Acute Kidney Injury: Biomarkers, Pathophysiologic Targets and Clinical Trial Designs

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Definitions of AKI need to use existing biochemical Parameters that clinically meaningful endpoints And predict short and long-term outcomes

Utilizing “road-map” biomarkers to design trials with interventions based upon the current and predominant pathophysiology

Proper study drug dosing studies: “One dose Does Not a Treatment Make”

Use of Observational studies-Prior to intervention Trials-reducing variability between Sites
Investigational Drugs for AKI: Considerations in Trial Design

- Which drug, What Dose, When & How Long?

- Dose response effects
  “Do you know how much to give?”

- When to Initiate Administration of Agent: Prophylactic administration?

- Duration of Study Drug Administration
  Arbitrary duration of treatment

- How to Administer Study Drug
  Intra-renal drug administration
AKI Definitions: Do they predict clinical Outcomes?
AKI Definitions: Good & Bad

- Over 30 AKI definitions have been published
  - Effectively all are based on absolute or Delta changes in serum creatinine

- Recent evolutions of AKI Definition:
  - Include severity scoring and correlation with CKD and 90 day all cause mortality

- Absence of Biomarkers to identify prevailing pathophysiology
  - Guide to determining the What and Where Question
Three Dominant AKI Definitions:

- RIFLE
- AKIN
- KDIGO
RIFLE Criteria: AKI

**Risk**
- Increase Serum Cr-1.5X OR \( \downarrow \) GFR > 25%

**Injury**
- Increase Serum Cr-2X OR \( \downarrow \) GFR > 50%

**Failure**
- Increase Serum Cr-3X \( \downarrow \) GFR > 75%
  OR Cr > 4.0

**Loss**
- Persistent AKI requiring renal replacement therapy X > 4 Weeks
- Persistent AKI requiring renal replacement therapy X > 12 Weeks (ESRD)

**ESRD**
- High Specificity
- Low Specificity

**Urinary Output**
- Urine Output < 0.5 mls/kg body Wt. X 6 hours
- Urine Output < 0.5 mls/kg body Wt. X 12 hours
- Urine Output < 0.3 mls/kg X 12 hours OR Anuria

**Persistent AKI requiring renal replacement therapy**
- X > 4 Weeks
- X > 12 Weeks (ESRD)
AKIN Criteria: AKI

Stage -I
Increase Serum Cr-1.5X OR
0.3 mg/dl

Stage-II
Increase Serum Cr-2X

Stage-III
Increase Serum Cr-3X OR Cr ≥ 4.0

Urine Output < 0.5 mls/kg body Wt.
X 6 hours

Urine Output < 0.5 mls/kg body Wt.
X 12 hours

Urine Output < 0.3 mls/kg body Wt.
X 24 hours OR
Anuria X 12 hrs

Start of Renal Replacement Therapy

Low Specificity

High Specificity
<table>
<thead>
<tr>
<th>Stage</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage-I</td>
<td>Increase Serum Cr-1.5-1.9X Baseline OR ↑0.3 mg/dl</td>
</tr>
<tr>
<td></td>
<td>Urine Output &lt; 0.5 mls/kg body Wt. X 6-12 hours</td>
</tr>
<tr>
<td>Stage-II</td>
<td>Increase Serum Cr-2.0-2.9 X Baseline</td>
</tr>
<tr>
<td></td>
<td>Urine Output &lt; 0.5 mls/kg body Wt. X 12 hours</td>
</tr>
<tr>
<td>Stage-III</td>
<td>Increase Serum Cr-3X OR Cr ≥4.0</td>
</tr>
<tr>
<td></td>
<td>Urine Output &lt; 0.3 X 24 hours</td>
</tr>
<tr>
<td></td>
<td>Start of Renal Replacement Therapy</td>
</tr>
</tbody>
</table>

KDIGO Criteria: AKI

Low Specificity

High Specificity
How effective are our current Definitions of AKI in Predicting Clinical Outcomes
Long-term Effects of Post-CABG AKI in Patients with Normal Perioperative Renal Function

OBJECTIVES: To determine the effect of pre-operative GFR and RIFLE criteria Risk, Injury, Failure or ESRD on long-term survival of Post CABG pts.

