirosis. In a rat model of unilateral ureteral obstruction, reduced numbers of sodium transporters were detected in all regions of the nephron, in both the obstructed kidney and the contralateral kidney. In leptospirotic humans, downregulation of sodium transporters and aquaporins in the proximal tubule predominate, but there is also evidence of dysfunction of the NKCC2 cotransporter in the thick ascending limb of the loop of Henle. Additional mechanisms for recovery phase polyuria include a natriuresis/diuresis in response to volume expansion and accumulation of solutes that occur during oliguria. In cases of leptospirosis, systemic vascular resistance is decreased and there is increased vascular permeability, both of which may contribute to administration of larger amounts of fluids during volume resuscitation. Once vasomotor tone, vascular permeability, and renal function have been restored, the volume load must be excreted. During renal recovery, there is a milieu of growth factors promoting repopulation of the tubular epithelium, one of which is insulin-like growth factor-1. This growth factor has been shown to increase renal blood flow and glomerular filtration rate in healthy human subjects, and may contribute to recovery phase polyuria. Finally, decreased circulating concentrations of anti-diuretic hormone, secondary to hyponatremia and/or volume expansion in the oliguric phase may contribute to polyuria. Alternatively, renal responsiveness to anti-diuretic hormone may be reduced, as demonstrated in an animal model of leptospirosis in which water and urea permeability was unchanged in the presence of anti-diuretic hormone.

The polyuric recovery phase presents a challenge to the clinician because the urinary losses of sodium, chloride, and water lead to the necessity for administration of large volumes of intravenous fluids. Fluid administration in excess can activate neurohumoral mechanisms for excretion of a volume load, making the distinction between recovery phase polyuria and iatrogenic polyuria difficult. In our hospital, we have observed that serial measurement of urinary electrolytes, in combination with daily urine volume quantification, may be a practical and useful means of determining when sodium and chloride-conserving mechanisms have been restored and intravenous fluid rates can be tapered. Sodium, and more frequently chloride, can be excreted in the urine in concentrations equal to or even
exceeding that of the plasma during recovery phase polyuria, so serial measurement of both urinary and plasma electrolytes may allow for assessment of renal reabsorptive capacity to guide intravenous fluid therapy. However, prospective studies evaluating this method are needed before it can be recommended as a routine practice.

References

Endothelial Function, Regulation, and Its Role in Kidney Disease

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Introduction
The endothelium has been broadly defined by most authors as the inner (luminal) cell layer of the vasculature. This definition is both limiting and limited in several ways. First, the inner layer of the vasculature can include non-endothelial cells (e.g. trophoblasts) in a process known as vascular mimicry. Second, this definition erroneously excludes the inner cellular lining of both the lymphatic vessels and the endocardium both of which are endothelial in origin. That being said, the author will hereafter use the term endothelium to refer solely to the vascular endothelial cells which line the luminal aspect of assorted types of blood vessels.

The endothelium should, by nearly every definition of the term, qualify as an organ system in its own right, but is seldom discussed in such terms. In the last four decades, our perception of the endothelium has changed from one in which it served a passive role to one in which these cells serve as central regulators of hemostasis, vasomotor tone, inflammation, and intravascular/interstitial barrier function. It would not be incorrect to say that there are no disease states in which the endothelium is not altered to some degree either as contributor or injured bystander. In addition to the modern recognition that the endothelium is a highly metabolically active tissue, it has also come to be accepted that the endothelium is incredibly diverse with each organ (or organ sub-region) creating a unique microenvironment which individualizes the endothelium to serve varied functions. It is this very heterogeneity that now makes the endothelium such a difficult organ system to study or discuss in broad strokes. This lecture will attempt to present an overview of some of the key features of endothelial biology with a special emphasis on the renal endothelium. In addition, the role of the endothelium in AKI and in the progression to more chronic renal disease states will be discussed in detail.

Endothelial Heterogeneity
Overview
To develop a useful understanding of the epithelium, one must first abandon the idea that all endothelial cells are created alike. None of the classic ultrastructural features of endothelial cells (e.g. Weibel-Palade bodies) are present in every epithelial cell, nor are there any protein or mRNA markers that are uniformly detectable. Certain features such as primary cilia may be present on only 25% of endothelial cells. The endothelial cells at a vascular branch points display different phenotypes than those away from such sites even when the general characteristics of the vessel remain unchanged. This heterogeneity reflects the wide variety of primary functions that endothelial cells serve. An arteriolar endothelial cell may expend considerable cellular energy on the modulation of vasomotor tone while a venular endothelial cells predominantly occupies itself with the regulation of leukocyte trafficking. Large-scale research efforts (both proteomic and genomic) are underway to attempt to identify region-specific vascular zip codes which identify the endothelium in different parts of the vascular tree, but such efforts are ongoing and their success is by no means certain. Endothelial heterogeneity is the product of both microenvironmental (reversible) and epi-genetic (largely irreversible) forces. The phenotypic diversity of the endothelium is highly evolutionarily conserved and this is an important fact to remember when considering disease states. A given property of the endothelium that was once long ago adaptive in a population of young, fit, lean, non-sedentary animals with short life expectancies may be maladaptive in modern pet populations that often fit few or none of those criteria.

Renal specific vascular beds
Just as the renal tubular epithelial cells exhibit functional diversity between nephron segments, the vascular endothelium of the kidney also displays functional and morphologic compartmentalization. This compartmentalization reflects both the roles these cells serve as well as a response to the environment to which they are exposed. For example, the glomerular endothelial cells are involved in the creation and maintenance of a barrier predominantly serving to filter fluids and solutes rather than exchange oxygen and nutrients as the endothelium of other capillary beds do. Another example would be the endothelium of the efferent arteriole which is exposed to blood with a much higher viscosity than other endothelia. At each step along the path from renal artery to renal vein endothelial cells are exposed to a different microenvironment and have developed to serve different functions. Some of the differences that have been described to date are outlined in Table 1 below. This list is by no means exhaustive and is meant to serve as only an introduction to the diversity of the renal endothelium.

<table>
<thead>
<tr>
<th>Marker/Phenotype</th>
<th>Variations within Renal Endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS immunoreactivity</td>
<td>Adult: Higher in medullary vessels than in cortical</td>
</tr>
<tr>
<td></td>
<td>Embryonic: Higher in cortical vessels than in medullary</td>
</tr>
<tr>
<td>Gap junction proteins (Connexin 37, 40, and 43)</td>
<td>Connexin 37 and 40 are expressed in afferent but not efferent arterioles</td>
</tr>
<tr>
<td></td>
<td>Connexin 43 expressed in both afferent and efferent arterioles</td>
</tr>
<tr>
<td></td>
<td>None of the three are expressed in glomerular capillaries</td>
</tr>
<tr>
<td>Claudin-10 and -15</td>
<td>Expressed in endothelium of the vasa recta, but not afferent or efferent arterioles</td>
</tr>
<tr>
<td>Urea transporter UT-B1</td>
<td>Expressed only in descending vasa recta not ascending vasa recta</td>
</tr>
<tr>
<td>Water channel AQ1</td>
<td>Expressed only in descending vasa recta not ascending vasa recta</td>
</tr>
<tr>
<td>Endothelial organization/structure</td>
<td>Descending vasa recta: Continuous</td>
</tr>
<tr>
<td></td>
<td>Ascending vasa recta: Fenestrated</td>
</tr>
</tbody>
</table>

**Endothelium in Health and Disease States**

*General*

Two terms are commonly used to describe the role of the endothelium in disease relative to the healthy state: *activation* and *dysfunction*. The definition of these terms has changed over the past 30 years since they were first introduced. Studies in the early 1980’s were the first to demonstrate *in vitro* and *in vivo* that inflammatory stimuli (e.g. cytokines, bacterial products, etc.) could induce the expression of new *activation antigens* on the surface of endothelial cells. The expression of these new antigens correlated with the expression of pro-adhesive, pro-coagulant, and antigen-presenting activities of the cells. An unintended and incorrect result of these early studies was that a paradigm evolved in which the endothelium was thought of as having an “on-off” switch. It was either activated or it wasn’t. In the activated state it was felt to be pro-coagulant, pro-adhesive, and vasoconstrictive with the quiescent state being one in which the opposite properties were present. More recent work has clearly demonstrated that this model does not reflect endothelial physiology well. Rather, the endothelium is now thought of as having a “dimmer” switch type of phenotypic continuum. It is now realized that the endothelium is always active, but not always activated. Endothelial activation can be thought of as a response to inflammatory (and non-inflammatory) stimuli that has the following features: (1) graded response, not all-or-none, (2) response that varies in time and location, (3) typically
features some combination of pro-inflammatory, pro-coagulant, and “leaky” phenotype, and (4) activation may be adaptive or maladaptive.

Endothelial dysfunction is a more difficult term to define. Early on endothelial dysfunction was used to describe a state of hyper-adhesiveness of endothelium to platelets. Paradoxical vascular responses to acetylcholine and the discovery of nitric oxide as a signaling molecule in the mid-1980’s led to defects in endothelial vasomotor regulation also falling under the heading of endothelial dysfunction. In 1994-95, a series of definitions of endothelial dysfunction were proposed by Gimbrone and colleagues. The most recent one is provided below and seems to capture most of the salient features of the condition:

“[Endothelial dysfunction represents]...non-adaptive changes in endothelial structure and function, provoked by pathophysiological stimuli, (resulting in) localized, acute and chronic alterations in the interactions with the cellular and macromolecular components of circulating blood and the blood vessel wall.”

While this definition serves the veterinary scientist from an intellectual standpoint, it doesn’t really lend itself to teaching trainees or communicating with clients. In this regard, the definition offered by William Aird in his many writings on the subject may serve better and is as follows:

“Endothelial dysfunction is a term for states in which the endothelial phenotype represents a net liability to the host.”

This definition seems less cumbersome and allows the clinician to focus on the net effects of endothelial function as a whole within a given setting. Von Willebrand’s Disease (vWD) and non-cardiogenic edema would both be clinical scenarios in which endothelial dysfunction would appear to be unequivocally present, while a localized reaction to a bee sting might represent either endothelial activation alone or endothelial dysfunction depending on the impact on the patient (e.g. stung in the mouth and experiencing airway obstruction or merely experiencing an adaptive local response in the dermis). In the veterinary critical care setting, endothelial dysfunction is most often acknowledged/appreciated in the context of SIRS/sepsis and capillary leak syndrome, ARDS and other non-cardiogenic edema states, acquired and congenital thrombotic and hemostatic disorders (vWD, PTE, DIC, among others), systemic and pulmonary hypertensive crises, septic and anaphylactic shock, heatstroke, heartworm disease, and hemangiosarcoma patients. Increasingly, the role of changes in endothelial phenotype is being appreciated in the pathophysiology of chronic liver disease as well. In liver fibrosis, the sinusoids undergo a process called capillarization which encompasses the progressive loss of fenestrae and the formation of a continuous basement membrane.

Before moving on to the role of the endothelium in acute kidney injury, the author will stop to make one key point regarding “vasculitis”. Vasculitis and endothelial dysfunction are not the same thing. Vasculitis is an immune response directed at components of a vessel wall and leads to vessel remodeling, vessel destruction, and/or granuloma formation. Dry FIP, heartworm disease and many glomerular diseases would be classic examples of vasculitis. Non-cardiogenic edema (e.g. ARDS), exercise-induced pulmonary hemorrhage, and capillary leak syndrome are all examples of veterinary disease states with profound endothelial dysfunction, but a distinct lack of histopathologic evidence of vasculitis. It is this author’s impression that true vasculitis is quite uncommon in small animal practice, while most if not all critical illnesses are associated with some degree of capillary “leakiness”. Reserving the term vasculitis for conditions in which there is targeted inflammation of the vessel wall with resultant remodeling would seem to be the more appropriate usage. For the past 18 years, the author has waged an unsuccessful campaign to try to get veterinary radiologists to stop using the term vasculitis when capillary hyperpermeability is meant. To date, he has only successfully convinced his wife (Linda Mellema DVM DACVR) and he suspects that even she may be patronizing him.

Endothelial Dysfunction in Acute Kidney Injury (AKI)
While there has been a great deal published about the role of the endothelium in AKI, a large proportion of the literature is framed within the context of renal ischemia/reperfusion injury (IRI). This may be a byproduct of the fact that AKI is largely a disease of hospitalized populations in humans and ischemia due to hypotension or sepsis is the most common mechanism of AKI in that setting. In addition, renal hypoperfusion is a common pathway for renal injury due to diverse factors (e.g. many toxins). This discussion will focus on the role of the endothelium in a similar context, but the endothelium’s role in other forms of renal injury will be highlighted as well at times. The role of the endothelium in AKI really began to be appreciated following Flores’ description of the “no reflow” phenomenon in AKI. Since that time a number of studies using animal models have extended our understanding of why renal hypoperfusion may persist even after whole body hemodynamics have been stabilized.

Animal models-Endothelial dysfunction in AKI

The most widely used animal models of renal IRI involve either vascular clamping or the infusion of potent vasoactive compounds. The models utilizing vasoactive agents serve to highlight the importance of circulating and local regulators of vasomotor tone. A brief discussion of such agents and the role of endogenous vasoactive compounds in AKI will be included in the presentation accompanying these notes. The interested reader is referred to a “not quite recent but still relevant” book chapter on for more detailed coverage of the topic (see Conger J, 2001, in the recommended readings). A concise statement of the role of relative or absolute renal ischemia in AKI has been put forward by Bonventure (2008) and is as follows:

“AKI is a state often characterized by enhanced intrarenal vasoconstriction; it is also associated with enhanced renal nerve activity and increased tissue levels of vasoconstrictive agents, such as angiotensin II and endothelin. A decreased responsiveness in the resistance vessels to vasodilators, such as acetylcholine, bradykinin, and nitric oxide(NO), as well as lower production levels of some vasodilators can enhance the impact of these vasoconstrictive agents. These effects on the resistance vessels are complemented by endothelial damage, enhanced leukocyte-endothelial adhesion (particularly in the postcapillary venules), and activation of coagulation pathways; together, these processes result in small-vessel occlusion and further activation of the leukocytes causing increases in inflammation and providing a positive-feedback network.”

