Using Biomarkers in Cardio-Renal Syndromes
NGAL and Cardio-Renal Syndrome

• Cardio Renal Syndrome and its mechanisms
• AKI and its consequences
• Current issues in AKI and CRS
• The importance of an early diagnosis
• Structural versus functional biomarkers
• Practical use of NGAL in CRS
• NGAL in CRS and AKI: emerging concepts
BIOMARKERS

- Sensitive (early appearance)
- Easy to detect
- Specific (typical of organ injury)
- Correlate with severity (prognosis)
- Quantitatively describing the level of injury
- Capable to indicate treatment initiation and discontinuation
- Predicting organ recovery
- Predicting progression to CKD

Management of Cardio-Renal Syndromes
Cardio-Renal Syndrome Type 1

Hemodynamically mediated damage

Exogenous Factors
Contrast media
ACE inhibitors
Diuretics

Decreased CO

Decreased perfusion

Increased venous pressure

Toxicity
Vasocostriction

Humorally mediated damage

RAA activation, Na + H2O retention, vasoconstriction

Humoral signalling

Immune mediated damage

Cytokine secretion

Caspase activation
Apoptosis

Monocyte activation

Endothelial activation

Hormonal factors

Natriuresis

BNP

Acute Heart Disease or Procedures

Acute decompensation
Ischemic insult
Coronary angiography
Cardiac surgery

Acute Kidney Injury

Renal hypoperfusion
Reduced oxygen delivery
Necrosis / apoptosis
Decreased GFR
Resistance to ANP/BNP

BIOMARKERS
KIM-1
Cystatin-C
N-GAL
Creatinine
NGAL and Cardio-Renal Syndrome

• Cardio Renal Syndrome and its mechanisms

• AKI and its consequences
Definition of Acute Kidney Injury (AKI)

- Previously called “acute renal failure“ (ARF)
- Defined as a rapid decline in renal function
- Until recently no unified definition, leading to >30 different interpretations
- Even mild dysfunction is associated with adverse clinical outcomes

- In the New Classification new term **AKI** replaces **ARF** to represent the entire spectrum from mild to severe renal injury
Acute Kidney Injury Represents an Independent Risk Factor of Mortality


Increasing incidence
Increased RRT demand
Increasing management costs
AKI in the ICU: a further risk for mortality

* Mortality of isolated ARF has decreased from 80% in 1974 to 14% in 2003

--

Star RA; Kidney Intern (1998); 54: 1817-1831
Mortality in CRS

% Mortality

0  20  40  60  80  100

Number of failing organs

Kidney  Kidney + Heart  K + 2  K + 3

CRS
NGAL and Cardio-Renal Syndrome

• Cardio Renal Syndrome and its mechanisms

• AKI and its consequences

• Current issues in AKI and CRS:
  
  • *Is it possible to prevent AKI in HF? What would be needed?*
  
  • *How can we manage HF without harming the kidney?*
  
  • *Can we help recovery of renal function in a failing kidney?*
“Nephroprotective” drugs

- Loop diuretics
- Mannitol
- Dopamine
- ATP with MgCl
- Thyroxine
- ANFs
- Urodilatin
- Calcium channel blockers

- Prostaglandins
- Allopurinol
- Clonidine
- Vitamin E
- ILGF-I
- “Vessel dilator”
- Epidermal growth factor
- Fenoldopam - Levosimendan

Very Nice .... but nothing works
Reasons for failure of prevention measures

- AKI is a complex syndrome and multiple causative factors are involved: tackling one pathway might not be sufficient
- Different drugs have specific actions that might not cover the entire pathophysiological process
- The time window between insult and development of AKI can be different in different patients
- The way drugs are tested in laboratory may not reproduce their possible use in real life
- In the clinical settings, AKI is often diagnosed too late, when the effects of the insult become evident (WRF)
Current issues in AKI management

- Repair and differentiation
- Apoptosis
- Inflammation
- ATP depletion
- Cellular injury
- Hemodynamic

GFR

Prerenal  Initiation  Extension  Maintenance  Recovery

Days

0  2  4  6
Time windows for AKI management

- Fluids
- Drugs
- Diuretics
- Nephroprotection?