METHODS: Univariate & multivariate analysis of GFR on patient survival of 4029 CABG patients at 150 months

CKD Stage-1 (46.5%) (GFR ≥90 ml/min/1.73 m²),
CKD Stage-2 (50.4%) (GFR 60-89 ml/min/1.73 m²)
CKD Stage-3 (3.1%) (GFR 30–59 ml/min/1.73 m²)

Outcomes: Long-term patient survival

Progressive AKI Mortality: Utility of RIFLE RISK, Injury and Failure in Post CABG AKI
Validation of KDIGO Criteria for AKI and Comparison to AKIN and RIFLE Criteria

General Consensus: Staged KDIGO Definitions Of AKI Predict Short and Long-Term Clinical Outcomes
Can Known AKI Risk Factors Be used to Enrich AKI Studies?
Relative Risk for Post-CABG AKI
Contribution of Specific Co-Morbid Variables

Incidence of Post-Op Renal Replacement Therapy as Function of Peak Serum Creatinine

% Incidence of Dialysis

- 1.6: 2%
- 2.0: 13.6%
- 2.5: 33%
- 3.0: 40%
- 3.5: 58%
- 4.6: 38%

References:
NephroNet Experience: Enriching AKI Populations Using Pre-Interventional Observational Studies
Site Qualification and Estimation Study Populations

- Hospital Based AKI Studies: Few centers have access to accurate AKI event rates

- AKI event rates vary:
  - Type of AKI definition
  - Time frame for AKI inclusion
  - Between Individual surgeons
  - Between individual centers

- Inclusion Criteria for AKI
  - No. of and type of co-morbid risk factors
  Note: AKI is a summation of intensity of renal insult in combination with co-morbidities that increase susceptibility to AKI
Primary Objective:
Determine incidence of Post CABG Acute kidney Injury (AKI) as defined by the KDIGO criteria in cardiac surgery patients with multiple pre-defined risk factors

Secondary Objectives:
- Incidence-Stage of AKI defined by AKIN or RIFLE R criteria
- Changes in Renal function over observation period
- Percent patients requiring renal replacement therapy
- Percent patients with renal dysfunction on POD-28, 60, or 90
- Relationship between # and type of risk factors to outcome
- Duration of AKI

Tumlin et.al. presented CRRT-2014, San Diego CA, March 2014
Inclusion Criteria: Patients scheduled for On-Pump cardiothoracic surgery (off-pump excluded)

- All CT patients with 2 or more concurrent risk factors:
  - CKD stage 3 or stage 4 (eGFR<20mls/mi excluded)
  - Insulin requiring diabetes
  - Non-insulin requiring diabetes and +2 proteinuria
  - Chronic obstructive pulmonary disease (COPD)
  - Cardiomyopathy: LVEF < 40%
  - Pre-operative anemia-Hgb < 10.0 mg/dl
  - Iodinated contrast exposure within 7 days of surgery

Tumlin et.al. presented CRRT-2014, San Diego CA, March 2014
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Demographics

- 100 patients enrolled
  - 73 men & 27 women
  - Average age (SD): 66 (10) years old
  - 86 Caucasian

- Average creatinine (SD) day of surgery: 1.2 (0.5) mg/dl

- Average eGFR (SD): 66 (26) ml/min/1.73m²

- Risk factors
  - CKD: 48%
  - Diabetes: 48%
  - COPD: 45%
  - LVEF <40%: 46%
  - Anemia: 48%
  - Contrast Agent: 11%

Tumlin et.al. presented CRRT-2014, San Diego CA, March 2014
Percent (%) Patients AKI Rate

Tumlin et al. presented CRRT-2014, San Diego CA, March 2014
Staging of AKI Severity