Much of the understanding which is summarized in the statements above are derived from animal models in general and rat models in particular. It was first demonstrated in rats that following renal ischemia, renal vascular resistance increases immediately. This is in contrast to how most other tissues respond to ischemia (i.e. immediate vasodilation). This reduction in blood flow exacerbates hypoxia and may lead to widespread cell death in the outer medullary tubules in particular which are particularly vulnerable. This critical period of altered vascular reactivity has been referred to as the “extension phase” by Sutton and others. The extension phase is ushered in by two significant events: (1) persistent hypoxia following the ischemic event and (2) an inflammatory response. Both events seem to be most evident in the regions of the outer medulla and corticomedullary junction. Many feel the extension phase is a crucial therapeutic window in which interventions may spare the kidney from chronic repercussions of acute injury. The role of the endothelial cell in the extension phase will be highlighted in this discussion.

Following transient renal IRI, morphologic and functional evidence of renal endothelial dysfunction can be identified even a week after the IRI event. Swelling of renal endothelial cells has long been speculated to contribute to reduced intrarenal blood flow since Flores first introduced the concept of “no reflow” several decades ago. However, consistent clear experimental evidence of such is generally lacking. In many studies endothelial cell size appears unaltered. Similarly, overt evidence of
endothelial cell death is lacking however increased numbers of circulating endothelial cells have been observed and it has been assumed that they represent damaged, dead, and/or detached endothelial cells. The bulk of the evidence supports altered function in intact, viable endothelial cells. Loss of normal endothelial nitric oxide synthetase (eNOS) function can be demonstrated even in the face of unchanged NOS protein levels. Rather, the functional capacity of eNOS appears to be impaired. In the experimental setting, enhancement of eNOS activity or the administration of NO donors has been shown to attenuate AKI following IRI. Other studies using video microscopy have demonstrated that perfusion in peritubular capillaries becomes compromised within minutes of reperfusion. The endothelium’s role in modulating leukocyte traffic likely contributes to this observed effect. Increased monocyte and macrophage adhesion to renal endothelium is observed early in renal IRI. This can lead to erythrocyte trapping and hemostasis which prolongs the period of ischemia and worsens tubular injury. The formation of microthrombi has also been described. A number of prothrombotic stimuli are present in the setting described above (some or all of which may contribute) including but not limited to the following: (1) adhered monocytes may be expressing tissue factor on their surface, (2) tissue factor release from activated, leaky endothelium would be expected to be increased, (3) activated platelets and endothelia release microparticles which are prothrombotic, (4) blood stasis enhances intravascular fibrin generation, and (5) the activated endothelium would be expected to have reduced antithrombotic membrane activities (e.g. Protein C, Protein S, thrombomodulin). Each of these factors represent plausible (and testable) hypotheses, however little or no research investigating the precise role of each in renal microvascular thrombosis in IRI has been published to date to the author’s knowledge.

Animal models-Endothelial dysfunction in AKI leading to CKI

Many patients with AKI might be expected to recover full (or near full) renal function if they survive the initial insult. However, it has been reported in humans that up to 13% of patients with AKI will progress to end-stage renal disease within 3 years. This proportion more than doubles (28%) if pre-existing renal disease is present at the time of AKI. These findings and others suggest that AKI predispose to chronic renal injury. A body of work by Basile, Sutton, and colleagues has led to accumulating evidence that the endothelium of peritubular capillaries is chronically dysfunctional following ischemic injury. Using a number of different methodologies these researchers have demonstrated a roughly 40% reduction in peritubular capillary density following AKI due to IRI. Importantly, a similar reduction in peritubular capillary density has been shown in other rodent models including AKI induced by folate, ureteral obstruction, and AKI due to inhibition of nitric oxide synthetase. Several recent follow-up studies by these authors have shed light on the mechanisms and consequences of this loss of capillary density. Specifically, they have shown that many renal endothelial cells undergo transition to a mesenchymal fibroblast phenotype following AKI due to IRI, that these mesenchymal fibroblast type cells cause interstitial expansion promoting cellular hypoxia, that angiogenic factors (e.g. VEGF) can attenuate vascular loss in the absence of an effect on proliferation, and that renal endothelial cells demonstrate limited capacity for regeneration following IRI. These findings raise several important issues: (1) that the renal endothelium as a whole has a much more limited capacity for repair than does the epithelium, and (2) that following an ischemic event, renal endothelial dysfunction may exacerbate tubular hypoxia long after total renal blood flow has been restored due to. Increased capillary permeability and edema accumulation may also contribute to tissue hypoxia by lengthening the diffusion distance for oxygen. It is also interesting to note that other models of progressive renal disease in which peritubular capillary dropout is characteristic (e.g. aging and hypokalemia) also manifest increased tissue hypoxia and reduced VEGF expression. The mechanism by which VEGF reduces vascular dropout is unclear. Studies have been unable to demonstrate an increase in endothelial cell proliferation in response to exogenous VEGF, but have still observed reduced vascular dropout. Primary cultures of endothelial cells from the kidney also demonstrate reduced expression of at least one VEGF receptor (VEGFR2) relative to culture of endothelial cells from other sites (brain, heart, liver).
**Therapeutic goals for the future**

If dysfunction of the endothelium in the extension phase of AKI is an important determinant of short or long-term outcomes, then there may be a number of therapeutic options worth exploring in the laboratory and clinical settings. In a way, it is encouraging that similar findings have been found in many different models of renal injury. It suggests that successful modification of the extension phase may yield positive results in patients with diverse forms of acute renal injury.

The intuitive approach one might reach for initially would be to suggest that we administer angiogenic factors such as VEGF to patients with AKI. Parenteral administration of VEGF by either the intracoronary or intravenous route has been the subject of Phase I and Phase II trials in the setting of coronary artery disease, but has not yielded promising results in regards to increasing neovascularization.

In the laboratory setting the administration of autologous CD34+ endothelial progenitor cells (EPCs) has been shown in some studies to ameliorate AKI. The mechanism remains unknown and does not appear to be due to repopulation of the renal vasculature by these stem cells. Rather a paracrine effect promoting endothelial function appears more likely. Circulating endothelial progenitor cell levels are varied in AKI with studies suggesting that uric acid and other factors may contribute to their mobilization. Mobilization of EPCs by exogenous agents has also been described including GM-CSF and erythropoietin. In contrast, EPC levels are reportedly low in chronic hemodialysis patients, but increase during dialysis sessions. Whether the early mobilization of increased numbers of EPCs in AKI patients can improve long term functional outcomes remains to be seen. Similarly, the isolation of autologous EPCs, expansion of their number in vitro, and administration of them to patients is an attractive but unproven approach at present. Efforts to conduct such studies in veterinary patients are hampered by a paucity of immunologic reagents required for the identification and isolation of EPCs in dogs and cats. In addition, it has been well documented that uremic toxins inhibit EPC function and thus the effectiveness of EPC treatment may be limited in those patients who might benefit most. However, if a paracrine effect is found to be the primary mechanism of benefit as is suspected then the exogenous administration of these paracrine factors may be able to recapitulate the treatment effect.

Modification of nitric oxide bioavailability is another potential therapeutic avenue worthy of exploration. The author has several ongoing studies underway examining nitric oxide bioavailability and partitioning in veterinary patients including uremic dogs and cats. Several uremic toxins are known to interfere with nitric oxide generation and therapeutic efforts to reduce levels of those toxins (e.g. ADMA) may prove beneficial. Alternatively, enhancing NO bioavailability via administration of precursors (e.g. L-arginine), donors, or NOS cofactors (e.g. tetrahydrobiopterin) may prove to be of benefit. Novel NO scavengers are being developed with kinetics that result in the scavenging of NO at excessive levels, but not at physiologic levels. Such agents may help to normalize nitric oxide bioavailability in settings like hemodialysis treated uremia patients where levels may fluctuate markedly.

Many of the uremic toxins that most strikingly alter endothelial function are proly dialyzable due to protein interactions. Modification of renal support methods to improve elimination of these compounds may prove beneficial in restoring long term function.

Lastly, one can only approach modification of endothelial function with a healthy dose of respect for the impact of aging. The author will present some data demonstrating altered endothelial responses in dogs younger versus older than eight years of age. The clinician must bear in mind that therapies that seem encouraging in young research animals may be of limited benefit in older patients. Treatment and lifestyle changes that slow or attenuate the aging process may yield benefits, but always seem to be just on the horizon.

**Conclusions**

The endothelium may best be considered as an organ system in and of itself. It is a functionally and morphologically diverse tissue type that is active in a number of key physiologic processes. Within
the kidney, the endothelium is functionally diverse with different portions of the renal vascular system displaying different phenotypes and serving varied roles. Animal models of AKI particularly those using IRI as the source of initial injury have demonstrated persistent endothelial dysfunction even after renal perfusion is restored. Endothelial dysfunction may promote renal hypoxia by a number of mechanisms and vascular dropout may be an important factor in AKI progressing to more chronic forms of renal dysfunction. The “extension phase” is one in which endothelial dysfunction appears to be key and therapeutic intervention in this time period may yield benefits to patients.

**Suggested readings**

The Role of the Kidney in Multiple Organ Dysfunction Syndrome (MODS)

Matt Mellema DVM PhD DACVECC
University of California, Davis

Introduction

Approximately 50 years ago physicians began to appreciate that critically ill patients may develop a wide array of dysfunctional organ systems following a seemingly isolated insult to a single site. This recognition was assisted by the fact that many members of the medical corps of the US Armed Forces (including the author’s father) were returning from the Vietnam War and applying what they had observed on the battlefield to civilian practice. Suddenly, “DaNang Lung” was being recognized as ALI/ARDS back in the states, for example. In particular, these physicians were observing in civilian patients a phenomenon that was seen all too frequently in military hospitals: patients who seemed to recover from an initial injury (often trauma) only to die due to progressive dysfunction of remote organ systems. Further, it became better recognized around this same time that certain types of insults are not limited to a single organ in their scope. Examples of non-focal diseases or syndromes that can lead to widespread organ dysfunction include heatstroke, systemic autoimmune diseases, shock, polytrauma, sepsis, uremia, and many toxins. This process in which there is parallel or sequential development of organ dysfunction in critically ill patients has become known as the Multiple Organ Dysfunction Syndrome (MODS). The earlier term Multiorgan Failure (MOF) has fallen out of favor for the same reason that ARF has given way to AKI as the preferred nomenclature. A recognition of a potential degree of reversibility and variable level of compromise is inherent in the term MODS, but lacking in MOF. The kidney is an important organ in determining the outcome in MODS. In one veterinary study of patients with sepsis secondary to gastrointestinal tract leakage it was found that 12.3% of the patients had AKI and that only 14% of these patients survived to discharge. The kidneys were one of four organ systems in which dysfunction was found to carry an increased odds ratio for mortality.

Ultimately MODS is fundamentally a process of detrimental organ system interaction and/or failure to contain a disease process to its initiation site. Processes that were adaptive at the local level frequently become maladaptive at the global level. Perhaps most commonly, MODS is a sequelae to a poorly controlled inflammatory response that has become widespread (the Systemic Inflammatory Response Syndrome; SIRS) although as mentioned above in some cases multiple organs are damaged from the onset (e.g. heatstroke, shock, etc...) by the same primary process. SIRS likely reflects global manifestations of the same archetypal processes that are used to recognize local inflammation: (1) calor becomes fever, (2) dolor becomes diffuse myalgia and arthralgia, (3) tumor becomes capillary leak syndrome with widespread tissue edema, (4) rubor becomes vasodilation with hyperdynamic cardiovascular performance, (5) function laesa (loss of function) becomes MODS.

SIRS was a more contentious subject prior to 1992 when the first consensus definition was put forward by the ACCP and SCCM. This consensus statement emphasized that immune dysregulation was felt to be the core process underlying SIRS. As such, it was noted that any physiologic stressor if it was of sufficient severity (duration, amplitude, or both) to activate inflammatory pathways systemically could: (a) lead to SIRS and (b) pose a risk for the development of MODS. A very similar set of criteria have been put forward for the recognition of SIRS in dogs and cats and are outlined below. It is proposed that dogs meeting two out of four and cats meeting three out of four of the criteria should be considered as exhibiting clinical signs consistent with SIRS.
**Table 1**: Proposed criteria for the recognition of SIRS in companion animals

<table>
<thead>
<tr>
<th></th>
<th>Canine criteria</th>
<th>Feline criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature (F)</td>
<td>&lt;100.6 or &gt;102.6</td>
<td>&lt;100 or &gt;104</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>&gt;120</td>
<td>&lt;140 or &gt;225</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>&gt;20</td>
<td>&gt;40</td>
</tr>
<tr>
<td>White blood count or %</td>
<td></td>
<td></td>
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<tr>
<td>band neutrophils</td>
<td>&lt;6 or &gt;16; &gt;3%</td>
<td>&gt;19 or &lt;5</td>
</tr>
</tbody>
</table>

These criteria serve as a reasonable starting point for a discussion of SIRS in companion animals, but have serious limitations. First, it is important to note that the findings need to be *persistent* in nature. Nearly any patient who is transiently agitated, excited, hyperthermic, or exerts themselves would fulfill the criteria. Failing to include persistence in the discussion would mean that visits to dog parks represent the leading cause of SIRS in dogs. Moreover, a number of disease processes that are generally considered unlikely to cause SIRS may lead to two or three of these criteria being met. Examples would include endocrine disorders like hyperthyroidism and pheochromocytoma as well as any cause of strongly regenerative anemia. Moreover, in the case of the canine criteria there is no allowance for breed variation. The heart rate changes with SIRS in a Chihuahua are unlikely to be of the same absolute magnitude as those in a Great Dane. This author is by no means opposed to these criteria, but in his own practice they are used more as *exclusion* criteria rather than *inclusion* criteria. What is meant by that statement is that the author feels is extremely unlikely that a canine or feline patient would be experiencing SIRS without manifesting two or three of these changes while recognizing that there are a fair number of patients that may meet the criteria in the absence of SIRS. The issue of uremia complicates the matter even further. Uremia is highly likely to alter the degree of change in response to systemic inflammation for a number of the criteria (e.g. body temperature). No “adjusted criteria” for SIRS in uremic patients have yet been put forward.
There have been a number of models of MODS proposed including the widely favored “two hit” model. In this paradigm, an initial injury primes the innate and acquired immune systems for a massive inflammatory response following a second activating process (the “second hit”). An example might be a period of profound hypotension (as the priming hit) followed by increased gastrointestinal translocation with resultant bacteremia (as the second hit). Alternatively, the “one-hit” model may be more representative of situations in which a single massive insult causes concurrent injury in multiple organ systems. Heatstroke and catheter-related sepsis would serve as examples of such. The “sustained-hit” model is invoked when it is suspected that a persistent insult has been unaddressed for a significant period of time (e.g. persistent pneumonia with multi-drug resistant organisms for which appropriate antimicrobials have yet to be administered). Any or all of these models may lead to MODS in patients and the three are not meant to be competing theories per se, but rather alternate paths to the same endpoint.