GFR (%)

Prerenal
Initiation
A
B
C

Extension
Maintenance
Recovery

Days

RRT
Biomarkers of Acute Kidney Injury

• Acute Kidney Injury and its consequences
• Current issues in Acute Kidney Injury
• The importance of an early diagnosis
RIFLE max and AKI outcomes

Days after hospital admission

P<0.001 (Log Rank)

The RIFLE criteria and mortality in acute kidney injury: A systematic review

Z Ricci¹, D Cruz²,³ and C Ronco²,³

¹Department of Pediatric Cardiosurgery, Bambino Gesù Hospital, Rome, Italy; ²Department of Nephrology, Dialysis and Transplantation, S Bortolo Hospital, Vicenza, Italy and ³International Renal Research Institute Vicenza (IRRIV), Vicenza, Italy

Increase in All-Cause Mortality with worse RIFLE Class

N=71,527 patients
Biology of AKI by Time-Zones

MOLECULAR
CELLULAR
BIOMARKER
CLINICAL

The clinical clock is always late

Multiple Timezone Organ Damage Clock Display

Ischemia/reperfusion
Toxicity
Damage
Necrosis
Apoptosis
Cell death

GFR

Delayed biomarkers for kidney injury

↑ Serum creatinine
↑ Blood urea nitrogen
Biomarkers of Acute Kidney Injury

- Acute Kidney Injury and its consequences
- Current issues in Acute Kidney Injury
- The importance of an early diagnosis
- Structural versus functional biomarkers
Clinical Continuum of AKI

Antecedents
Intermediate stage
AKI
Outcomes

Complications

Normal ➔ Risk ➔ Damage ➔ GFR ➔ Kidney failure ➔ Death

Biomarkers of structural injury
NGAL, IL-18, L-FABP, KIM-1

Biomarkers of functional injury
Serum cystatin C, serum creatinine

Devarajan, Biomarkers Med 4:265-80, 2010
Structural VS Functional Biomarkers

Biomarkers

Normal epithelium

Ischemia/reperfusion

Toxicity

Ischemia

Damage

Necrosis

Apoptosis

Cell death

\[ \downarrow \text{GFR} \]

Potential urinary biomarkers for early diagnosis of AKI

- NAG
- \( \beta_2 \text{M} \)
- \( \alpha_1 \text{M} \)
- RBP
- Cystatin C
- KIM-1
- Clusterin
- Microalbumin

NGAL

CYR-61

IL-18

OPN

FABP

NHE3

Fetuin A

Delayed biomarkers for kidney injury

\[ \uparrow \text{Serum creatinine} \]

\[ \uparrow \text{Blood urea nitrogen} \]
Structural AKI Biomarkers

• Early diagnosis of evolving AKI could result in prevention and/or earlier changes in management:
  – Prevention of disease progression either stopping harmful interventions or mitigating/avoiding exposure to the insult
  – Early therapeutic interventions designed to protect the kidney

• More accurate differential diagnosis of AKI could direct appropriate therapy of AKI (pre-renal vs renal)

• More accurate staging of AKI could help prognostic stratification and therapy of AKI
  – Serial staging of phases of AKI (evolution of the syndrome)
  – Assessment of current and future severity of injury
Using cDNA microarray as a screening technique, a subset of genes whose expression is up-regulated within the first few hours after renal injury can be discovered. (Or early ↓ GFR)
NGAL mRNA Is Markedly Induced in the Early Postischemic Kidney


Ischemic kidneys synthesize NGAL!
Induction of mouse kidney NGAL protein after unilateral or bilateral ischaemia
NGAL following cisplatin

Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery

Jaya Mishra*, Catherine Dent*, Ridwan Tarabishi*, Mark M Mitsnefes, Qing Ma, Caitlin Kelly, Stacey M Ruff, Kamyar Zahedi, Mingyuan Shao, Judy Bean, Kiyoshi Mori, Jonathan Barasch, Prasad Devarajan

71 children undergoing cardiopulmonary bypass surgery
Single ED measurement of NGAL was useful to identify AKI and to distinguish it from other morbid conditions in which creatinine was altered. NGAL was highly predictive of clinical outcomes including nephrology consultation, ICU admission and need for dialysis.
Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population

Dinna N. Cruz
Massimo de Cal
Francesco Garzotto
Mark A. Perazella
Paolo Lentini
Valentina Corradi
Pasquale Piccinni
Claudio Ronco
AKI Biomarkers

McIlroy et al, Anesthesiology 2010; 112: 998-1004
Ngal-reporter mouse was generated inserting a double-fusion reporter gene encoding luciferase-2 and mCherry into the NGAL locus. The NGAL-reporter accurately recapitulated the endogenous message and illuminated injuries in vivo in real time correlating with the level of NGAL.
No Kidney NGAL in PreRenal Mice

NGAL: Settings of application

➢ Early biomarker for ischemic and nephrotoxic kidney injury (ATN, CIN and ICU setting).

➢ Rises significantly in AKI patients but not in controls.

➢ Rises in AKI 24-48 h before the rise of creatinine

➢ NGAL levels on the day of transplant predicts delayed graft function and dialysis requirement (2–4 days later).

➢ Urine NGAL predicts the severity of AKI and dialysis requirement in children.