- **Stage-I**: 70%
- **Stage II**: 18%
- **Stage III**: 12%

**KDIGO Stage 1**
- SCr 1.5 to 1.9 times baseline, or
- SCr ≥ 0.3 mg/dl increase

**KDIGO Stage 2**
- SCr 2.0 to 2.9 times baseline

**KDIGO Stage 3**
- SCr 3 times baseline, or
- increase in SCr to ≥ 4.0 mg/dl
- or initiation of RRT

Tumlin et al. presented CRRT-2014, San Diego CA, March 2014
KDIGO AKI Stage Severity

- CKD
- Diabetes
- COPD
- LVEF <40%
- Pre-Op anemia
- Contrast media

Risk factors

- 2 risk factors: 7%
- 3 risk factors: 35%
- 4 or more risk factors: 58%

n = 58, 58%
More CKD patients developed AKI as compared to non CKD patients: 71% vs. 44% (P<0.01)
AKI Rate
+/- Presence CKD

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Percent (%) Clinical Endpoint

- CKD

Transient Stage I AKI
25%

Sustained Stage I AKI-6%

Stage II-III AKI-13.5%

+ CKD

Transient Stage I AKI
18.8%

Sustained Stage I AKI-31.5%

Stage II-III AKI-21%
Serial Cr at 90 Days:
No CKD

- No AKI
- Transient AKI
- Sustained AKI
- Stage II-III AKI
- AKI 2,3: Severe

Note: 90 Day permanent injury does not occur in the absence of prior CKD.
Serial Cr at 90 Days: With CKD

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Note: AKI at 48hrs with prior CKD contributes to 90 sustained renal injury

- No AKI
- AKI1: Transient
- AKI1: Sustained
- AKI 2,3: Severe
Outcome:
Canadian Pharmaceutical Company Changed Corporate Strategy: Conducting Prophylactic Post-CABG Study
Why is The Need for Validated Biomarkers in AKI So Important?

- Biomarkers provide the opportunity for early intervention with the prospect of disrupting the pathophysiologic cascade that culminates in progressive renal failure/dialysis.

- Appropriately selected biomarkers can inform the clinician WHERE in the cascade of AKI the patient is clinically.

- Biomarkers can assist in determining what combination to treatments are required prevent Further decline in AKI.
Furosemide Stress Test
“A Functional Biomarker”
Validation of an Old Idea
Development of a Furosemide Stress Test to Predict the Severity of Acute Kidney Injury

- **Study Objective:** To determine the predictive efficacy of the urine response to Bolus Furosemide in patients with non-oliguric and oliguric AKI.

- **Study Methods:** 77 subjects with primary endpoint of AKIN-III were examined for urine response to a 1.0 mg/kg bolus furosemide.
  - Hourly urine response
  - Progression to death or dialysis
  - Receiver operator curve analysis

- **Study Results:** Urine output cutoff < 200 mls/2 hours
  - ROC value-0.87
  - Progression to AKIN-III 32%

Development of a Furosemide Stress Test to Predict the Severity of Acute Kidney Injury

Development of a Furosemide Stress Test to Predict the Severity of Acute Kidney Injury

Koyner et. al. SAKINET Group: In Press
Positive Furosemide Stress Test:

- More likely to progress to AKIN Stage III
- More likely to require Dialysis or CRRT
- Higher likelihood of 90 Day Mortality
“Just Because You Are in Rome Does Not Mean You Are On the Right Road to the Vatican”
The Ideal Biomarker:
Should exhibit high sensitivity & specificity but also the ability to locate where a patient is in the pathologic continuum of AKI
“What Do We Do Now Captain?”
What Time is it?
Physiologic Targets of Drug Therapy in AKI

- Restoration of Corticomedullary blood flow
- Disruption of tubular apoptotic pathways
- Inhibition Neutrophilic-monocytic infiltration
- Stem Cell repopulation
- Prevention of CKD and ESRD progression
Pathologic Continuum of AKI: Early Tubular Injury-Reduction of Corticomedullary Blood Flow: 6-18 hours