### Table 2: Pathologic processes implicated in MODS and representative examples of how these processes may manifest

<table>
<thead>
<tr>
<th>Pathologic process implicated in MODS</th>
<th>Examples of markers or manifestations of this process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwhelming/uncontrolled infection</td>
<td>Persistent infection</td>
</tr>
<tr>
<td></td>
<td>Nosocomial infection</td>
</tr>
<tr>
<td></td>
<td>Endotoxemia</td>
</tr>
<tr>
<td></td>
<td>“Superinfection” (new development of reduced antimicrobial susceptibility and/or polymicrobial infection)</td>
</tr>
<tr>
<td>Profound systemic inflammation</td>
<td>Increased cytokinemia (IL-6, IL-8, TNFα especially)</td>
</tr>
<tr>
<td></td>
<td>Leukocytosis</td>
</tr>
<tr>
<td></td>
<td>Increased capillary permeability</td>
</tr>
<tr>
<td>Immune paralysis (anergy)</td>
<td>Nosocomial infection</td>
</tr>
<tr>
<td></td>
<td>Increased cytokinemia (IL-10 especially)</td>
</tr>
<tr>
<td></td>
<td>T-helper population shift (Th1 → Th2)</td>
</tr>
<tr>
<td>Tissue hypoxia</td>
<td>Elevated blood lactate levels</td>
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<tr>
<td></td>
<td>Decreased central/mixed venous oxygen content</td>
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<tr>
<td></td>
<td>Increased oxygen extraction ratio</td>
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<tr>
<td>Diffuse microvascular coagulopathy and endothelial dysfunction</td>
<td>Increased procoagulant activity (e.g. TEG changes)</td>
</tr>
<tr>
<td></td>
<td>Decreased anticoagulant activity (e.g. decreased AT)</td>
</tr>
<tr>
<td></td>
<td>Increased levels of fibrin derivatives (FDPs, D-dimers)</td>
</tr>
<tr>
<td></td>
<td>Increased vWF</td>
</tr>
<tr>
<td></td>
<td>Increased capillary permeability (tissue edema, non-cardiogenic edema, larger distribution volume of albumin)</td>
</tr>
<tr>
<td>Dysregulated apoptosis</td>
<td>Enhanced apoptosis: epithelial and lymphoid cells</td>
</tr>
<tr>
<td></td>
<td>Diminished apoptosis: neutrophils</td>
</tr>
<tr>
<td>Altered organ crosstalk</td>
<td>Gut-liver axis alterations (increased infection with gut-derived microbes, endotoxemia, Kupffer cell activation)</td>
</tr>
</tbody>
</table>
Many different pathologic processes have been proposed over the years to explain the development of MODS. Several of these are summarized in Table 2 and Figure 1. It is important to remember that more than one of these processes may be present concurrently in a given patient or may occur sequentially as well (e.g. there may be a period of massive systemic inflammation followed by a period of relative immune paralysis).

The Kidneys in MODS
The kidney as “the victim”

There is little doubt that the kidneys represent an organ system that both frequently becomes dysfunctional in MODS and also is an important determinant of outcome. In humans, AKI occurs in approximately 36-67% of critically ill patients. The large range in the proportion reflects different authors/groups using varied AKI criteria. At either end of the spectrum this would represent a huge problem. In humans, 5-6% of ICU admissions will require renal replacement therapy. While the mortality associated with AKI in the critically ill depends widely on the patient population studied, in the majority of studies mortality increases proportionally with the severity of AKI. Even small increases in serum creatinine are associated with significant mortality risk in critically ill humans. Both the RIFLE and AKIN classification schemes have been used to stage AKI in the intensive care setting. Both schemes focus on abrupt and sustained changes in serum creatinine and urine output in assessing severity. More than 30 other definitions of AKI may be found in the literature as well. Diagnosis of AKI in the intensive care setting is a challenge because serum creatinine values are unlikely to represent a stable steady-state. Most of the determinants of serum creatinine concentration (rate of production, volume of distribution, and rate of elimination) are all highly labile in the critically ill patient. Creatinine values also lag behind reductions in GFR and fail to provide the sort of real-time information that would be most highly prized in this setting. Accepting the limitations in our ability to identify significant reductions in renal function at the moment they occur, it is important to recognize the impact of AKI in critically ill patient populations. The most common causes of AKI in critically ill humans are outline in Table 3 and is taken from work published by Uchino and colleagues in 2005.

Many of these common causes are also found in companion animals and likely represent similar risk factors. What may be important to take away from this list is an understanding of those factors that represent insults distinct from SIRS. It is crucial that when AKI develops in a critically ill companion animal that the SIRS/MODS process not be the only differential diagnosis considered. Factors such intra-abdominal hypertension and abdominal compartment syndrome, nephrotoxic medications, obstruction, and pre-renal azotemia all represent etiologies that are potentially far more easily addressed than SIRS and subsequent MODS types of renal dysfunction.

General preventative strategies are typical of those in all critical illnesses: (1) Avoid primary injury by limiting exposure to nephrotoxins such as radiocontrast agents, chemotherapeutics, and certain antibiotic classes whenever possible, (2) Avoid secondary injury by [a] recognizing significant risk

<table>
<thead>
<tr>
<th>Table 3: Common causes of AKI in critically ill human patients</th>
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<tbody>
<tr>
<td><strong>Five most common:</strong></td>
</tr>
<tr>
<td>- Sepsis (#1)</td>
</tr>
<tr>
<td>- Major surgery</td>
</tr>
<tr>
<td>- Low cardiac output</td>
</tr>
<tr>
<td>- Hypovolemia</td>
</tr>
<tr>
<td>- Medications</td>
</tr>
<tr>
<td><strong>Other common causes:</strong></td>
</tr>
<tr>
<td>- Hepatorenal syndrome</td>
</tr>
<tr>
<td>- Trauma</td>
</tr>
<tr>
<td>- Cardiopulmonary bypass</td>
</tr>
<tr>
<td>- Abdominal compartment syndrome</td>
</tr>
<tr>
<td>- Rhabdomyolysis</td>
</tr>
<tr>
<td>- Obstruction</td>
</tr>
</tbody>
</table>
factors such as diabetes, advanced age, CKD, hypertension, cardiac dysfunction, and liver dysfunction and considering those factors in the development of diagnostic and therapeutic plans and [b] maintain renal perfusion and again avoid nephrotoxins. The avoidance of hyperglycemia is a strategy widely used in human patients that has received far less attention to date in veterinary critical care nephrology. The relative risk of AKI posed by hyperglycemia in critically ill dogs and cats remains unknown at present. Similarly, the risk of the use of colloids such as hetastarch continues to be an active area of research. It is entirely possible that current formulations of starch-based colloids will be banned from use in humans in the US within the next half decade. Reports of histologic changes consistent with “colloid induced AKI” have been increasing in veterinary medicine. The very name “colloid-induced AKI” appears to be a misnomer, however. A recent meta-analysis (CI Wiedermann, et al, 2010) concluded that hyperoncotic albumin actually reduced the relative risk of AKI whereas hetastarch increased it. The role of current formulations of hetastarch in veterinary critical care medicine is also likely to undergo a great deal of scrutiny in the coming decade.

Sepsis is the most studied cause of AKI in MODS. The mechanisms of AKI in this setting seem to be multifactorial and may be summarized as follows: (1) Hemodynamic [altered renal blood flow, intra-renal redistribution of blood flow, and altered bioenergetics], (2) Immunologic/toxic [cytokines and bacterial products], and (3) Apoptotic [controversial; see below]. These three major factors are also summarized graphically in Figure 1. Altered renal blood flow is an oft cited, but poorly documented cause of AKI in sepsis and MODS. It is simply not known if renal blood flow decreases in septic patients in the presence of normal or increased cardiac output (hyperdynamic state, “warm shock”). Determination of renal blood flow requires invasive measurements that are not made continuously in patients due to the risk posed by attempting to do so. The issue has been studies in animal models however and it has been shown in several studies that renal blood flow does not decrease in septic animal models, but rather it remains stable or increases. It has been proposed that septic AKI may represent a unique form of AKI which has been termed “hyperemic AKI”. By contrast, in septic shock patients (i.e. decompensated septic patients that are hypotensive despite fluid resuscitation) renal blood flow does decrease substantially. So when one asks whether renal ischemia plays a role in AKI in MODS one must ask which type of patient is being considered, one with hyperdynamic or hypodynamic cardiovascular performance. One hypothesis that has been put forward is that in hyperdynamic septic patients the efferent arteriole may dilate to a greater degree than the afferent. Thus, renal blood flow may be enhanced why glomerular filtration pressure is reduced.

Intrarenal hemodynamics and bioenergetics (i.e. cellular energy depletion) have been studies in septic AKI models as well. These studies were undertaken to determine if intra-organ shunting of flow may lead to AKI in the face of normal or increased total renal blood flow. Those few studies that have been undertaken have failed to show significant medullary ischemia during hyperdynamic sepsis. Moreover, these studies have also shown that cellular ATP levels are preserved in renal cells following injection of E. coli. Taken together, these findings suggest that renal ischemia may not be the prevalent mechanism of AKI in sepsis-induced MODS.

Immunologic and endogenous toxic mechanisms of AKI in MODS have been investigated in several animal models. It has been shown that endotoxin stimulates the release of TNFα from mesangial cells. TNFα has been shown to have an important effect in LPS-induced AKI in mouse models. Neutralization of TNFα protects against LPS-induced AKI in murine models (GFR reduced by 30% rather than 75%). TNFα receptor knockout mice (TNFR1 k/o) demonstrated less tubular apoptosis and fewer infiltrating leukocytes than wild type mice in an LPS-induced AKI model. Lastly, when TNFR1 k/o kidneys are transplanted into wild type mice and vice versa only TNF wild type kidneys developed LPS-induced AKI. As will be discussed in one of the author’s other lectures at this symposium, the kidney may be particularly sensitive to cytokine-induced injury.
The role of apoptosis in septic AKI and MODS remains somewhat controversial. In cell culture models, the addition of high dose TNFα does increase tubular cell apoptosis. Time and dose-dependent increases in glomerular endothelial cells in culture have been demonstrated in response to both LPS and TNFα. However, in vivo models of sepsis in sheep demonstrated that sepsis increases the expression of both pro- and anti-apoptotic genes.

Organ crosstalk is a broad term that refers to the various means by which the performance of one organ impacts upon that of another. The kidney can certainly be negatively impacted by “crosstalk” from other organ systems. One of the most striking examples organ crosstalk negatively impacting the kidneys is the effects of mechanical ventilation on renal function. Low tidal volume ventilation strategies are widely used in the managements of patients with the Acute Respiratory Distress Syndrome (ARDS). This approach has gained widespread acceptance and has become standard of care because reduced hospital mortality has been demonstrated with this technique relative to standard ventilation strategies. One question that has lingered involves the basis for the improved outcomes. It has been proposed that low tidal volume ventilation strategies may actually affect the well being of other organ systems including the kidney. Imai and colleagues using a rabbit model demonstrated that injurious ventilator settings led to renal and gastrointestinal epithelial apoptosis and renal dysfunction. Plasma from these animals also induced renal cell apoptosis relative to control plasma. This effect could be attenuated by Fas-ligand blockade. It has subsequently been shown that Fas-ligand levels in human ARDS patients correlates with serum creatinine. Other means by which is has been suggested that mechanical ventilation leads to renal dysfunction include: (1) ventilation strategies such as permissive hypercapnia or permissive hypoxemia may compromise renal blood flow, (2) mechanical ventilation associated reductions in cardiac output may reduce renal blood flow, (3) redistribution of blood flow from cortical to juxtamedullary nephrons may occur with mechanical ventilation with PEEP and (4) mechanical ventilation may alter hormonal and sympathetic nervous system regulation of renal blood flow and distribution.

Numerous other examples of organ crosstalk involving the kidneys have been described and space and time constraints limit our ability to discuss them all. There are numerous syndromes that have been described some or all of which may be involved in altering renal function in MODS. The hepatorenal syndrome is development of renal failure in patients with advanced chronic liver disease. The cardiorenal syndromes (CRSs) are a group of clinical and pathophysiological entities which have been defined as “the concomitant presence of renal and cardiovascular dysfunction”. Ronco and colleagues identified five subtypes of the syndrome. The first (Type 1) CRS is defined as acute renal failure secondary to an abrupt worsening of cardiac function. Type 2 CRS involves a progressive and permanent chronic kidney dysfunction which is caused by chronic worsening in cardiac function. The third type (i.e. Type 3 CRS) consists of an acute cardiac dysfunction (e.g., heart failure, arrhythmia, and ischemia) secondary to an abrupt worsening of renal function (e.g., acute kidney ischemia or glomerulonephritis). Type 4 CRS involves a state of chronic kidney disease causing decreased cardiac function, cardiac hypertrophy, and/or increased risk of adverse cardiovascular events. The final type (Type 5 CRS) reflects concomitant cardiac and renal dysfunctions in the setting of a systemic condition which primarily affect both organs (e.g. sepsis). Thus one needs to bear in mind that context matters when considering AKI as a component of MODS. The five distinct forms of CRS make it clear that the renal system can serve not only as the victim in MODS, but also as the instigator.

