NGAL in AKI diagnosis

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Fu Jen Catholic University Hospital

輔大醫院急重症醫學部
哈多吉 主任

Objectives

1. Describe the need for biomarkers in AKI
2. Describe the role of biomarkers in AKI
3. Discuss examples of promising AKI biomarkers

Take Home Messages

- AKI is a common problem, with serious short-term and long-term consequences
- The diagnosis of AKI is frequently delayed
- Potentially effective preventive and therapeutic measures are available, but frequently delayed due to lack of early predictive biomarkers
- Novel technologies are providing early, non-invasive, tools for the prediction of AKI

AKI: A Common, Serious Problem

- AKI is present in 5% of all hospitalized patients, and up to 30% of patients in ICUs
- The incidence is increasing at an alarming rate
- Mortality rate >50% in dialyzed ICU patients
- 25% of ICU dialysis survivors progress to end stage renal disease within 3 years

Diagnosis of AKI is Often Delayed

- Elevation in serum creatinine is the current gold standard, but this is problematic
- Normal serum creatinine varies widely with age, gender, diet, muscle mass, muscle metabolism, medications, and hydration status
- In AKI, serum creatinine can take several days to reach a new steady state
- Up to 50% of kidney function may be lost before serum creatinine even begins to rise

Biomarkers: AMI versus AKI

Period	Acute Myocardial Infarction	Acute Kidney Injury
1960s	LDH	
1970s	CPK, myoglobin	
1980s	CK-MB	
1990s	Troponin T	
2000s	Troponin I	

⇩

Multiple Therapies
50% ↓ Mortality

Biomarkers: AMI versus AKI

Period	Acute Myocardial Infarction	Acute Kidney Injury
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1970s	CPK, myoglobin	Serum creatinine
1980s	CK-MB	Serum creatinine
1990s	Troponin T	Serum creatinine
2000s	Troponin I	Serum creatinine
	⇩ Multiple Therapies 50% ↓ Mortality	⇩ Supportive Care High Mortality

Need early biomarkers of AKI for improved understanding, early treatment and better outcomes

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Role of Biomarkers in AKI

- Early prediction and diagnosis of AKI (before increase in serum creatinine)
- Identify the primary location of injury (proximal tubule, distal tubule, interstitium, vasculature)
- Pinpoint the duration (Prerenal, AKI, CKD) and severity
- Identify the etiology of AKI (ischemic, septic, toxic, combination)

Devarajan, Semin Nephrol 27:637-651, 2007
Devarajan, Contrib Nephrol 160:1-16 , 2008

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Role of Biomarkers in AKI

- Differentiate from other types of kidney disease (UTI, glomerulonephritis, interstitial nephritis)
- Predict the outcome (need for RRT, length of stay, mortality)
- Monitor response to intervention and treatment
- Expedite the drug development process

Devarajan, Semin Nephrol 27:637-651, 2007
Devarajan, Contrib Nephrol 160:1-16 , 2008

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Biomarkers: From Bench To Bedside

- Discovery phase (Phase 1)
  - Identification of candidate biomarkers using basic science technologies
- Translational phase (Phase 2)
  - Development of robust assays for the candidate biomarkers, and testing in limited clinical studies
- Validation phase (Phase 3)
  - Testing the assays in large clinical trials

Devarajan, Semin Nephrol 27:637-651, 2007
Devarajan, Contrib Nephrol 160:1-16 , 2008

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Discovery Phase: Promising AKI Biomarkers

- The adaptive response of the stressed kidney itself is providing us with biomarkers that inform early diagnosis, and outcomes:
  - Neutrophil gelatinase-associated lipocalin (NGAL)
  - Interleukin 18 (IL-18)
  - Kidney injury molecule 1 (KIM-1)

