Biomarkers in Acute Kidney Injury

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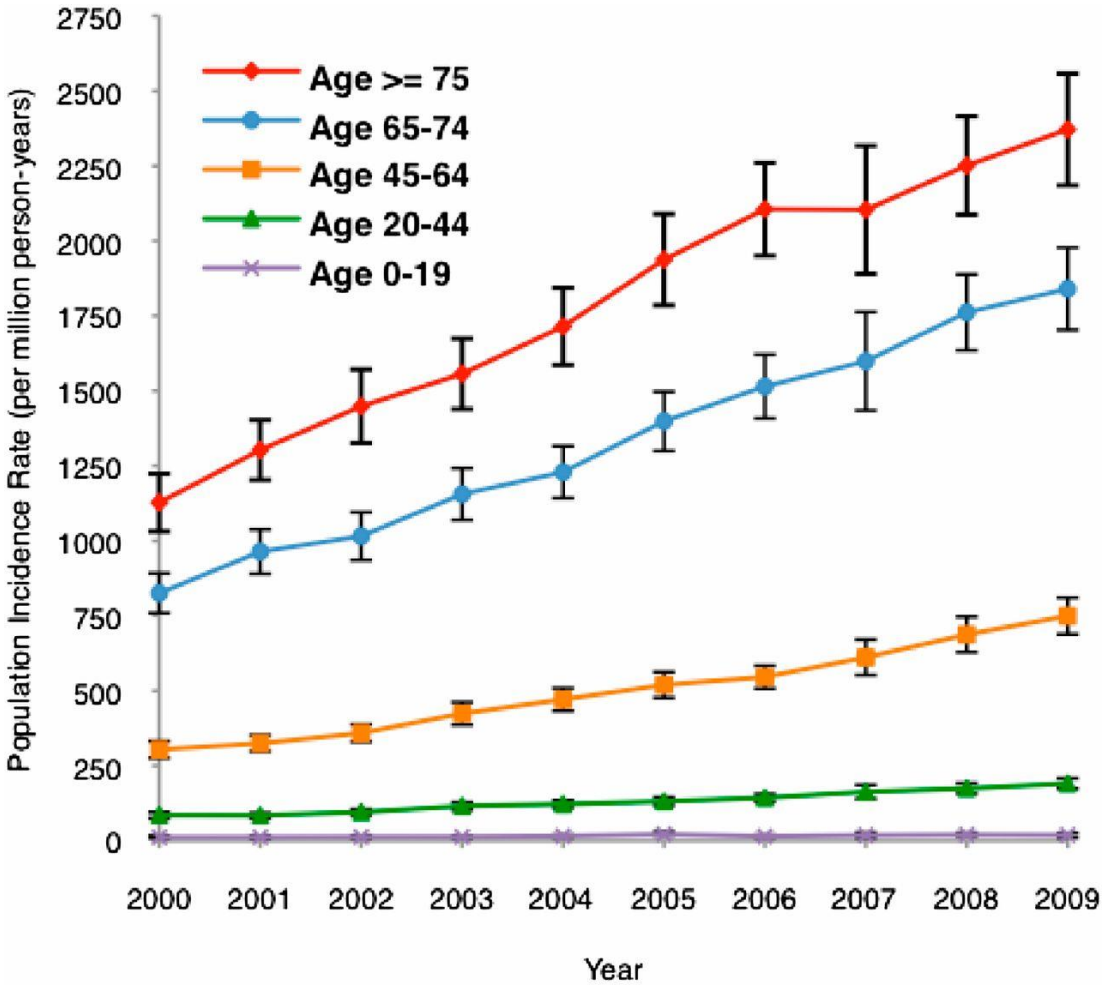
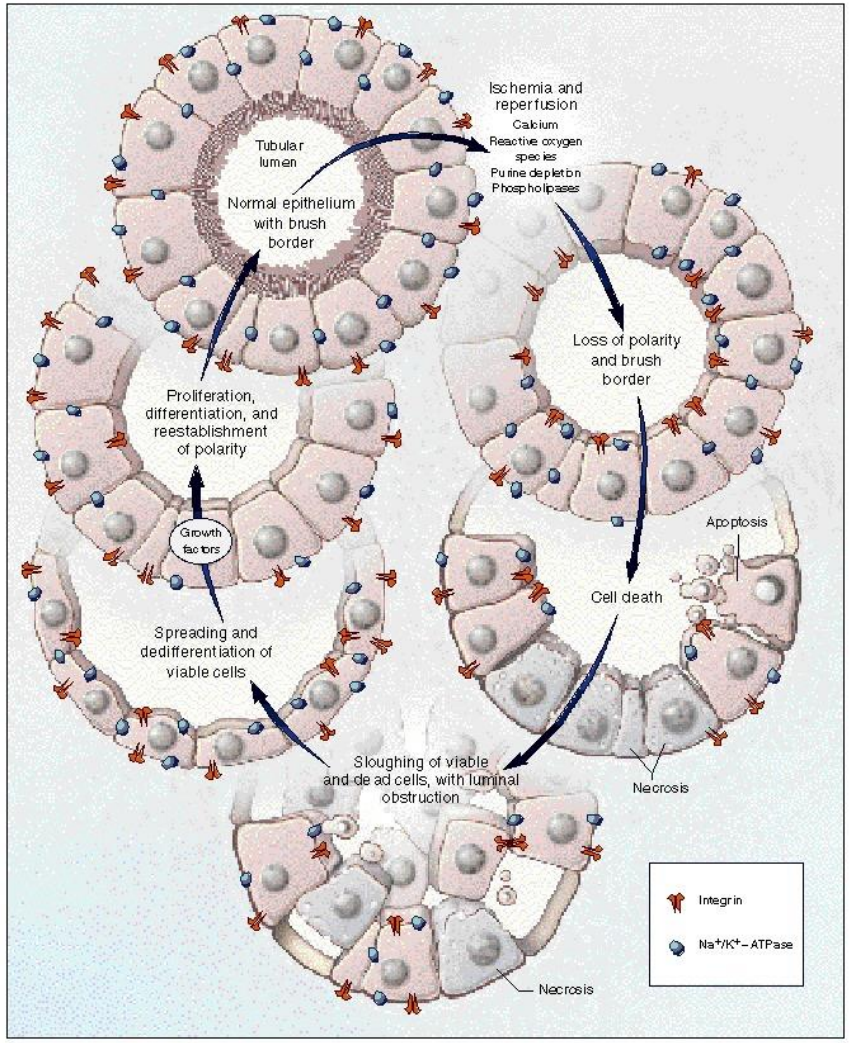
Population incidence of dialysis-requiring AKI in the United States by age groups from 2000 to 2009