The kidney as “the perp”

There are certainly cases where the kidneys can be clearly implicated as the primary source of sepsis, SIRS, and or MODS. Pyelonephritis leading to sepsis would seem to be one relatively unambiguous example. However, renal disease with accumulation of uremic toxins and/or poorly controlled, unrelenting systemic hypertension is another example in which a primary renal disorder can lead to widespread endothelial dysfunction and subsequent distant organ disease. This author will
spend another entire session during this symposium on uremic lung which will serve to further illustrate the point that the kidneys can be the driving force behind some cases of MODS. However, some contributions of the kidneys to distant organ dysfunction are more subtle. A fairly recent paper by Viera and colleagues demonstrated in a population of critically ill patients that kidney injury prolonged the time that mechanical ventilation was required. The attached editorial commentary that accompanied this manuscript was entitled “Relation between acute kidney injury and multiple-organ failure: The chicken and the egg question”. This editorial raises many of the points that we are gathering to discuss in this session of this symposium, but the answer to the chicken/egg controversy is quite clear cut. When it comes to MODS the kidney is a chicken, an egg, or both. It can serve as the initiating source of SIRS, sepsis, and MODS. It can also be one of the distant organs damaged in MODS that originated via injury to a different organ. Reductions in GFR can cause reduced clearance of cytokines and amplify inflammatory cytokine levels thus leading to further dysregulation of inflammatory and hemostatic responses.

In closing, the kidney plays a central role in MODS and there seems little doubt that strategies that prevent reductions in GFR are likely to result in not only improved renal functional outcomes, but also in improvements in the function of non-renal organ systems as well.

Recommended readings
Writing a Dialysis Business Plan

Cathy Langston, DVM, DACVIM
Animal Medical Center

The number of veterinary dialysis units is burgeoning, with almost twice as many in existence in 2012 compared to 2010. The demand for specialized veterinary care is expanding, and more and more referring veterinarians and clients are accepting of these advanced offerings. Starting a dialysis unit provides many benefits to the practice. It offers a therapy for when standard treatment has failed, which is one of the main motivating factors for starting a unit. Veterinary dialysis units do not appear to typically generate substantial revenue beyond the cost of providing care. Starting a dialysis unit requires a commitment from the entire hospital. Items to consider prior to opening a unit include equipment and facilities, staffing, and training, to name a few. Below are some notes about some of the items that need consideration. The numbers are based on the program at the Animal Medical Center, and much of this is my personal opinion.

### Dialysis Business Plan

**Mission Statement:** The goal of the dialysis unit are:

1) Provide an advanced level of care
2) Function in a revenue-neutral or revenue-generating fashion
3) Increase the visibility of the medical team, critical care unit, and emergency department that are involved in managing the dialysis unit

**Resources needed:**

**Facilities**

1) A. Dialysis machines for intermittent hemodialysis program
   a. Phoenix intermittent dialysis delivery system (need 2) $13,500 each
   b. Water purification system $5-10,000
   It is highly recommended that 2 dialysis machines are available. Although all the machines I have worked with have been very reliable, many times lives are on the line if there is even a 12 hour delay in getting machine service.
   The capacity of the water treatment system will depend on how many dialysis machines it needs to supply.
   B. Dialysis machines for continuous renal replacement therapy program
      a. Prismaflex (need 2) $35,000 each
      b. NxStage – leasing option available or can purchase outright (need 2) $35,000 each
   C. Facilities modification. The dialysis room will likely require
      a. Tap water supplying hot and cold water at 5 gallon/min
      b. Floor drain
      c. Sink with tap water
      d. 120V 60 Hz outlets; 240V 50/60Hz for Phoenix for heat disinfect cycle
      e. Oxygen supply to each dialysis station
2) Ancillary monitoring equipment (dialysis unit will need access, many may be shared with other departments depending on patient volume)
   a. Coagulation monitor, such as ACT II (Medtronics) or Coag Dx $600-5,000
   b. Blood pressure monitor

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i. The specific blood pressure monitor is up to the individual unit preferences, but a multi-function machine with EKG and pulse oximetry may be less expensive than purchasing each component separately.

c. CritLine Hematocrit monitor $3500
   i. This machine is invaluable in monitoring patient volume status to avoid precipitous volume changes and hemodynamic instability. Need one during each treatment (thus, 2 CritLines if you will be treating two patients simultaneously).

d. Chemistry analyzer $17,000
   i. Access to a chemistry analyzer is necessary, although the equipment may be housed in the ICU unit or available as an on-call lab. The technology must be able to get accurate BUN measurements on severely uremic patients (i.e., typically by dilution), as patients may have BUN in excess of 300 mg/dl.

e. Micropipetter
   i. This is necessary to ensure accurate dilution of samples.

f. Centrifuge for serum/plasma samples (for chemistry analyzer) and for hematocrit tubes

g. Refractometer

3) Space to perform dialysis. The Phoenix dialysis machine needs to be connected to the water system. A small water system may be portable (attaches to the back of the dialysis machine). It can be taken to the bedside, but it is advantageous to have a designated area for dialysis that is near ICU (for easy patient transport and for emergency back-up if the patient is unstable), but in a low traffic area that is conducive to keeping the patient still (as excess motion may trigger catheter flow related alarms).

4) Storage space for dialysis related supplies

Table 1. List of Durable Goods for the dialysis unit

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Dialysis Machine</td>
<td>Cushions for exam tables</td>
</tr>
<tr>
<td>Water Treatment System</td>
<td>Harnesses</td>
</tr>
<tr>
<td>CritLine</td>
<td>Clippers</td>
</tr>
<tr>
<td>Hemoclamps</td>
<td>Cat Carry Kennel</td>
</tr>
<tr>
<td>Oscillometric Blood Pressure Monitor</td>
<td>Laryngoscope</td>
</tr>
<tr>
<td>EKG Machine</td>
<td>Ambu bag</td>
</tr>
<tr>
<td>Pulse oximeter</td>
<td>Syringe pump</td>
</tr>
<tr>
<td>Doppler Blood Pressure Monitor</td>
<td>Fluid pump</td>
</tr>
<tr>
<td>ACT Machine</td>
<td>Chairs</td>
</tr>
<tr>
<td>Scale for &lt; 10 kg</td>
<td>Micropipetter</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>Step stool</td>
</tr>
<tr>
<td>Refractometer</td>
<td>File cabinet</td>
</tr>
<tr>
<td>Heating pad</td>
<td>Desk and chair</td>
</tr>
<tr>
<td>Supply Cart/ Crash Cart</td>
<td>Scale for &gt; 10 kg</td>
</tr>
<tr>
<td>Computer</td>
<td>Dry Chemistry Machine</td>
</tr>
</tbody>
</table>

Disposables
In addition to the fixed cost of equipment and facilities, and the variable cost of manpower, each dialysis treatment will involve disposable supplies. For intermittent hemodialysis, these supplies cost around
$100 per treatment. For CRRT, each cartridge (good for 2-72 hours) costs around $200, and fluids may cost $35-350/day. Specific price depends on volume and discount – you may be able to get a deal by working with the human medical side of your university. We pay close to “retail” as we are not affiliated and are low volume.

Catheters
- Temporary
- Permanent

Intermittent Hemodialysis Disposables
- Dialyzer
- Extracorporeal Circuit Tubing
- CritLine Chamber
- Bicart
- Acid Concentrate per gal ($1.5/gal in drum = $2.35) $3.75 x 1.5 = 7.5
- Saline for Priming (approx 300-600ml) $1
- Heparin $0.05
- ACT cartridges $2 x 6
- Syringes, Gloves, Surgical Masks, Scrub $1-5

Total disposables per treatment $66-100

Continuous Renal Replacement Therapy
- Cartridge (filter and tubing)
- CritLine Chamber $3.25
- Saline for Priming (1 L) $1
- Citrate $10
- Calcium $10
- Ionized calcium monitoring $10-200
- Syringes, Gloves, Surgical Masks, Scrub $1-5
- Dialysate (5 L bags) $35 x 1-6 = $35-210

Total disposables per 24 hours $265-670

Service contracts
- Dialysis Machine $4000/yr per machine
- Water treatment system up to $10,000 ($1000-2000 for supplies for “do-it-yourself” plan)

Manpower Costs
- Intermittent Hemodialysis
  - Dialysis Consultation (nephrologist) – Once familiar with dialysis, patient assessment and client communication takes about 2 hours (as little as 30 minutes in clear cut cases with avid owners)
  - Dialysis Catheter placement (nephrologist and anesthetist) – a temporary catheter can be placed in 7 minutes, but plan on 30-60 minutes when taking into account anesthesia and procedure set-up time.
  - Writing the dialysis prescription (nephrologist) – Once familiar with dialysis, writing the prescription for the initial one to three dialysis prescriptions will take about 15 minutes each. Subsequent prescriptions for the same patient usually take less than 5 minutes to write.
Performing the treatment – At our center, the nephrologist is on-site and typically in the dialysis unit for the first dialysis treatment, and on-site for the second and any treatments when the patient is hemodynamically unstable. For the majority of treatments, the dialysis technician performs the treatment, with phone support for problems, once the technician is proficient (estimated to take 6 months for this level of proficiency). The treatment can be performed by one person in most situations. If the patient will rest quietly with a catheter that performs well, one technician could set up and treat a second patient, which would prolong the day by about an hour.

Machine set-up and patient preparation – 1 hour
Treatment time – Typically 4-5 hours, may range from 3 to 8-12 hours
Discontinuing treatment, machine cleaning, wrap-up – 30-60 minutes

Continuous Renal Replacement Therapy
Consultation – same as above
Catheter placement – same as above
Prescription writing – same as above
Performing the treatment –
Machine set-up and patient preparation: 30 minutes. This will require someone familiar with CRRT

Treatment time – because treatment is (theoretically) 24 hours a day, someone will need to man the machine during the entire treatment duration. This person may be a resident or technician who has attended a 4-8 hour training class, with the dialysis nephrologist or technician on-site for emergency troubleshooting. If the patient is on the ventilator, an additional person will not be necessary if the vent tech has had the minimum dialysis training. In a stable patient, the resident or tech may have time for other duties (i.e., paperwork, assisting with other patient treatments), but availability for those tasks is unpredictable.

Discontinuing treatment – 15 minutes

Personnel
1) Doctors. At least one doctor will need specialized training in dialysis to open a unit. The amount of training is not firmly established. One guideline (proposed by Cowgill and Langston) is that a 6-12 month fellowship or 100 dialysis treatments should provide adequate experience to open a unit (although further phone support will likely still be necessary).
   a. Because of the propensity for dialysis cases to present as emergency cases, and the time involved per case, running a unit will likely require at least two doctors to provide adequate emergency coverage. Because of the opportunity for in-house training, not all doctors on the team will need to complete an off-site fellowship training program.

2) Technicians. At least one technician whose primary responsibility is hemodialysis will be needed to run an intermittent hemodialysis program. It will take approximately one month of training to learn to use the Phoenix (and most other intermittent machines) with the skill needed for the average veterinary dialysis unit, presuming the dialysis nephrologist is machine proficient and on-site during treatments. Because of the propensity for dialysis cases to present around the clock, having two expertly trained technicians is preferable. When dialysis is not needed (and there may be weeks between cases, depending on the practice), this technician typically will perform other duties in the hospital.
a. If the program is predominantly a CRRT program with a robust number of people participating, a dedicated dialysis technician may not be needed, in lieu of having a dedicated dialysis nephrologist.

**Training Costs**

1) Dialysis nephrologist - variable  
2) Dialysis technician - variable

**Revenue**

Pricing schemes. There is no standard method of charging for dialysis treatments.  

Intermittent hemodialysis. We have two levels of treatment - simple (4-6 hours, performed mainly by technician) and complex (> 6 hours, emergency, or requiring significant attention from the nephrologist), and we charge $700 and $1100, respectively. 

Continuous Renal Replacement Therapy. We currently charge $1100 per 12 hours (or fraction thereof), or $2200 per day. We do not charge a filter setup fee or charge for additional filters if one becomes clotted and must be replaced, but some units do.

**Anticipated caseload**

Caseload may vary greatly from unit to unit, and from season to season. I think it reasonable to hope for a minimum of 12 cases per year (with sufficient outreach to referring veterinarians). We currently treat about 24 cases a year.

Number of treatments per patient –average at our unit is 3 for cats (about half of our feline dialysis patients get a ureteral stent when metabolically stabilized by dialysis) and 6-7 for dogs, for a total of about 50-100 IHD treatments per year. For calculation purposes, I would consider each 24 hours on CRRT equivalent to one IHD treatment.

Sample Revenue Estimates per year

<table>
<thead>
<tr>
<th>Service</th>
<th>Rate</th>
<th>Qty</th>
<th>Revenue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysis consultation</td>
<td>$200 each</td>
<td>12</td>
<td>$2,400</td>
</tr>
<tr>
<td>Catheter placement</td>
<td>$250 + $150 anesthesia</td>
<td>12</td>
<td>$4,800</td>
</tr>
<tr>
<td>First treatment</td>
<td>$1100</td>
<td>12</td>
<td>$13,200</td>
</tr>
<tr>
<td>Subsequent treatments</td>
<td>$700</td>
<td>40</td>
<td>$28,000</td>
</tr>
<tr>
<td>Total Dialysis Specific Revenue</td>
<td></td>
<td></td>
<td>$48,400</td>
</tr>
</tbody>
</table>

**Ancillary Revenue**

Radiographic Confirmation of Catheter Placement  
Esophagostomy tube placement (90% of patients)  
Lab fees (pre and post dialysis renal panel)  
ICU and other hospitalization fees  
Diagnostics

**References**


Coagulation

Carrie White, DVM, DACVIM
Animal Medical Center

Coagulation consists of primary and secondary hemostasis. Primary hemostasis involves platelets and the vascular endothelium, while secondary hemostasis involves coagulation factors. Dysfunction of either primary or secondary hemostasis can result in clinical bleeding. Strict regulation of coagulation is required to ensure that clot formation is adequate but not excessive. There are several mechanisms that act to ensure that this occurs, including anticoagulant pathways, fibrinolytic processes, and inhibitors of fibrinolysis.

Primary Hemostasis:
Platelets

Platelets are released from megakaryocytes in the bone marrow at a rate of $10^{11}$ platelets per day. The rate of platelet production can increase up to ten fold when there is an increased need for platelets. Megakaryocyte precursors are programmed, through the action of transcription factors and thrombopoietin, to form platelet specific organelles and express platelet surface proteins. Megakaryocytes will become a mass of proplatelets, which fragment into platelets that are released into the circulation. Platelets will circulate as quiescent, nonadhesive anucleate cells for an average lifespan of 6-8 days.