Devarajan, NEJM 358;3:312, 2008

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NGAL: Discovery Phase

- Neutrophil gelatinase-associated lipocalin
- First identified as a neutrophil granule protein
- Normally very small amounts in kidney tubules
- The most upregulated gene in the kidney by gene chip analysis, very early after ischemic or nephrotoxic AKI in animals

Supavekin et al, Kidney Int 63:1714-24, 2003 (ischemia)
Kieran et al, Kidney Int 64:480-492, 2003 (ischemia)
Amin et al, Environ Health Perspect 112:465-479, 2004 (cisplatin)
Yuen et al, Physiol Genomics 25:375-386, 2006 (ischemia & HgCl)
Hung et al, Food Chem Toxicol 45:1123-1130, 2007 (cisplatin)
Grigoryev et al, J Am Soc Nephrol Jan 30, 2008 (ischemia)

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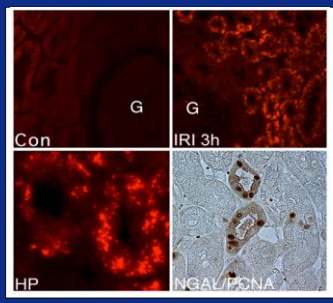
Phase 1: Kidney NGAL in Ischemic AKI



- Mouse Ischemia
- 30 min ischemia
- S creat ↑ 24 h
- Kidney NGAL ↑ 3 h
- Colocalize with PCNA (proliferating cell nuclear antigen)

Mishra et al, JASN 14:2534-43, 2003
Mishra et al, JASN 15:3073-82, 2004

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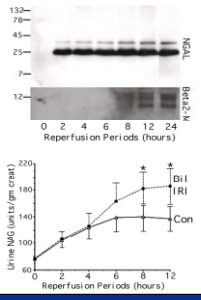
Phase 1: Urine NGAL in Ischemic AKI



- Mouse Ischemia
- 30 min ischemia
- S creat ↑ 24 h
- Urine NAG ↑ 8 h
- Urine β2M ↑ 8 h
- Urine NGAL ↑ 2 h

Mishra et al, JASN 14:2534-43, 2003
Mishra et al, JASN 15:3073-82, 2004

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Phase 2: Human NGAL ELISA

- Sandwich monoclonal ELISA for human NGAL
- Inter- and intra-assay coefficient variations 5%
- Linear relationship in the 1-1000 ng/ml range
- Excellent correlation with Western blots
- Still, only a research tool, long turnaround time, not practical in the clinical setting

Mishra et al, Lancet 365:1231-1238, 2005

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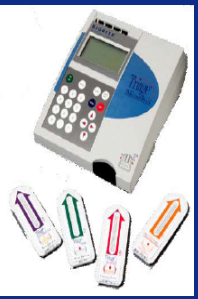
Phase 3 Transition: Plasma NGAL Kit

*TRIAGE® NGAL KIT**
Biosite Inc.

- Tests for cardiac markers
- Adapted for NGAL testing
- 15 min results - whole blood
- Compact, portable
- Simple, easy to use
- Undergoing clinical testing



** In development. Currently not for sale in US*

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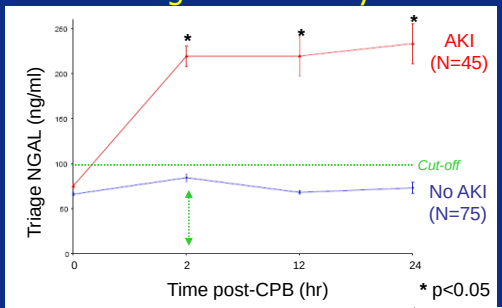
Triage Kit for Plasma NGAL in CPB: Longitudinal Study



** In development. Currently not for sale in US*
Dent et al, Crit Care 2007 Dec 10;11(6):R127

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Triage Kit for Plasma NGAL in CPB: Longitudinal Study