➢ Measurements may be influenced by coexisting variables such as systemic or urinary tract infections and pre-existing renal diseases.
NGAL applications

• NGAL is an early marker of AKI in a wide spectrum of clinical conditions:
  – after CPB in children and adults
  – in pediatric and adult ICU
  – In emergency departments
  – In trauma patients
  – In contrast induced nephropathy and toxic AKI
  – NGAL is an early predictor of clinical outcomes in AKI
  – NGAL enable us to distinguish AKI from other conditions
  – NGAL correlates with severity of AKI
  – NGAL is predictive of AKI in CRS discriminating pre-renal AKI
  – NGAL can drive therapy in heart failure patients
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RAA SYSTEM

Kidney hypoperfusion congestion

ANP - BNP SYMPATHETIC NS VASOPRESSIN
**Cardio-Renal Syndrome Type 1**

**Hemodynamically mediated damage**

- Decreased CO
- Decreased perfusion
- Increased venous pressure

**Diuretics**

**Humorally mediated damage**

- RAA activation, Na + H2O retention, vasoconstriction

**Immune mediated damage**

- Caspase activation
- Apoptosis

**Humoral signalling**

- Monocyte activation
- Endothelial activation

**Cytokine secretion**

**Natriuresis**

**Hormonal factors**

- BNP

**Acute Heart Disease or Procedures**

- Acute decompensation
- Ischemic insult
- Coronary angiography
- Cardiac surgery

**Acute Kidney Injury**

- Renal hypoperfusion
- Reduced oxygen delivery
- Necrosis / apoptosis
- Decreased GFR
- Resistance to ANP/BNP

**BIOMARKERS**

- KIM-1
- Cystatin-C
- N-GAL
- Creatinine
Time Course of worsening of renal function (Creatinine increase) in hospitalized HF patients

Gotlieb et Al, JACC 2008
CRS and Diuretics

Diuretics

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image-url" alt="Graph image" /></td>
<td>Creatinine</td>
<td>BNP</td>
<td>Diuresis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Creatinine**
  - Day 0: 0.8
  - Day 1: 1.2
  - Day 2: 1.4
  - Day 3: 1.6
  - Day 4: 1.8

- **Diuretics**
  - BNP
  - Diuresis
CRS and Ultrafiltration

Creatinine

Day 0  Day 1  Day 2  Day 3  Day 4  Day 5

Ultrafiltration

0  500  1000  1500  2000

Ultrafiltration

BNP  Ultrafiltration
HF, Ultrafiltration and NGAL

Stop Ultrafiltration «5B»

NGAL Warning

Creatinine

Day 0  Day 1  Day 2  Day 3  Day 4  Day 5

NGAL  BNP  Ultrafiltration
Body weight & volumes

Assessment of volume status

Components

• Hydration (Amount of fluid)
  – Total body water

• Space (Container size)
  – Intravascular
    • Arterial, Venous, Capillary
  – Interstitial
  – Intracellular

• Content (Composition)
  – Osmolality and tonicity
  – Electrolytes and Acid Base
BALANCE (of fluids)

Why is it so important?
Why is it so difficult?

Components of fluid balance

- How much in? Which fluid? Why?
- How much out? How? How Fast?

Infusions
Diuretics
Extracorporeal ultrafiltration
  - Blood Volume and hemodynamics
  - Fluid balance prescription
  - Fluid exchange prescription
  - Fluid balance errors
Blood Volume

Transcellular water flux

Osmolality

Interstitium

Intravascular Refilling

Starling Forces
Cardiovascular Conditions

Vascular Space

Blood Volume

Extracorporeal Uf
Blood Volume + B I V A

Uf / Refilling rate related hypotension

Overall ECFV related hypotension

Blood Pressure

Blood Volume

UF beginning

UF end
THE PATHWAY FOR FLUID BALANCE
The 5 “B” approach

- Drugs & SCUF
- Blood Volume
- Body Weight, Body Volumes
- BIOMARKERS
- B.I.V.A.
- HEART
- KIDNEY
- BALANCE
Combined BNP/ BIVA sequential measurements help to achieve adequate fluid balance status in patients with ADHF and can be used to drive a “tailored therapy,” allowing clinicians to identify high-risk patients and possibly to reduce the incidence of complications secondary to fluid management strategies.

Let’s add NGAL to prevent AKI
Diuretic Therapy in HF

1. Loop Diuretics induce an enhanced diuresis but significantly decrease eGFR and cause creatinine increase which is apparent after 72 hours as a peak. They do not improve acute kidney injury (AKI) or renal recovery after an ischemic insult.

2. We can’t make an early detection of AKI the use of serum creatinine and there would be a need of a timely diagnostic tool able to address the clinical injury while it is happening (see different clocks).

3. We need to couple the diagnosis of HF and AKI in the early phases: a kidney marker to couple with the natriuretic peptides (NGAL + BNP).

4. It would be ideal to make it available a panel including cardiac and renal biomarkers building specific pathophysiologically-based molecular profiles.