Tissue injury results in endothelial disruption, leading to rapid platelet activation. Platelets will adhere to subendothelial collagen via platelet glycoprotein (GP) VI receptor or to von Willebrand factor (vWF) via GP Ib receptor. Platelet activation can be triggered by many substances, in addition to endothelial disruption, including thrombin, collagen, epinephrine, ADP (specifically under high shear conditions) and thromboxane A2 (TXA2) (specifically under low shear conditions). Thrombin is the most potent physiologic platelet activator, while ADP and epinephrine are considered weak agonists. All of these substances initiate a pathway that increases the calcium concentration within the platelets, leading to platelet activation. Activated platelets release granule contents, synthesize platelet activating factor (PAF) and TXA2, which recruit and activate additional platelets.

Platelets contain dense and alpha granules, and lysosomes. Dense granules contain nucleotides (ATP, ADP), histamine, epinephrine, calcium and serotonin, while alpha granules contain fibrinogen, coagulation factors V and VIII, platelet derived growth factor, vWF, fibronectin, β-thromboglobulin, heparin antagonist (PF 4), and thrombospondin. Platelet granules are secreted into an open canalicular system, and, along with TXA2, act to recruit additional platelets to the site of injury. Thrombin, which is the product of the coagulation cascade, acts as a powerful platelet agonist. Thrombin cleaves fibrinogen, strengthening the platelet plug and resulting in the formation of a fibrin-platelet meshwork. Thrombin’s actions on platelets occur independent of the endothelial disruption of vWF. Late stage platelet activation involves the exposure of phosphatidylserine (PS), which is moved from its usual location inside the platelet to the outer platelet membrane. PS increases the speed of coagulation and acts as a procoagulant membrane. Activated platelets act as a platform for the assembly of coagulation factors and for the formation of crosslinked fibrin, which involves secondary hemostasis.

Platelets have several receptors. The most abundant receptor is αIIbβ3 (also known as GP Ila/IIIa), which functions as a receptor for fibrinogen, fibronectin and vWF. The binding of fibrinogen to this receptor is essential for platelet aggregation and clot retraction. Activation of the αIIbβ3 receptor acts as the final common pathway for all platelet agonists. Receptor αGβ1 (GP Ib/IIa) acts as a receptor for collagen. Platelets have receptors for endoperoxidases, which are synthesized by endothelial cells and
released into the vascular space. Prostaglandins $\text{PGE}_2$ and $\text{PGD}_2$ and prostacyclin ($\text{PGI}_2$) have an inhibitory effect on platelet aggregation and adhesion. Binding of these substances to platelets increases cAMP levels and lowers calcium concentrations. Thromboxane A2 has the opposite effect on platelets, and promotes platelet aggregation and adhesion via decreased cAMP levels and increased calcium concentrations.

**Platelet Inhibition**

There are several substances that act to inhibit platelet function, including nitric oxide (NO), $\text{PGI}_2$, platelet endothelial cell adhesion molecule 1 (PECAM-1), and healthy endothelium. NO is constitutively released from endothelial cells, macrophages and platelets. It acts to inhibit platelet activation, adhesion and aggregation, and promotes vasodilation. NO is inhibited by reactive oxygen species, which is the mechanism by which antioxidants can decrease platelet activity. $\text{PGI}_2$ is synthesized by endothelial cells and acts to inhibit platelet function and induce vasodilation. NO and $\text{PGI}_2$ act synergistically to inhibit platelet function. PECAM-1 is a transmembrane protein expressed on endothelial cells, which inhibits platelet activation by collagen. Healthy endothelium expresses ADPases, heparin sulfate and thrombomodulin which act to degrade and inhibit platelet agonists.

**Von Willebrand Factor (vWF)**

vWF plays a significant role in primary hemostasis. vWF is synthesized by megakaryocytes and endothelial cells, and is stored in Weibel-Palade bodies in endothelial cells and alpha granules in megakaryocytes and platelets. vWF release from platelets and endothelial cells is stimulated by thrombin, fibrin, vasopression, collagen, PAF, epinephrine and histamine. vWF is involved in platelet adhesion and aggregation; it mediates platelet adhesion to extracellular matrix and to other platelets. It also circulates bound to factor VIII, protecting it from being cleared from the circulation. Following the release of vWF from endothelial cells, it may either bind to collagen in the subendothelium or enter the circulation. At a site of endothelial injury, platelets will roll along the endothelium and attach via vWF and collagen. This leads to a change in platelet conformation, causing exposure of the integrin $\alpha_{\text{IIb}}\beta_3$ receptor, which acts as a binding site for vWF. There is a second vWF receptor, GP Ibα, which is a surface integrin located on cell surfaces that, following activation, undergoes a shape change to express a binding site for fibrinogen.

**Platelet Aggregation**

Platelet aggregation occurs through the cross-linking of platelets through active $\alpha_{\text{IIb}}\beta_3$ receptors with fibrinogen bridges (platelet stimulation will increase the number of $\alpha_{\text{IIb}}\beta_3$ receptors). A platelet plug bridges the gap between endothelial cells, and the endothelial cells adjacent to this plug will release $\text{PGI}_2$. $\text{PGI}_2$ induces vasodilation and decreases platelet aggregation, which act to control platelet plug growth beyond the site of injury.

There is a close relationship between the coagulation and the inflammatory cascade, both at the level of platelets and coagulation factors. Platelets release substances, including cytokines, platelet activating factor (PAF) and serotonin, which can induce inflammation. Additionally, circulating platelets can be activated by bacteria and other infectious agents, as well as immune complexes.

**Secondary Hemostasis:**

**The Cascade Model of Coagulation**

The traditional model of coagulation involves a cascade of enzymatic reactions that result in fibrin formation. This model adequately explains in vitro coagulation testing, however it does not address the role of cellular components in the process of coagulation.
The cascade model suggests that the extrinsic and intrinsic pathways operate as independent and redundant pathways. However, clinical manifestations of coagulopathies prove that this isn’t true. A good example of this is factor XII deficiency in cats. While this deficiency does not result in clinical bleeding, this model would suggest that factor XII deficiency would not allow for an intact intrinsic pathway, which would inhibit clot formation.

Secondary Hemostasis:
Cell-Based Model of Coagulation

The cell-based model of coagulation more accurately reflects in vivo coagulation. There are 2 main paradigm shifts from the cascade model: tissue factor (TF) is the primary physiologic initiator of coagulation (not contact), and coagulation is localized to, and controlled by, cellular surfaces. There are three main phases of the coagulation: initiation, amplification and propagation.

Initiation Phase

TF, an integral membrane protein, acts as the main initiator of coagulation. TF is predominantly expressed by extravascular cells and fibroblasts, which ensures that coagulation is not initiated outside of the vasculature. There are some exceptions to this, however, and some circulating cells (macrophages, tumor cells) can also express TF. Endothelial damage allows for contact between plasma and TF-bearing cells. TF binds to factor VIIa (FVIIa), which activates more FVII, FIX and FX. It is important to note that FVIIa is the only coagulation protein that circulates in the blood in its active enzyme form; all other coagulation proteins circulate as zymogens that must be cleaved by enzymes to become...
activated. As a result, if any FVIIa manages to leave the vasculature through small breaks in the endothelium, it may bind to TF and initiate coagulation. This rarely progresses past the formation of a small amount of fibrin, though, unless platelets and larger proteins also leave the vascular space. FIXa can dissociate and move to nearby cells and platelets, while FXa is restricted to the surface of the TF-bearing cell. If any FXa diffuses away from the cell, it is rapidly inactivated by tissue factor pathway inhibitor (TFPI) or antithrombin (AT). FXa combines with FVa to produce small amounts of thrombin.

Amplification Phase
Thrombin, which is generated during the initiation phase, moves away from the TF-bearing cells, and carries out several actions. Thrombin will bind to platelets at the site of vessel injury, resulting in platelet activation. It also cleaves factor XI to Xla, and activates FV to FVa on the platelet surface. Thrombin cleaves vWF off of factor VIII, releasing vWF so that it can function in platelet adhesion and aggregation. The released FVIII is activated by thrombin to FVIIIa. Thus, thrombin amplifies the initial signal, activates platelets and sets the stage for procoagulant complex assembly on the platelet surface.

Propagation Phase
Once a few platelets are activated during the amplification phase, they release their granules, which recruits additional platelets to the site of injury. The propagation phase occurs on the surfaces of these activated platelets, where coagulation complexes will become assembled. Activated platelets express high affinity binding sites for coagulation factors. FXI binds to the platelet surface, and is activated by thrombin to FXIa; FXIa generates FIXa. FIXa complexes with FVIIIa, which in turn activates FX. FXa binds to FVa, which cleaves prothrombin to thrombin. There is a “burst” of thrombin generation, which cleaves fibrinopeptide A from fibrinogen, produces large quantities of fibrin. Soluble fibrin molecules polymerize into a stable fibrin clot.
Regulation of Hemostasis

Strict regulation of coagulation is required to ensure that clot formation is restricted to the site of vessel injury, and that the clot is sufficient to impede bleeding, but not to be excessive so as to obstruct blood flow. One of these regulatory mechanisms is restriction of the initiation and propagation steps of coagulation to cell surfaces—particularly to TF-bearing cells. Platelets do not express TF, and only activated platelets express the pro-coagulant membrane that is essential in order for coagulation to occur. Normal, resting endothelium expresses three platelet inhibitors: PG12, ectoadenosine diphosphatase and NO. PG12 limits platelet responsiveness to thromboxane. Ectoadenosine diphosphatase metabolizes ADP (which is a platelet agonist). NO decreases intracellular calcium, reduces the number and affinity of fibrinogen binding sites, and inhibits the release of vWF from endothelial cells.

There are three anticoagulant pathways that act to restrict the effect of thrombin to the site of injury: antithrombin (AT), activated protein C (APC), and tissue factor pathway inhibitor (TFPI). AT is a circulating α2 globulin that is produced by the liver. AT inactivates coagulation proteins that escape into the circulation from the site of injury. Specifically, AT binds to and inactivates thrombin (FIIa) and FXa, and neutralizes FXa, FXla, FXIIa and kallikrein. The effects of AT are potentiated when AT is bound to heparin sulfates in the endothelial matrix. Protein C is a vitamin K-dependent serine protease that is activated when free thrombin binds to thrombomodulin, which is an endothelial surface receptor. Activation of protein C is augmented when protein C is bound to an endothelial protein C receptor (EPCR). APC inactivates FVa and FVIIIa, which leads to decreased thrombin formation. It achieves this action with protein S (another vitamin K-dependent protein) as a cofactor. APC also enhances fibrinolysis via inactivation of plasminogen activator inhibitor 1 (PAI-1). APC has other actions, including anti-inflammatory effects, and acts to decrease endothelial cell apoptosis in response to cytokines and ischemia. TFPI is synthesized and expressed by endothelial cells, and regulates early phases of coagulation. TFPI accumulates at the site of injury caused by local platelet aggregation, and has anticoagulant actions, including inhibition of TF, inhibition of the initiation complex of FVIIa-TF, and inhibition of FXa. TFPI also has antiangiogenic and antimetastatic actions.

Fibrinolysis

Fibrinolysis, the enzymatic dissolution of fibrin, is the normal hemostatic response to vascular injury. Plasminogen, a β globulin proenzyme found in blood and tissue fluid, is converted to the protease plasmin either intrinsically (by activators from the vessel wall) or extrinsically (from the tissues). There are two main types of plasminogen activators: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). t-PA is synthesized and secreted by endothelial cells. Fibrin acts as both a cofactor for plasminogen activation and a substrate for plasmin. In the presence of fibrin, the efficacy of t-PA increases by 1000 fold; this helps to localize the generation of plasmin, and thus fibrinolysis, to the fibrin clot. As fibrin is degraded, the activation of plasmin will decrease, which helps to keep the system in check so that fibrinolysis is not excessive. t-PA plays an important role in fibrinolysis in circulation. u-PA is released as an inactive glycoprotein, and is activated by plasmin or kallikrein. u-PA is important in fibrinolysis in tissues. Plasmin acts to degrade fibrin into fibrinogen degradation products (FDPs).

There are inhibitors of fibrinolysis, including PAI-1, α2 antiplasmin, α2 macroglobulin, and thrombomodulin. PAI-1 is stored in platelet α granules, and is released with platelet activation. PAI-1 inhibits both t-PA and u-PA. α2 antiplasmin is synthesized in the liver, and inhibits free circulating plasmin. α2 macroglobulin inhibits plasmin following antiplasmin consumption. Thrombomodulin binds to thrombin, activating thrombin activatable fibrinolysis inhibitor (TAFI).
Go With the Flow: Anticoagulation Strategies for Renal Replacement Therapy

Cathy Langston, DVM, DACVIM
Animal Medical Center

A variety of methods of anticoagulation can be used for extracorporeal therapies. Unfractionated heparin is the most commonly used method in both intermittent and continuous renal replacement therapy. It is simple and effective, but increases the risk of patient bleeding, as the patient is systemically anticoagulated. Systemic heparinization is generally avoided in patients with pre-existing coagulopathy or in patients that recently have had or will have surgery. Regional citrate anticoagulation with calcium infusion is more complicated to use, but prevents dialyzer clotting more effectively than heparin and does not anticoagulate the patient. Citrate is relatively contraindicated in patients with liver failure. Regional citrate anticoagulation is used in just under half of human CRRT programs. Citrate is the most common anticoagulant for apheresis procedures. Dialysis can be performed with no anticoagulation, with intermittent saline flushing of the dialyzer, but the treatment frequently must be stopped due to clotting within a few hours. Other anticoagulant options are available, but are not in widespread use.

Heparin

Unfractionated heparin is usually administered as an intravenous loading dose followed by a constant infusion. The infusion is adjusted to maintain clotting times within a specified range. Additional boluses of heparin may be needed if the clotting time is far below the target range. Activated clotting time is the most commonly used measure (due to availability of automated equipment early in the history of dialysis). The Medtronic ACT II machine is the most commonly used device. For most ERRT treatments, the target range is 160-200 seconds (1.6-2 times normal). If there are concerns about systemic heparinization, a tighter range, generally 160-180 seconds may be prescribed. PTT might be an alternative measure, if accurate bedside machines that require a minimal blood volume are available. Many dialysis machines have an integrated syringe pump for heparin administration.