Using the 2 hr Triage NGAL cut-off value of 100 ng/ml for prediction of AKI

Sensitivity	100%
Specificity	75%
PPV	100%
NPV	75%
ROC AUC	0.96

** In development. Currently not for sale in US*
Dent et al, Crit Care 2007 Dec 10;11(6):R127

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Triage Kit for Plasma NGAL in CPB: Longitudinal Study

2 hr plasma NGAL values correlated with:

Creatinine change	(r=0.46, p<0.001)
Duration of AKI	(r=0.57, p<0.001)
Length of stay	(r=0.44, p<0.001)
Mortality	(r=0.48, p=0.004)

** In development. Currently not for sale in US*
Dent et al, Crit Care 2007 Dec 10;11(6):R127

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Phase 3 Transition: Urine NGAL Platform

- Abbott Diagnostics
- ARCHITECT: Standardized clinical platform

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Phase 3 Transition: Urine NGAL Platform

- NGAL Immunoassay* for ARCHITECT platform
- Results in 30 minutes with 150 μl urine
- No processing needed
- Undergoing multicenter clinical testing

** In development. Currently not for sale in US*

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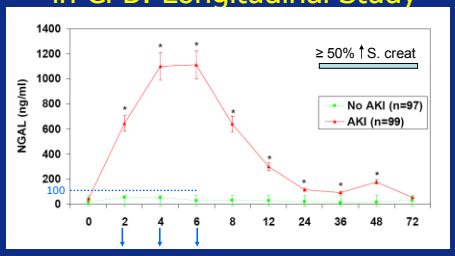
ARCHITECT Assay for Urine NGAL in CPB: Longitudinal Study



Sensitivity	0.82	0.91	0.89
Specificity	0.90	0.91	0.95
AUC	0.95	0.96	0.98

NGAL: an excellent early predictive AKI biomarker

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ARCHITECT Assay for Urine NGAL in CPB: Longitudinal Study

2 hr urine NGAL values correlated with:

Severity of AKI (r=0.66, p=0.001)
Duration of AKI (r=0.73, p=0.001)
Length of stay (r=0.42, p=0.001)
Dialysis requirement (r=0.48, p=0.01)
Mortality rate (r=0.53, p=0.01)

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Phase 2/3: NGAL as an Early AKI Biomarker

Biomarker Name	Cardiopulmonary Bypass (CPB)	Contrast induced Nephropathy	Sepsis or ICU Setting	Kidney Transplant (tx)
NGAL	2 hr post CPB 2 days pre AKI	2 hr post contrast 1-2 days pre AKI	2 days pre AKI	12 hr post tx 2-3 days pre DGF
(ROC AUC)	0.91-0.99	0.92	0.78	0.90
(ref)	(1-5)	(6, 7)	(8, 9)	(10, 11)

AKI = 50% or greater increase in serum creatinine from baseline
DGF = dialysis requirement within the first week after transplant

(1) Mishra et al, Lancet 2005, 365:1231-1238 (U+P)
(2) Dent et al, Crit Care 2007, 11(6):R127 (P)
(3) Bennett et al, CJASN 2008, 3:665-73 (U)
(4) Portilla et al, KI 2008, 4:465-72 (U)
(5) Wagener et al: Anesthesiol 2006, 105: 485-491 (U; AUC 0.78)
(6) Mitsnefes et al, Ped Nephrol 2007, 22:101-8 (U+P)
(7) Bachorzewska-Gajewska et al, NDT 2007, 22:295-6 (U+P)
(8) Zappitelli et al, Crit Care 2007, 11(4):R84 (U)
(9) Wheeler et al, Crit Care Med 2008, 4:1297-303 (P)
(10) Parikh et al, Am J Transplant 2006, 6:1639-45 (U)
(11) Kusaka et al, Cell Transplant 2008, 17:1-6 (P)

U=Urine P=Plasma

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NGAL as a Predictor of AKI Outcomes