- Normal Tubular Epithelia
- Ischemia
- Re-Perfusion
- Loss of Brush Border & Polarization
- Apoptosis
- Necrosis
- Apoptotic Blebs
- Vasa Recta & Luminal Neutrophil Invasion
- Release of MPO O$_2^*$ Radicals
- Formation "Muddy Brown Casts"
- Anuria Tubular Occlusion
- Loss of Cell Adhesion & Sloughing Epithelial Cells

Intervention:
- Renal directed vasodilator therapy
- Fenoldopam
- Anaritide
- PGI$_2$

Early Biomarkers:
- NGAL, KIM-1, IL-18
AKI: Impaired Vasodilation and Autoregulation

Initiation Phase: 6-18 hrs.

Maximized Vasoconstriction
Increased Vascular Reactivity

- ET-1
- ET-α receptor
- ET-β receptor
- Thromboxane A₂
- Angiotensin II
- Adenosine

Vascular Congestion
Occluded Vasorectas

Impaired Vasodilation

- i NOS
- e NOS

Blunted Vasodilator Response

- Acetylcholine
- Bradykinin
- Nitroprusside
Allgren Anaritide Trial
1st Major Attempt
Anaritide in Acute Tubular Necrosis

- **Study Objective:** To determine the efficacy of the Atrial Natriuretic peptide (Anaritide) on the 21 day rate of dialysis at in patients with established ATN.

- **Study Design:** Multicenter, randomized, double-blind, placebo-controlled trial of 504 patients with ATN.

- **Diagnosis of ATN:** 1.0 mg/dl rise Cr < 48 hrs
  Predefined oliguric group < 400 mls/24 hrs

- **Exclusion criteria:** (1) > 3.0 mg/dl or initiation dialysis

- **Study Treatment:** ANP titrated 200 ng/kg/min X 24 hrs
  Dose held at maximum BP tolerated

- **Primary Endpoint:** 21 day dialysis-free survival

Anaritide in Acute Tubular Necrosis

WWW: What Went Wrong?

- **Study Dose:**
  - Anaritide infused at 200 ng/ml
  - 46% of Anaritide developed hypotension

- **Study Drug Infusion:**
  - Anaritide infusion started 48 hrs
  - Baseline Cr: 40% Anaritide > 4.0 mg/dl
    - 50% Placebo > 4.0 mg/dl

- **Dialysis Endpoint:**
  - "Floating Endpoint"-left to the discretion or primary attending

- **Differential Etiology AKI:**
  - Nephrotoxic: 59% Anaritide vs. 66% Placebo
  - Ischemic: 28% Anaritide vs. 41% Placebo

Lewis Anaritide Trial
Round-1
Atrial Natriuretic Factor in Oliguric Renal Failure

- Multicenter, randomized, double-blind, placebo controlled trial of 222 patients with ATN.

- Diagnosis ATN: (1) FeNa+ > 1.0%
  (2) 0.5 mg/dl rise Cr over 48 hrs
  (3) < 400 mls / 24 hrs

- Exclusion criteria: (1) >3.0 mg/dl or initiation dialysis
  (2) renal transplant
  (3) previous dialysis

- Randomized: (1) ANP titrated by 50 ng/kg/min to maximum dose 200 ng/kg/min X 24 hrs

  Primary Endpoint: 21 day dialysis-free survival

- Secondary endpoints: All cause mortality

- Note: 42% Developed symptomatic Hypotension

Lewis et.al Am J. Kid Dis. 36(4), 767-774, 2000
Atrial Natriuretic Factor in Oliguric Renal Failure: 14 and 21 Day Dialysis Rates

Lewis et.al Am J. Kid Dis. 36(4), 767-774, 2000
WWW: What Went Wrong?