Table 1. Medtronic ACT II Reference Ranges for Normal Dogs and Cats

<table>
<thead>
<tr>
<th>Dogs (n = 28)</th>
<th>Cats (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-103 sec</td>
<td>52-108 sec</td>
</tr>
</tbody>
</table>

Heparin Protocol #1

The following protocol has been in use, with modifications, for over 20 years in veterinary intermittent hemodialysis. It is based on clinical observations. The initial heparin bolus depends on the starting ACT. Adjustments to the heparin infusion rate are made to maintain the ACT around 1.6 to 2 times normal. Clinical experience suggests that patients with CKD tend to be more hypercoagulable and need more heparin, whereas those with AKI tend to be hypocoagulable and need less heparin. Infusing a small amount of heparin (i.e., 10 u/hr) into the venous chamber may help decrease clotting at that interface. Clinically apparent clotting in the dialyzer and extracorporeal circuit seem more common if a blood transfusion is administered during dialysis, and thus we routinely increase the heparin infusion rate if giving a transfusion. Most IHD machines can be programmed to discontinue the heparin infusion at a set time prior to the end of the treatment (commonly 30 minutes) to allow the effects of heparin to partially dissipate before the patient leaves the unit.
Table 2. Initial Heparin Bolus

<table>
<thead>
<tr>
<th>Pre-treatment ACT</th>
<th>Heparin bolus (u/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 120 sec</td>
<td>50</td>
</tr>
<tr>
<td>120-160 sec</td>
<td>25</td>
</tr>
<tr>
<td>&gt; 160 sec</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Initial Heparin Infusion Rate

<table>
<thead>
<tr>
<th>Species</th>
<th>Body Weight</th>
<th>Heparin Rate (U/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cats, Dogs</td>
<td>&lt; 6 kg</td>
<td>50-100</td>
</tr>
<tr>
<td>Cats</td>
<td>&gt; 6 kg</td>
<td>50-100</td>
</tr>
<tr>
<td>Dogs</td>
<td>6-12 kg</td>
<td>100-300</td>
</tr>
<tr>
<td>Dogs</td>
<td>12-20 kg</td>
<td>400-600</td>
</tr>
<tr>
<td>Dogs</td>
<td>20-30 kg</td>
<td>1000</td>
</tr>
<tr>
<td>Dogs</td>
<td>≥ 30 kg</td>
<td>1000-1200</td>
</tr>
</tbody>
</table>

A retrospective review of 459 IHD treatments in 56 dogs and 167 IHD treatments in 34 cats found that dogs were within the target range 44% of the time (and above the target range 42% of the time), whereas cats were within the target range 23% of the time (and above 74% of the time).

Moderate to severe clotting of the dialyzer occurred in 41% of treatments in dogs and in 9% of treatments in cats. Bleeding was recorded during dialysis in 26 treatments in 10 dogs and 4 treatments in 3 cats. Bleeding episodes were not correlated to ACT measurements.

Table 4. Alternate Heparin Protocol (Ross 2011)

<table>
<thead>
<tr>
<th></th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial heparin bolus (U/kg)</td>
<td>25-50</td>
<td>10-25</td>
</tr>
<tr>
<td>Constant infusion rate</td>
<td>50-100 U/kg/hr</td>
<td>20-50 U/cat/hr</td>
</tr>
<tr>
<td>Target ACT (sec)</td>
<td>160-180</td>
<td>150-180</td>
</tr>
</tbody>
</table>

Heparin Protocol in CRRT

The following protocol for systemic heparinization is adapted from the CRRT literature.

Heparin Prime: 25 u/kg, repeat if ACT < 180 sec
Heparin CRI: Start 10-20 u/kg/hr

Table 5. Adjustments to Heparin Rate in CRRT

<table>
<thead>
<tr>
<th>ACT (sec)</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;200</td>
<td>Decr by 1 u/kg/hr</td>
</tr>
<tr>
<td>180-200</td>
<td>No change</td>
</tr>
<tr>
<td>150-180</td>
<td>Incr by 1 u/kg/hr</td>
</tr>
<tr>
<td>&lt;150</td>
<td>Bolus 15 u/kg &amp; incr CRI</td>
</tr>
</tbody>
</table>

Heparin complications

Besides the obvious risk of patient hemorrhage, another complication of heparin to consider is heparin induced thrombocytopenia (HIT). HIT affects about 10% of human dialysis patients. HIT is an immunologically mediated disease that causes a decrease in platelet count of about 30-50% from baseline. It does not typically cause the profoundly low platelet counts seen with other drug reactions or ITP. Patients with HIT are at risk for thromboembolic complications. This complication has not been recognized in veterinary medicine, although platelet counts are not routinely monitored as part of the dialysis protocol.
Regional Citrate Anticoagulation

Calcium is an important factor in multiple steps of the coagulation cascade. Citrate binds to calcium, preventing activation of coagulation. With regional citrate anticoagulation, citrate is infused into the extracorporeal circuit as the blood is being withdrawn from the body, and anticoagulates the blood in the extracorporeal circuit. Calcium is infused into the patient (generally through a separate catheter) to prevent hypocalcemia. Some portion of the citrate and bound calcium is removed in the effluent (dialysate and ultrafiltration). Any citrate (and the calcium bound to it) not removed in the dialyzer circulates to the liver where each citrate molecule is metabolized to 3 molecules of bicarbonate and the calcium is released.

Citrate Protocol for CRRT

A separate fluid infusion pump to administer the citrate will be needed, as the heparin infusion pump incorporated into most machines will not be able to provide the flow rates necessary. The citrate line can be attached to the heparin infusion line (leaving the heparin syringe pump empty, and the heparin infusion rate set at 0). A stopcock between the catheter port and extracorporeal circuit line is another option, but may interfere with rapid blood flow if the internal lumen of the stopcock is smaller than the surrounding tubing.

Citrate is available in several concentrations. The following recommendations are based on 3.2% Sodium Citrate (anticoagulant citrate dextrose A, ACD-A). Start the citrate infusion (in ml/hr) at 1.5 to 2 times blood flow rate in ml/min (Example: If Qb = 100 ml/min, start citrate at 150-200 ml/hr). Start at 3X blood flow rate for first 30 minutes. Titrate the citrate infusion to maintain extracorporeal iCa++ 0.25 to 0.45 mmol/L.

<table>
<thead>
<tr>
<th>Post filter iCa++ (mmol/L)</th>
<th>Citrate Infusion Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 5 kg</td>
</tr>
<tr>
<td>&lt; 0.25</td>
<td>Decrease rate by 3 ml/hr</td>
</tr>
<tr>
<td>0.25-0.35</td>
<td>5 ml/hr</td>
</tr>
<tr>
<td>0.36-0.45</td>
<td>Increase rate by 3 ml/hr</td>
</tr>
<tr>
<td>&gt;0.45</td>
<td>5 ml/hr</td>
</tr>
<tr>
<td>Notify Dr. if citrate infusion &gt; 200 ml/hr</td>
<td></td>
</tr>
</tbody>
</table>

Calcium chloride will cause sloughing if given perivascularly; make sure that the catheter used for infusion is in a central vein and has not dislodged. Calcium gluconate is not irritating if given SQ, but it does not have the same concentration of elemental calcium as calcium chloride, and doses should be adjusted accordingly.

Make a 0.8% calcium chloride solution (2 gm CaCl₂ in 250 ml 0.9% saline). Administer the calcium chloride infusion at 0.4 times the citrate infusion rate. Titrate the calcium infusion rate to maintain patient iCa++ at 1.1-1.3 mmol/L. Use calcium free dialysate.
Table 7. Calcium Infusion rates using 0.8% calcium chloride

<table>
<thead>
<tr>
<th>Patient iCa++ (mmol/L)</th>
<th>Calcium Infusion Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 5 kg</td>
</tr>
<tr>
<td>&gt; 1.3</td>
<td>Decrease rate by 3 ml/hr</td>
</tr>
<tr>
<td>1.1-1.3</td>
<td>No adjustment</td>
</tr>
<tr>
<td>0.9-1.1</td>
<td>Increase rate by 3 ml/hr</td>
</tr>
<tr>
<td>&gt;0.9</td>
<td>Increase rate by 5 ml/hr</td>
</tr>
<tr>
<td></td>
<td>Notify Dr. if calcium infusion &gt; 200 ml/hr</td>
</tr>
</tbody>
</table>

An abstract on using regional citrate anticoagulation in intermittent hemodialysis is published elsewhere in these proceedings. Protocols using calcium-containing dialysate, to replenish patient calcium without the need for an intravenous infusion, thus simplifying the procedure, have been evaluated. Predictably, this leads to greater clotting in the dialyzer and circuit. Dialysate containing citrate has been investigated, and appears to allow lower heparin infusion rates, but does not completely prevent clotting.

Complications of Citrate

The use of regional citrate anticoagulation may be associated with several complications. Hypocalcemia may occur if the calcium replacement rate is too low in relation to the citrate infusion rate. Typical symptoms of hypocalcemia, including facial pruritis (tingling lips), tremors, and tetany, can be induced. Careful monitoring of the circuit and patient ionized calcium concentrations should allow appropriate treatment adjustments. Because citrate is metabolized to bicarbonate, long term administration of citrate (i.e., with CRRT) may lead to metabolic alkalosis. After the first day or two of CRRT, switching to a lower bicarbonate dialysate (i.e., 25 mEq/L instead of 35 mEq/L) may be prudent.

Development of a “citrate gap” is an interesting phenomenon. Citrate-calcium complexes are delivered to the liver, where citrate is metabolized to bicarbonate and the calcium is released. If liver dysfunction is present, the rate of citrate metabolism may be severely curtailed. In that situation, citrate-calcium complexes circulate for longer. Total calcium concentrations count this bound calcium, although the ionized calcium concentration is low. This discrepancy between total and ionized calcium is the citrate gap. If the total calcium is being monitored instead of the ionized, the high concentrations will lead to a decrease of the calcium infusion rate, risking symptomatic ionized hypocalcemia.

No Anticoagulation

In some settings, systemic anticoagulation should be avoided, including patients with pre-existing critical bleeding (i.e., pulmonary hemorrhage, CNS hemorrhage), or within 24 hours of surgery or invasive procedures (e.g., renal biopsy, feeding tube placement). Regional citrate anticoagulation may be an alternative to systemic heparinization, but if liver failure is also present, citrate is relatively contraindicated. Some human units routinely avoid anticoagulation in CRRT treatments and replace the dialyzer 2 to 3 times a day when it clots.

Intermittent hemodialysis can be performed without anticoagulation. A fast blood flow rate should be used to decrease thrombosis. Every 30 minutes, the dialyzer is flushed with 100-200 ml saline to disrupt any thrombi that are forming, and to visually assess the amount of clotting present. Smaller volumes of saline (25-50 ml) may be used every 15-30 minutes, but this volume will not visually clear the system. The ultrafiltration rate is adjusted to remove this fluid administration. In my experience, even with these modifications, thrombosis is severe enough to require discontinuation of treatment between 1 and 1.5 hours of treatment, unless the patient has a severe coagulopathy (i.e, full-blown DIC). The use
of anticoagulant impregnated dialyzers has been investigated to decrease the need for systemic anticoagulation, with some success.

**Regional Heparinization with Protamine**

Administration of protamine to counteract the effects of heparin can be used in a fashion similar to regional citrate anticoagulation. One milligram of protamine reverses 100 units of heparin. Calculating the protamine dose should account for the administered heparin and its natural half-life of heparin of 1 to 1.5 hours. Because it is difficult to regulate the combined effects of heparin and protamine, this protocol is rarely used.

**Other Anticoagulation Strategies**

Other anticoagulants have been investigated in human dialysis fields, but there is no veterinary experience as yet. Low molecular weight heparin can be effective, and is preferred by some for people at high risk of bleeding, but is not considered cost-effective in the average patient. Prostanooids have been used for dialysis in people, but not in animals. Argatroban has been used for dialysis in people, but not in animals. Namfostat is not a suitable anticoagulant for CRRT in dogs.

**References**


How to Choose The Right Dialysis Modality

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TERMINOLOGY

There are a variety of dialysis modalities that can be used for renal replacement therapy. Extracorporeal therapies are those that take blood out of the body for purification. Peritoneal dialysis, in which the purification takes place inside the body, is considered intracorporeal therapy. The extracorporeal therapies have conventionally been divided into intermittent or continuous modalities, but the lines between those have become blurred recently. Chronic hemodialysis, for people with end-stage kidney disease, has traditionally been intermittent treatments of 3 to 4 hours duration 3 days a week. Whether this is an appropriate dosing scheme is debatable, but because the vast majority of veterinary dialysis is provided for acute kidney injury, recommendations for treating CKD in people lack validity in this setting.

Intermittent hemodialysis (IHD) is usually provided with a dialysis delivery machine that produces the dialysate from purified water. The dialysate is not sterile, but modern machines have several filtration steps that can render the dialysate ultrapure, meaning almost free of bacterial contamination and with very low concentrations of endotoxins. The dialysate flow rate can be varied, but typically in the range of 300-800 ml/min (18-48 L/hr). Blood flow rates are maximized (up to 500 ml/min). This very fast dialysate flow rate compared to the blood flow rate provides highly efficient diffusive clearance of small solutes such as urea, creatinine, and potassium.

Continuous renal replacement therapy (CRRT) is typically performed with a different type of machine using pre-packaged sterile fluids for dialysate. The traditional schedule for CRRT is an intention to treat 24 hours a day until the patient recovers or is stable enough to transition to IHD. This may be a few days up to a few weeks. In practice, disruptions in treatment (i.e., because of filter clotting, scheduled filter changes, for procedures such as imaging, etc.) limit treatment to something less than 24 hours a day. Dialysate flow rates may vary from 0 to 8 L/hr (0-133 ml/min). CRRT typically relies more heavily on convective clearance compared to diffusive clearance. Convective solute clearance removes solutes dissolved in fluid that is extracted from the patient (using hydrostatic forces with extracorporeal therapies and osmotic forces with peritoneal dialysis). To avoid volume depletion, replacement fluid is administered intravenously to the patient. Although not approved by the FDA, many units use dialysate and replacement fluids interchangeably, as both are sterile. Replacement fluid rates may range from 0 to 8 L/hr. The decrease in efficiency is overcome by a much longer treatment time. CRRT typically uses slower blood flow rates (up to 200 ml/min) than IHD.