Biomarker Name	Cardiopulmonary Bypass (CPB)	Intensive Care Setting	Kidney Transplant (tx)
NGAL	Predicts AKI duration, AKI severity, dialysis, death (1, 2)	Predicts AKI duration, AKI severity, and dialysis (3, 4)	Predicts AKI duration (5) and tubulitis (6)

(1) Bennett et al, CJASN 2008, 3:665-73
(2) Dent et al, Crit Care 2007 Dec 10;11(6):R127
(3) Trachtman et al, Ped Nephrol 2007, 21:989-94
(4) Zappitelli et al, Crit Care 2007 Aug 2;11(4):R84
(5) Parikh et al, Am J Transplant 2006, 6:1639-45
(6) Schaub et al, Transplant 2007, 84:104-12 (trend)

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NGAL as a Discriminator of AKI in Unselected ED Patients

- Single measurement of urinary NGAL at ED presentation distinguishes between AKI, prerenal azotemia, and CKD (AUC 0.95)
- It is also highly predictive of subsequent nephrology consultation, dialysis requirement, and ICU admission

Nickolas et al, Annals of Internal Medicine 2008, 148:810-19

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Urinary AKI Biomarkers: Confounding Factors

Marker	UTI	CKD	Protein-uria	PKD	SLE nephritis	IgA nephrop	Chronic allograft nephrop
NGAL	Yes	Yes	Yes	Yes	Yes	Yes	Yes
IL-18	No	No	?	?	Yes	?	?
KIM-1	No	Yes	Yes	Yes	Yes	Yes	Yes

In general, much lower amounts when compared to levels in AKI

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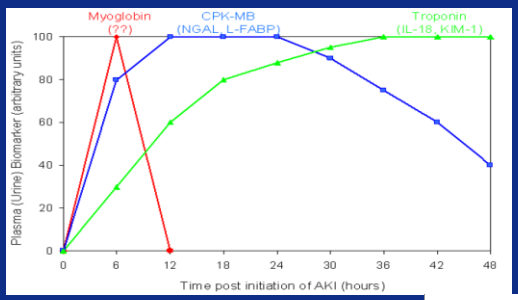
Urinary Panel for Early Diagnosis of AKI after temporally defined events



In analogy with cardiac markers

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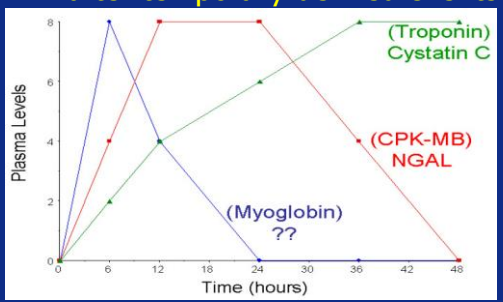
Plasma Panel for Early Diagnosis of AKI after temporally defined events



In analogy with cardiac markers

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Response to an Early Biomarker

Be Warned, Be Watchful

- Monitor intensively
- Monitor fluid balance, urine output
- Monitor blood pressure, cardiac function
- Monitor electrolytes, kidney function

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Response to an Early Biomarker

Do No Harm

- Avoid and treat hypotension
- Avoid and treat hypovolemia
- Avoid and treat oliguria
- Avoid contrast agents
- Avoid nephrotoxic medications

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Response to an Early Biomarker

Early Intervention with CRRT

- Early fluid overload
- Cytokine removal in sepsis
- Toxin removal after contrast administration

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Response to an Early Biomarker

Other Specific Therapies

Paradigm	*Before Injury*	*After Injury*
Vasodilators	Diuretics, Dopamine, Fenoldepam, Calcium Channel Blocker	ACE inhibitor, ANP
Growth Factors	IGF-1, EGF, HGF IGF-1	
Antioxidants	N-acetylcysteine	

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Take Home Messages

- AKI is a common and serious problem
- The diagnosis of AKI is often delayed
- Preventive and therapeutic measures are delayed due to lack of early biomarkers
- Novel biomarkers are providing tools for the early prediction of AKI and outcomes, and for testing therapies

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