- **Study Dose**: Anaritide infusion 200 ng/ml
  - No change from Allgren
  - 95% of Anaritide SBP <90 mmHg
  - Mean delta SBP-37 mm Hg
  - Failure to use “Organ Directed Therapy”

- **Duration Drug Infusion**: Max duration: 24 hours

- **Dialysis Endpoint**:
  - “Floating Endpoint”-left to the discretion primary attending- No change from Allgren

- **Etiology AKI**:
  - Non-Ischemic: 41% Anaritide vs. 51% Placebo
  - Ischemic: 58% Anaritide vs. 49% Placebo

Lewis et.al Am J. Kid Dis. 36(4), 767-774, 2000
Sward Anaritide Trial
Round-3
Atrial Natriuretic Factor in Acute Renal Failure: A Second Look

- **Study Objective:** Determine the effect of reduced rate Anaritide infusions on the incidence of 21 day dialysis in patients with post-operative ATN.

- **Study Methods:** Randomized, double-blind, placebo controlled in 61 patients Post Bypass ATN.

- **Entry Criteria:**
  - Post cardiac bypass patients
  - Admission serum Cr < 1.7 mg/dl
  - Rise > 50% above admission Cr

- **Randomization:**
  - Control group: Normal saline
  - Treatment group: Anaritide 50 ng/kg/min

Sward et al. Crit. Care Medicine 32(6) 1310-1315 2004
Atrial Natriuretic Factor in Acute Renal Failure: A Second Look

Dialysis-free survival

Dialysis-21%

Dialysis-47%

p=0.017

Time (days)

ANP
Placebo

Sward et al. Crit. Care Medicine 32(6) 1310-1315 2004
Anaritide in AKI: Mystery of Goldilocks Porridge

Lewis Anaritide Bear

Sward Anaritide Bear

Allgren Anaritide Bear

Still Too HOT

Just Right!

Way to HOT
WWW: What Went Right?

- Study Dose: Anaritide infusion 50 ng/ml
  - 75% Reduction from Allgren-Lewis
  - 95% of Anaritide SBP <90 mmHg
  - Mean delta SBP-37 mm Hg

- Study Drug Infusion: Maximum duration
  - Anaritide: 5.3 days
  - Placebo: 4.3 days
  - Maximum: Allgren-Lewis 24 hours

- Uniform Etiology AKI:
  - Nephrotoxic AKI: excluded
  - Only Post CT surgery ischemia AKI

Intra-Renal Drug Delivery: “More Bang For Your Buck”
Intra-Renal Drug Delivery: Teaching an Old Drug a New Trick

Utilizing Targeted Organ Delivery for Pharmacologic Treatment of AKI
Superior Enhancement of Renal Blood Flow with Intra-Renal FNP Delivery

Intra-Renal Vasodilator Administration: Comparative Effects of Different Agents

Dopamine

Fenoldopam

Percent (%) Change Avg. Peak Velocity-Resistance

Renal Vasc Resistance

Ug/kg

Dose

0.1 0.3 0.8

5 15 30

5 15 30

Moral of the Story?

“One Dose Does NOT A Therapy Make!”
Physiologic Targets of Drug Therapy in AKI

- Restoration of Corticomedullary blood flow
- Disruption of tubular apoptotic pathways
- Inhibition Neutrophilic-monocytic infiltration
- Stem Cell repopulation
- Prevention of CKD and ESRD progression
Pathologic Continuum of AKI: Tubular Apoptosis Versus Necrosis: Time Unknown

- Normal Tubular Epithelia
- Loss of Brush Border & Polarization
- Apoptosis
- Necrosis
- Bleb Formation
- Early Biomarkers: Apoptotic Blebs, Caspase
- Thrasos 184
- Vasa Recta & Luminal Neutrophil Invasion
- Release of MPO $O_2^*$ Radicals
- Formation "Muddy Brown Casts"
- Anuria
- Tubular Occlusion
- Loss of Cell Adhesion & Sloughing Epithelial Cells
- Cellular Regeneration
Physiologic Targets of Drug Therapy in AKI

- Restoration of Corticomedullary blood flow
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Pathologic Continuum of AKI: Neutrophilic Infiltration into Corticomedullary Junction