CRRT can be further divided into modes. Continuous venovenous hemofiltration (CVVH) involves solely convective clearance via a combination of fluid removal by ultrafiltration and fluid replacement, without any dialysate for diffusive clearance. CVVHD involves diffusive clearance via dialysate, with minimal contribution from convective clearance from the fluid removed by ultrafiltration to control overhydration. CVVHDF involves a combination of replacement fluid and dialysate, in a proportion determined by the operator. Theoretically, CVVH improves middle molecule clearance compared to CVVHD. CVVH may be associated with more filter clotting compared to CVVHD.

“Hybrid” therapies are variations on a theme. Sustained low-efficiency dialysis (SLED) uses an intermittent hemodialysis machine, slow blood flow rates, and long treatment times (e.g., 6-12 hours). Sustained low-efficiency daily dialysis with filtration (SLEDD-f) adds in a component of convective clearance. Extended daily dialysis (EDD) involves intermittent treatments (usually 6-8 hours) 6 to 7 days
a week. Intermittent CRRT is, as the name suggests, treatment provided with a machine designed for CRRT but used for a shorter treatment duration. Other novel machine types are available, such as the Genius system, which generates a fixed amount of dialysate (75 L) from purified water for use during a single treatment. Another machine, the NxStage machine, uses pre-packaged sterile fluids, and is marketed for use either in a CRRT mode or for daily dialysis. As new models of the most popular IHD and CRRT machines are introduced, they are becoming more versatile, in that the ranges of dialysate flow rate, replacement fluid rate, and blood flow rate are expanded, blurring the distinction between types of machines. A suggested name for these methods of use is prolonged intermittent renal replacement therapy (PIRRT), which removes the connotation of type of machine used.

**CHOICE OF MODALITY**

In the absence of data, strong and animated debate about the virtues and perceived superiority of one modality over the other was a popular topic in nephrology. Informal surveys showed that nephrologists, who are most familiar with IHD for their CKD patients, tend to choose IHD for their AKI patients. Criticalists tend to choose CRRT. Because of a perception that CRRT maintains hemodynamic stability better than IHD, there tends to be a bias towards sicker patients preferentially being started on CRRT, which has confounded retrospective data review.

One possible criterium for choosing a modality would be the survival rate. In a prospective randomized multi-center study, Mehta et al found worse mortality rates in the CVVH arm (65.5%) compared to the IHD arm (47.6%, p = 0.02). However, despite randomization, the CVVH patients had higher APACHE III scores at entry. In a single center study by Augustine et al, there was no difference in mortality (67.6% in CVVHD vs 70.0% in IHD). These patients were equal in illness severity score. In a study by Uehlinger et al, again there was no difference in mortality (47% in CVVHDF vs 51% in IHD). In both the Hemodiafe and SHARF studies, survival rates were similar in CVVH vs IHD arms. A meta-analysis by Bagshaw et al found no difference in the overall survival. In all of these studies, length of hospitalization was similar between groups (mean, 21-42 days). All the studies mentioned so far compared CRRT to IHD prescriptions in which treatment duration was less than 5 hours and patients were treated 3 to 7 days a week. Marshall et al retrospectively evaluated mortality in 3 hospitals who converted from CRRT to PIRRT. They found no difference in the observed mortality with PIRRT. In a randomized prospective single-center study, Abe et al compared CVVHDF to SLEDD-f and found better survival in the SLEDD-f group.

Renal recovery rates also tend to be similar between CRRT and IHD groups. In the studies mentioned above, renal recovery rates ranged from 12.5-50% in the CRRT arm, and from 10-42% in the IHD arm. One thing to point out is that these are recovery rates for all patients. Because death is a competing outcome, studies that report renal recovery in only the survivors may have much higher recovery rates. In Jacka et al, the renal recovery rate of 87% in the CRRT arm compared to 36% recovery in the IHD arm was significantly different. However, more patients died in the CRRT arm, and when considering all patients, the magnitude of difference lessens, in that 32% of all CRRT patients recovered renal function, compared to 18% of IHD patients. Abe et al found a higher renal recovery rate in the SLEDD-f group (60% in CVVHDF vs 80% in SLEDD-f, p < 0.05).

Based on these data, survival, length of stay, and renal recovery rates are similar between CRRT and IHD. CRRT is preferred over IHD in hemodynamically unstable patients because of the perception that CRRT is superior in that setting. Several prospective studies specifically enrolled critically ill patients in a randomized manner to test that hypothesis. In studies by Uehlinger et al, Vinsonneau et al, and Augustine et al, there was no difference in mean arterial pressure between groups. Augustine et al found a decrease in MAP during treatment compared to pre-treatment in the IHD group but not in the CVVHD group. However, the average decrease was only 2.6 mmHg. Kielstein et al found no difference
in hemodynamic parameters (mean arterial pressure, heart rate, cardiac output, and systemic vascular resistance) between critically ill ventilated patients treated with CRRT compared to PIRRT.

Patients with AKI and absolute or relative oliguria may develop substantial fluid accumulation over the course of resuscitation and treatment before starting renal replacement therapy. CRRT may be chosen as the therapy in those cases, to allow for more gradual but sustained fluid removal. Because fluid is removed from the vascular space, and the vascular volume then refills from the interstitial space, a rate of fluid removal that exceeds the refill rate can cause intravascular volume depletion leading to hypotensive episodes. In the study by Augustine et al, patients on IHD had a net gain of fluid on day 2 (a non-dialysis day), and although they had a negative balance on day 3, the CRRT patients had a more negative net balance on day 3. This is despite a greater decrease in urine output in the CRRT group in that study, indicating that CRRT was more effective at fluid removal compared to IHD. Bouchard et al found a negative net balance at 10 days in the CRRT group, while the IHD group sustained a positive fluid balance by day 10. Improvement in fluid control may better protect cerebral perfusion in patients with fulminant hepatic failure, acute brain injury, or cerebral edema, and in that subset, CRRT may be superior to IHD.

**CVVH vs CVVHD vs CVVHDF**

CRRT can provide clearance via convection, diffusive, or a combination of the two. CVVH is a purely convective clearance mode. With CVVH, a large volume of fluid (i.e., 20-35 ml/kg/hr) is removed from the patient to clear the solutes dissolved in that fluid. The fluid used to replace that volume is a balanced polyionic solution. The replacement fluid can be delivered to the extracorporeal blood circuit before the blood reaches the filter. This dilutes the blood entering the filter and decreases the efficiency of solute removal. Alternately, the replacement fluid can be added after the filter, maintaining maximum efficiency of clearance. In that set-up, the large amount of plasma water removal as the blood passes through the filter hemoconcentrates the blood and increases the risk of clotting in the filter. With CVVHD, clearance is predominantly diffusive, and filter clotting may be less severe than with CVVH (in pre-dilution or post-dilution configurations). Diffusive clearance is excellent for small molecules (< 100 molecular weight), but clearance decreases as molecular weight increases, with almost no molecules over 1000 MW being cleared. Convective clearance can remove solutes up to 10,000 MW. Because cytokines and other inflammatory mediators fall in the middle molecule size range, theoretically convective clearance may be superior to diffusive. In practice, however, this does not translate into better survival or other outcome measures.

**Peritoneal Dialysis**

Peritoneal dialysis (PD) may seem like an attractive choice because it does not require a specialized dialysis machine. Phu et al compared PD to CVVH in patients with infectious causes of AKI (primarily malaria) and found the PD patients were 5 times more likely to die. The CVVH group had faster resolution of azotemia and acidosis and those patients were on renal replacement therapy for a shorter period of time. The cost of CVVH was half that of PD. However, in a study by Gabriel et al comparing PD to daily IHD, no difference was found in solute control, mortality, or renal recovery, and the IHD patients required longer courses of treatment than the PD patients.

**NON-MEDICAL ISSUES IMPACTING CHOICE OF THERAPY**

One of the main criticisms of CRRT is that it is labor intensive and therefore costly. CRRT roughly costs 2.5 times as much as IHD (Forni and Hilton, Manns et al, Rauf et al). The main costs of CRRT include the fluids, which are expensive to produce and ship, and the allocation of ICU nursing services. The main costs of IHD are nursing costs, which are determined by the frequency and duration of
treatment. The cost of providing renal replacement therapy is highly variable based on the institution, and the cost is only a part of the total cost of care for the AKI patient.

The abilities of the nursing staff are also a factor to consider in deciding about modality. If the nurses are comfortable with providing CRRT, that may be a great choice, but if the nurses are not adequately trained and lack sufficient clinical experience to have confidence, CRRT may be a poor choice for the unit and the patient. In addition to the level of training, the amount of support plays a role in deciding about modality. Small, low-volume units may be understaffed to provide continual care. Using PIRRT may alleviate some of the staffing issues by shifting treatment times to first and second shift time slots and avoiding the need for an overnight shift.

CONCLUSION

There is no right choice for RRT modality. From a medical perspective, no therapy has proven to be superior in survival, renal recovery, stability, or efficacy for people, and veterinary experience is far too limited at this stage to provide data. In human hospitals, the choice of therapy appears to be made based on availability and physician preference, and this is likely going to be the case in veterinary medicine. In a unit that can provide all of the types of therapy, each has advantages and disadvantages for an individual case that may play a role in choosing. For units that offer a limited number of therapies, developing a team that is proficient at providing that type of therapy is likely the best strategy for optimizing patient outcomes.

REFERENCES
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SPIRONOLACTONE ADMINISTRATION IN A RODENT MODEL OF CHRONIC ALLOGRAFT NEPHROPATHY
Schmiedt CW; Cogar SM, Brown CA; Vandenplas M; Hurley DJ

Aldosterone’s role in chronic allograft nephropathy (CAN), an important cause of renal allograft loss, is unknown. The purpose of this study was to evaluate spironolactone (SPIRO) on the development of CAN. We hypothesized SPIRO would result in a reduction of allograft damage and down regulation of proinflammatory and profibrotic genes.

A F344 to Lewis rat renal transplant (RTx) model was employed and rats were divided into 4 groups. 2 groups were nephrectomy controls (NEPH, n=4) and 2 underwent heterotopic RTx (n=8). 1 NEPH and RTx group received SPIRO (10 mg/kg/day) and the other 2 groups received water (0.25 ml/day). Serum creatinine and urine protein: creatinine (UP:UC) were measured before surgery and at time points throughout the study. After 16 weeks, rats were euthanized and renal cortex was harvested for RT-qPCR for TNF-α, TGF-β, collagen type 1, PDGF, Edn-1, CTGF.

4 RTx (1 water, 3 SPIRO) rats did not survive. Creatinine was not different between groups. UP: UC was significantly increased in RTx groups compared to baseline, but no difference between groups was observed. There was a significant increase in TNF-α gene expression in the RTx groups compared to the NEPH groups. However, no significant difference was noted between RTx groups for overall expression of any evaluated gene.

Histologically, when NEPH rats were compared to transplant recipients, there was a significant increase in acute interstitial inflammation, mesangial matrix expansion, and chronic tubular atrophy in RTx animals, but no difference was observed between RTx groups. SPIRO did not influence CAN progression in this model.
IN VIVO WARM RENAL ISCHEMIA AS A MODEL OF ACUTE TUBULAR INJURY IN CATS

Schmiedt CW, Brown CA, Hurley DJ, Brown SA

The objective of this study was to evaluate unilateral (UL) and bilateral (BL), warm ischemia-reperfusion kidney injury as a model of acute kidney injury in the cat. 13 adult healthy cats underwent 60 minutes of UL (n=6) or BL (n=4), in vivo renal warm ischemia or served as sham operated controls (n=3). In UL and BL cats the renal artery and vein was occluded. Kidney function was evaluated before and after ischemia using serum creatinine and BUN concentration, urine protein: creatinine, and iohexol clearance estimation of glomerular filtration rate (GFR). Renal biopsy specimens taken before injury, after ischemia, and at various intervals following reperfusion were evaluated histopathologically. Cats with BL ischemia suffered acute kidney injury and a significant decline in GFR. All BL cats became severely azotemic with GFR reduced to 2.7 to 11.8% of preoperative values. Renal pathology was broadly characterized by proximal acute tubular necrosis and thrombosis. In an effort to reduce uremia associated with BL, we evaluated the effects of UL in the remaining 6 cats. UL cats were euthanized on postoperative day 3 or 6. No UL cat experienced morbidity or azotemia, in spite of a 43% reduction in GFR on day 6. Histopathologically, severe acute tubular necrosis was observed on day 3 with signs of tubular regeneration observed on day 6. All control cats were normal post-operatively. 60 minute, UL ischemia is a functional model for acute kidney injury in cats and had a far lower level of morbidity than induced by BL ischemic injury.
Neutrophil gelatinase-associated lipocalin (NGAL) is a protein that is gaining utility in the diagnosis of kidney disease in human medicine. The purpose of this study is to investigate NGAL concentration in dogs with chronic kidney disease (CKD) by measuring serum and urine NGAL concentration in normal dogs and dogs with CKD.

Forty dogs were assessed to be free of kidney disease on the basis of a normal physical examination, complete blood count, serum biochemical profile, urinalysis, urine protein creatinine ratio (UPC) and blood pressure. Patients with urine specific gravity <1.030, serum creatinine >150 μmol/L, UPC >0.2, and/or urine leukocytes >5/hpf were excluded. Serum and urine NGAL concentrations were measured in 40 normal dogs using a commercially available canine-specific ELISA kit. Fifteen dogs with naturally occurring CKD based on clinical signs as well as consistent laboratory data (presence of renal azotemia, loss of urine concentrating ability, with or without proteinuria and/or hypertension) will be recruited for the study. These dogs will be followed for 6 months and have a glomerular filtration rate GFR measured by plasma technetium clearance (Tc99m-DTPA) at 0 and 6 months. Serum and urine NGAL concentrations were measured at 0, 3 and 6 months.

Data has been collected on 30 normal dogs and 6 CKD dogs at this time. The mean creatinine value of the normal dogs was 86.4 μmol/L (SD 29.02) with a mean urine specific gravity of 1.043 (SD 0.072). The mean urine NGAL concentration of normal dogs was 23.8 pg/ml (SD 32.4) with a mean serum NGAL concentration of 123.2 pg/ml (SD 72.8). The CKD dogs have a mean creatinine value of 195.3 μmol/L (SD 81.3), mean urine specific gravity of 1.017 (SD 0.009), and mean UPC of 1.76 (SD 2.59).