Biomarkers of Acute Kidney Injury

- Acute Kidney Injury and its consequences
- Current issues in Acute Kidney Injury
- The importance of an early diagnosis
- Structural versus functional biomarkers
- Biomarkers of AKI: molecules and meaning
- Biomarkers of AKI: emerging concepts
Biomarkers in AKI and CRS

The Kidney Injury Continuum

(Risk predictor)

(Severity of AKI)

(Early injury)

(Need of RRT)

Complications

(Recovery)

(Recovery)

(Recovery)

(Guide therapy)

(Progression to CKD)
NGAL: number of publications per year
Multiple AKI Biomarkers

<table>
<thead>
<tr>
<th>PHASES</th>
<th>POTENTIAL STUDY DESIGNS</th>
<th>STUDIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Cross Sectional/Case Control/Prospective Cohort</td>
<td>NGAL (n = 35) Cystatin C (n = 22) IL-18 (n = 17) NAG (n = 15) KIM-1 (n = 14) α/π GST (n = 9) L-FABP (n = 7) Plasma IL-6 (n = 6) GGT/AlkPhos (n = 4) Netrin-1 (n = 2)</td>
</tr>
<tr>
<td>Proof of Concept (AKI vs. no AKI)</td>
<td>Nested Case Control/Prospective Cohort</td>
<td>NGAL (n = 19) Cystatin C (n = 12) IL-18 (n = 9) KIM-1 (n = 4) Plasma IL-6 (n = 4) α/π GST (n = 3) NAG (n = 3) GGT/AlkPhos (n = 3) L-FABP (n = 1)</td>
</tr>
<tr>
<td>#2</td>
<td>Prospective Validation (Hard Outcomes)</td>
<td>NGAL (n = 22) Cystatin C (n = 11) IL-18 (n = 10) KIM-1 (n = 6) Plasma IL-6 (n = 5) NAG (n = 5) L-FABP (n = 3) α/π GST (n = 1) GGT/AlkPhos (n = 1)</td>
</tr>
<tr>
<td>#3</td>
<td>Incremental Value to Known Predictors</td>
<td>NGAL (n = 1) Netrin-1 (n = 1)</td>
</tr>
<tr>
<td>#4</td>
<td>Does it Change Management (Clinical Utility)</td>
<td></td>
</tr>
<tr>
<td>#5</td>
<td>Improve Clinical Outcomes?</td>
<td></td>
</tr>
<tr>
<td>#6</td>
<td>Cost-Effective?</td>
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Randomized Clinical Trial/Prospective

**Pathophys**

**Initiation:** vasoconstriction, ATP depletion, oxidant and labile iron generation

**Extension:** apoptosis and necrosis, inflammatory response

**Maintenance:** ongoing injury, dedifferentiation, regeneration, repair

**Therapy**

**Vasodilators, ATP donors, Anti-oxidants, Fe Chelator**

**Anti-inflammatory, Anti-apoptotic, Stem cells**

**Growth factors, Stem cells, RRT, renal devices**

**Hypothesis:** Biomarkers to Guide AKI Therapy
NGAL to predict successful RRT discontinuation and independence

A graph showing the levels of N-GAL (ng/ml) over time (T12, T24, D2, D3, D4, D5, D6, D7) for different patient IDs (ID1, ID2, ID3, ID4, ID5). The x-axis represents time in days, and the y-axis represents N-GAL levels in ng/ml. The graph highlights points where RRT was stopped and restarted, as well as periods labeled as 'STOP' and 'RECOVERY'.
NGAL to predict successful RRT discontinuation and independence
Predictive value of NGAL for renal recovery from RIFLE-F

Kellum J. et al. 2010

AUC (95% CI)
- Clinical model + pNGAL: 0.80 (0.73-0.87)
- Clinical model only: 0.78 (0.71-0.85)
- pNGAL only: 0.74 (0.66-0.81)
“Added Value”: Outcome of NGAL(+) Creat(-)
“Subclinical AKI” in ICU Subjects

Haase et Al,  JACC, 2011
What can we do today based on present knowledge on NGAL?
1. Time to intervention (Preventive, Protective, Therapy if any)
2. Admission and Length of stay in the ICU (identify patients to be admitted from emergency room or dischargeable from ICU)
3. Differentiate CKD from AKI
4. Guide therapy in heart failure to prevent CRS type 1
5. Use as a trigger for interventional trials

What are the barriers to implementation of NGAL in practice?
1. Specific therapeutic plans for NGAL patterns are lacking
2. Cost/effectiveness. Awareness of importance of AKI
3. Benefit/routine/benefit vicious cycle

How can we overcome these barriers?
1. Specific interventional trials (Phases 4-5-6 of the NGAL Story)
• Identify which population
• Incidence of AKI in this population
• Identify Candidate Biomarker
• Potential intervention
• Potential outcome of interest
• Define Solid End-Points