Time 24-36 hrs

Normal Tubular Epithelia → Loss of Brush Border & Polarization → Apoptosis

Ischemia → Re-Perfusion → Necrosis

Apoptotic Blebs → Bleb Formation

Anti-ICAM-1
Adenosine 2a Receptor agonist M

Early Biomarkers:
Myeloperoxidase
Elastase

Vasa Recta & Luminal Neutrophil Invasion → Formation “Muddy Brown Casts”

Release of MPO O₂⁻ Radicals → Anuria

Cellular Regeneration

Loss of Cell Adhesion & Sloughing Epithelial Cells

Time 24-36 hrs: Pathologic Continuum of AKI: Neutrophilic Infiltration into Corticomedullary Junction
Post-ischemic neutrophilic infiltration into corticomedullary junction: 24-36 hrs

Neutrophil Recruitment
- ↑ TNF-α 60 minutes
- ↑ IL-1 60 minutes
- ↑ MCP-1
- ↑ ICAM-1
- ↑ E-Selectin

Vasculature Occlusion
- ↑ iNOS leukocyte recruitment
- ↑ Outer medullary leukocyte infiltration
- ↑ Expression ICAM/VCAM
- ↑ Occlusion of vasarecta
Acute Tubular Necrosis: Inflammatory Phase and Neutrophilic Infiltration at Corticomedullary Junction

- β integrins
- I-CAM-1
- E selectins
**Alpha-MSH Blocks Interstitial Neutrophilic Infiltration of the Kidney Following Reperfusion Injury**

- **Study Objective:** To determine delayed infusion of the melanocortin peptide α-MSH is able to block ischemia-reperfusion induce AKI

- **Study Methods:** BALB-C mice & Sprague Dawley rats Bilateral renal pedicles cross clamping X 40”

- Animal sacrificed at 4, 24 and 72 hours

- **Renal Function:** Serum Cr 1-6 hrs. post ischemia

- **Neutrophil infiltration:** Naphthol-Chloroacetate esterase staining
  **Number Neutrophils:** Glomeruli, cortex, outer-inner stripe medulla

Alpha-Melanocyte Stimulating Hormone Protects Against Ischemic ATN

Alpha MSH Minimizes Ischemic Changes In Outer Medulla

α MSH Block Neutrophil Infiltration in Interstitium & Glomeruli Following Ischemia Reperfusion Injury

α MSH reduces interstitial PMN infiltration at 4 & 24hrs post AKI

α MSH reduces glomerular PMN infiltration at 4 & 24hrs post AKI

![Graphs showing neutrophil infiltration in interstitial and glomerular tissues after AKI with MSH treatment.](image)

Alpha-Melanocyte Stimulating Hormone Reduces PMN Migration in Ischemic ATN

Blockade of Neutrophil Extravasation Attenuates Tubular Apoptosis Following I/R Injury

Mizuno et.al. Am. J. Pathol. Vol. 166, No. 6, June 2005
α-MSH Analogue (AP214)
Alpha-Melanocyte-Stimulating Hormone (\(\alpha\)-MSH) analogue (AP214) Blocks Sepsis-Induced AKI

Dose-Dependent Effect

Equivalent Efficacy \(\alpha\)-MSH

Doi et.al. Kid Internal. Vol-73, 1266-1274 (June (1) 2008)
Alpha-Melanocyte-Stimulating Hormone (α-MSH) analogue (AP214) Blocks Sepsis-Induced AKI

AP214 administered 6 hours after CLP

Doi et.al. Kid Internal. Vol-73, 1266-1274 (June (1) 2008)
Summary and Conclusions

- Current definitions of AKI accurately reflect clinical outcomes in aggregate but not individually.

- Use of observational studies with pre-defined Inclusion criteria CAN enrich AKI populations.

- “Functional” Biomarkers such as FST are simple inexpensive tests that incorporate physiologic renal responsiveness.

- Serial Biomarkers that identify specific “points” in the pathologic sequence of AKI will be needed for proper management of patients.

- Target organ delivery may yet be method to maximize treatment while minimizing toxicity.