The results suggest that serum NGAL is greater on average than urine NGAL and that dogs with CKD have reduced GFRs as estimated by plasma technetium clearance.
Therapeutic plasmapheresis has been used in veterinary medicine for several pathological conditions but no data concerning the application of cascade filtration technique in the treatment of hyperviscosity syndrome are still available. A 12 year old, 38 kg, mix-breed, intact male dog was presented to the Haemodialysis and Blood Purification Unit of the Veterinary Teaching Hospital “Mario Modenato” with a 20 day history of clinical signs related with HVS secondary to multiple myeloma. The dog was anesthetized for the placement of a central venous catheter and submitted to three treatments of cascade filtration plasmapheresis. A Diapact® CRRT machine (BBraun, Avitum AG) was used in plasmapheresis modality. A 0.2 m$^2$ polyethylene plasma separator Plasmaflo™ OP-02 (©Asahi Kasei Kuraray Medical Co., Ltd), with maximum pore size of 0.3 µm, was used for sharing plasma, while a 2 m$^2$ ethylene vinyl alcohol copolymer plasma filter Cascadeflo™ EC-50 (©Asahi Kasei Kuraray Medical Co., Ltd) was used for plasma filtration. Blood flow (Qb) and plasma flow (Qp) were set at 70 ml/min and 20 ml/min respectively and the time of treatment was set at 2 hours. The pre- and post-treatment concentrations of total proteins, albumin, alpha1, alpha-2, beta and gamma-globulins were assessed by electrophoresis and compared through t-test (p<0.05). After plasmapheresis a significant reduction (p=0.008) in serum total proteins and a complete remission of the clinical signs of HVS were found. Cascade filtration seems to be a promising technique, which avoids high costs of plasma and albumin supplementation.
IHD TREATMENT AND BILATERAL URETERAL STENTING IN A 8YRO PITT BULL FEMALE WITH SEVERE BILATERAL HYDRONEPHROSIS

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Maybe, a 8yro Pitt Bull spayed female was referred for AKI development as consequence of bilateral hydronephrosis due to massive urolithiasis involving both renal pelvis, ureters and the bladder. The dog was under treatment with allopurinol for two years after diagnosis of leishmaniasis.

At the presentation the dog was depressed, vomiting, moderately anaemic (Hct 38%), uremic (Creatinine 13.09 mg/dl; Serum Urea 242 mg/dl; Pi 15,37 mg/dl) and hypertensive (175/110 mmHg), but not acidotic. IHD was prescribed to improve uremic status and allow to approach the massive urolithiasis and bilateral hydronephrosis by positioning double pig tail stents. The uroliths were radiolucent and detectable only by ultrasound: they were numerous in both renal pelvis, in the ureters and in the bladder. Maybe improved well, being treated with seven IHD in eleven days; on day 13 from hospitalisation the ureteral stents were positioned surgically because it was impossible to do it by cystoscopy. After the surgery, Maybe received two hours of dialysis, without heparin. She improved rapidly and was dismissed with renal diet only and after removal of bilume catheter and oesophageal feeding tube.

Three week later, the referring veterinarian informed that creatinine started to raise again and PU/PD was noted. The dog was rechecked and a relapse of the hydronephrosis of the right kidney was seen by ultrasound. An xRay film revealed that the stent was completely rolled up into the right pelvis. Creatinine was 8.65 mg/dl.

A surgery was planned for the following day. The right renal pelvis was very dilated as well as the right ureter in the proximal part. In the middle part uroliths were palpable. An incision was made in the pelvis to remove the rolled stent and all the uroliths that was possible to localize, a second incision was made in the middle ureter, to remove other stones and check the patency of the ureters in order to place a new stent of bigger size. No more hemodialysis was performed. Four days after the surgery, creatinine dropped to 2.77mg/dl and the dog was fine again. Both stents were in place and the hydronephrosis on the right kidney dramatically reduced.

In the mean time we received the report of uroliths quantitative analysis, from the Minnesota Urolith Center: 100% Xantine. A CT control of the uroliths with the ROI technique showed a mean Hounsfield Unit < 400.
ENDOSCOPIC SCLEROTHERAPY FOR THE TREATMENT OF IDIOPATHIC RENAL HEMATURIA

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**Background:** Idiopathic renal hematuria (IRH) results in chronic upper urinary tract bleeding. In humans, ruptured renal pelvic hemangiomas/angiomas are typically the cause. Although benign, anemia, ureteral and urethral obstruction(s) can ensue. With the advent of endourology, renal-sparing therapies like ureteropyeloscopic-guided electrocautery or sclerotherapy has replaced ureteronephrectomy.

**Objectives:** To describe the use of endoscopic-fluoroscopic-guided sclerotherapy for the treatment of IRH in dogs and report the first clinical outcomes.

**Methods and Materials:** Each UVJ was identified cystoscopically. Once the bleeding was confirmed a retrograde ureteropyelogram was performed. A ureteropelvic junction balloon was used for ureteral occlusion, and pelvis filling volumes were recorded. Four dwells were performed (2 5% povidone iodine mixture; and 2 sterile liquid 1% silver nitrate). A double-pigtail ureteral stent was placed.

**Results:** Seven dogs (n=9 renal units) had sclerotherapy. Five unilateral, 1 bilateral, and 1 developed contralateral bleeding. Five were right and 4 left-sided. There were 6 males and 1 female. The median age and weight was 6 years and 27.5kg, respectively. Median procedure time was 150 minutes. There was 1 complication of severe renal discomfort and pyelectasia in an unstented dog. Cessation of hematuria occurred in 6/9 renal units (median 12 hours). Two had recurrence within 3 weeks both resorting to intermittent and mild hematuria. Two failed treatment. Median follow-up time was 5 months (range, 1.5-19).

**Conclusions:** Overall, topical sclerotherapy for IRH can be safe and effective. This is the first report of local sclerotherapy for IRH in dogs and further investigation is required. Renal sparing therapies should be considered prior to ureteronephrectomy.
ENDOSCOPIC NEPHROLITHOTOMY FOR NEPHROLITHIASIS IN DOGS

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Objective: Percutaneous nephrolithotomy (PCNL) is considered the standard-of-care for removal of nephroliths >1.5 cm in people, minimizing morbidity and preserving renal function. Success rates are reported to be 90-100%. Most veterinary nephroliths remain clinically silent and removal is only recommended for complicated stones. Morbidity of nephrotomy can be severe. The objective is to describe endoscopic-guided nephrolithotomy (ENL) in canine and feline patients and report clinical outcomes, hypothesizing it is safe and effective.

Animals: Nine dogs and 1 cat were included.

Materials and Methods: Patients that had either PCNL or surgically-assisted endoscopic nephrolithotomy (SENL) were retrospectively evaluated. A renal puncture needle and balloon-dilation-sheath combination was used for tract formation. A nephroscope provided visualization for intracorporeal lithotripsy. Stone fragments were removed and a ureteral stent was placed.

Results: Four had PCNL and 6 SENL. Indications included recurrent UTIs (4), worsening azotemia (4), and ureteral-outflow obstructions (2). Median weight was 8.2 kg (3.1-26.9). Stone composition was calcium oxalate (6), mixed struvite (2), urate (1), and cystine (1). Median stone size was 2 cm (0.7-5). Median pre- and 3 month post-operative creatinine was 1.3 (0.8-9.1) and 1.1 mg/dL (0.6-6.1), respectively. The median procedure time was 165 minutes. Successful removal of all stones were documented in 11/12 (91.6%). Procedure-related complications occurred in 3 units, all were easily managed. Median follow-up time was 150 days (4-2007 days). Four patients are still alive. No patient died from the ENL procedure.

Conclusion: ENL can be safely performed in dogs and cats, yielding similar success rates to people. Advanced endourologic experience is recommended.
DETERMINATION OF EXTRACELLULAR FLUID VOLUME (ECFV) AND GFR/ECFV IN CATS

N.C. Finch, A.M. Peters, R. Heiene, H.M. Syme and J. Elliott

ECFV can be determined using bromide dilution or from the distribution volume of the plasma clearance marker, iohexol. By correcting for the one-compartment assumption, GFR/ECFV can be measured from slope-intercept iohexol clearance. The objectives of the present study were firstly, to validate a correction factor for the one-compartment assumption for determining GFR/ECFV from slope-intercept iohexol clearance in cats, and secondly, to compare ECFV calculated from slope-intercept GFR/ECFV with that determined using bromide dilution.

Client-owned cats with a range of renal function were studied. The dilutional space of bromide (ECFV\textsubscript{Bromide}) was calculated. GFR/ECFV was determined using multi-sample iohexol clearance and from slope-intercept clearance. The correction factor was obtained by regression analysis. ECFV was determined from slope-intercept iohexol clearance (ECFV\textsubscript{Cl}) using the GFR/ECFV data and expressed in L. The correction factor for slope-intercept GFR/ECFV was $1.027\times\beta$ ($\beta$ = elimination rate constant). Slope-intercept GFR/ECFV showed excellent agreement with multisample GFR/ECFV (n=18).

Mean ± SD of ECFV\textsubscript{Bromide} was 0.85±0.19L. Mean ± SD of ECFV\textsubscript{Cl} was 0.83±0.29L. ECFV\textsubscript{Bromide} and ECFV\textsubscript{Cl} were significantly correlated but agreement was poor (n=66).

A method for determining ECFV from slope-intercept clearance by applying a correction factor for the one-compartment assumption was validated in cats. Agreement between ECFV\textsubscript{Bromide} and ECFV\textsubscript{Cl} was poor which may be related to differing rates of penetration of ECFV by the markers resulting in different estimated distribution volumes.
REGIONAL CITRATE ANTICOAGULATION FOR INTERMITTENT HEMODIALYSIS IN DOGS.

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Extracorporeal blood purification techniques such as hemodialysis (HD) require anticoagulation of the circulating blood. The most common protocol uses systemic heparinisation that can however hinder urgent surgical procedures or worsen existing hemostatic disorders such as disseminated intravascular coagulation or leptospirosis-associated pulmonary hemorrhages. Regional anticoagulation techniques have thus been developed in humans for critical patients treated with continuous renal replacement therapy. One technique aims at reducing the ionized calcium concentration in the extracorporeal circuit (< 0.4 mmol/l) by infusing trisodium citrate in the arterial line and restoring normocalcemia (> 0.8 mmol/l) with calcium chloride administration in the venous line prior to returning the blood to the patient. The aim of this study was therefore to establish and evaluate the adequacy of a canine protocol of regional citrate anticoagulation (RCA) in intermittent HD for acute kidney injury (AKI).

The RCA protocol was based on established human protocols and on in vitro pilot experiments. 211 HD sessions have been performed with Gambro AK200 UltraS system in 55 dogs treated for acute leptospirosis (n=33), toxic nephrosis (n=14), or other causes of AKI (n=8) following individually adjusted standard HD protocols. The initial flow ratio of blood : citrate (102 mmol/l) : calcium (340 mmol/l) was 10 ml/min : 15 ml/h : 1.5 ml/h and it was adjusted based on the ionized calcium concentrations in the circuit and in the animal. Satisfactory anticoagulation was assessed based on successful completion of the procedure, change in the dialyzer pressure gradient, urea and creatinine reduction ratios (URR, CrRR), and visual scoring of the extracorporeal circuit after blood rinseback.

The initial citrate and calcium infusion rates required adjustments in 27% and 44% of the treatments, respectively. Anticoagulation was judged overall satisfactory in 93% of the treatments. Four HD treatments (2%) hindered by severe catheter malfunction had to be stopped early due to severe clotting. The dialyzer pressure gradient increased ≥25% from baseline in 14% of the treatments. The extracorporeal circuits were considered moderately and severely clotted in 3% and 1% of the treatments, respectively. URR and CrRR were ≥25% below the expected ratios in 10% and 17% of the treatments, respectively. No clinical or laboratory side effect were observed.

With the described protocol of RCA, extracorporeal circulation could be safely and efficiently performed in dogs without the need for systemic heparinisation, representing a major advance in the treatment of animals at risk of bleeding.
Helpful Resources

Websites:

www.vetcrrt.net This is the home page of the Veterinary CRRT Society, and this is one place to join the VetCRRT List Serve Discussion Group

www.queenofthenephron.com This is a listing of veterinary units performing renal replacement therapies

www-users.med.cornell.edu/~spon/picu/calc/cacalc.htm This is a helpful site when using regional citrate anticoagulation, to ensure your calcium dose is equivalent.

renalpharmacyconsultants.com/sitebuildercontent/sitebuilderfiles/DialysisofDrugsUS2011web.pdf This is a handbook of drug removal by dialysis, useful in determining if post-dialysis supplementation is necessary, and somewhat helpful in deciding if dialysis is appropriate for overdose.

www.tinkershop.net/nephro.htm This is a useful calculator for Kt/V

www.pcrrt.com Pediatric CRRT has many parallels to veterinary CRRT

Other Resources:

Wikispaces dialysis handbook This is the AMC dialysis handbook, available for review by invitation to veterinarians (ask cathy.langston@amcny.org). This is intended to be a collaborative effort, and you should include your viewpoint and observations here!

Printed Material (Veterinary Specific)


Equations for CRRT

CVVHD
Qd = 2000mL/1.72m2/hr
Kcalc = dialysate rate (mL/min)
Kdel = Postdialyzer dialysate urea (mg/dL) * dialysate rate (mL/min)/patient blood urea (mg/dL)

CVVH post-dialyzer
Kcal post = Qrep ml/min
Kdel post = Ultrafiltrate urea concentration (mg/dL) * Replacement fluid rate (mL/min)/ Patient urea concentration (mg/dL)
FF = (Qf * 100)/(Qb *(1-HCT))

CVVH pre-dialyzer
Kcalc pre = Qf (mL/min)/[1+(Qrep (mL/min) / Qb (mL/min)]
Kdel = UF urea (mg/dL) * Qf (mL/min)/ Patient urea (mg/dL)

CVVHDF post-dialyzer
Kcalc = Qf (mL/min) + Qd (mL/min)
Kdel = Ultrafiltrate urea (mg/dL) * (Ultrafiltration rate (mL/min) + dialysate rate (mL/min))/ Patient urea (mg/dL)

Provided by:
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Companion Animal Medicine
School of Veterinary Medicine
Louisiana State University
Baton Rouge, LA 70803-8410
### Dialysis Units

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