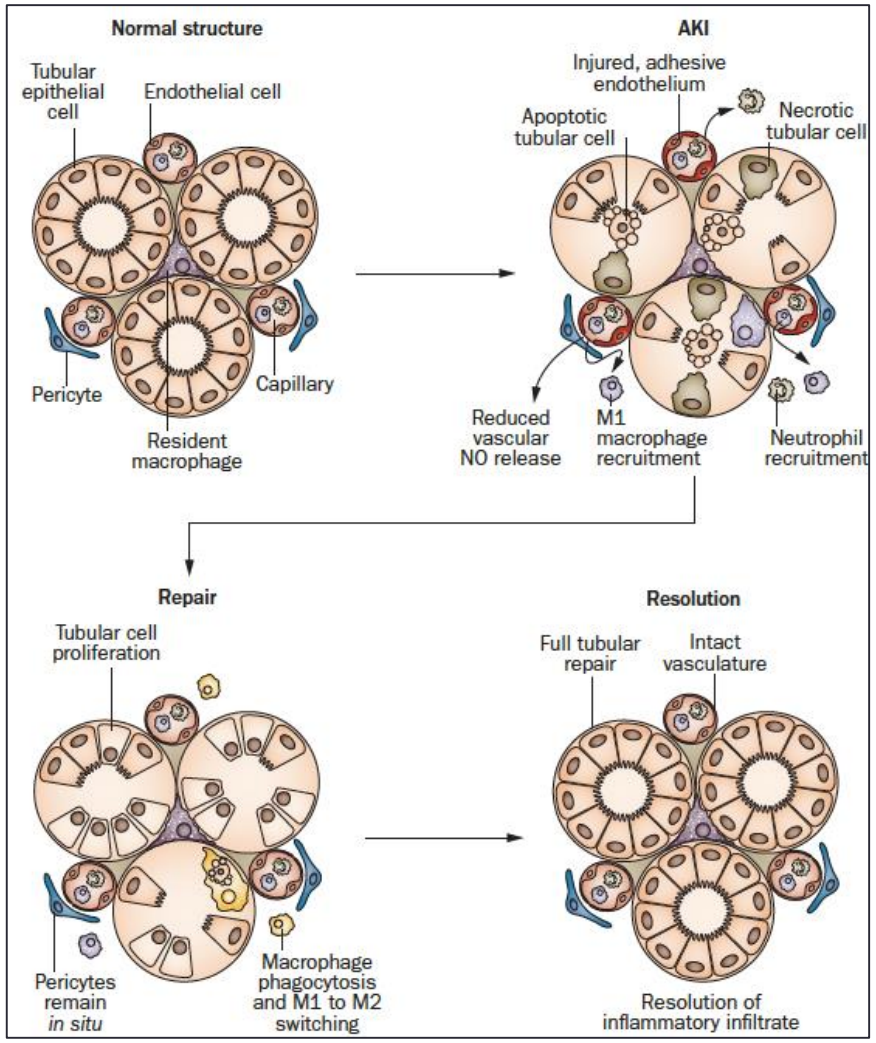


Hsu R K et al. JASN 2013;24:37-42

AKI Pathophysiology: 1996-2018



R. Thadhani, M. Pascual, JV Bonventre
N Engl J Med 334(22):1448-60, 1996

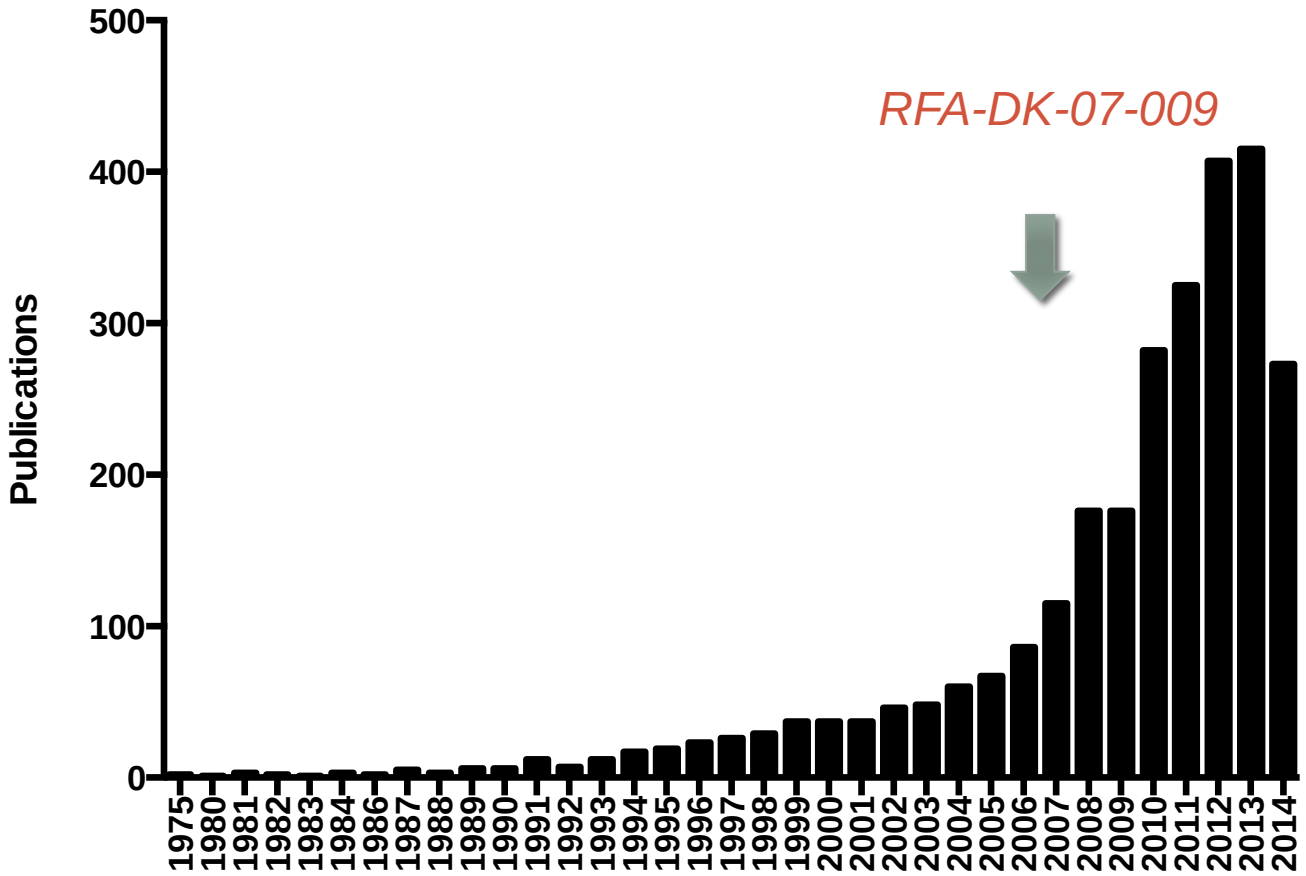


D.A Ferenbach and J.V. Bonventre
Nat Rev Nephrol 11:264-76, 2015

Shift in focus to AKI Biomarkers

"Despite some advances in our understanding of the pathophysiology and epidemiology of Acute Kidney Injury, real breakthroughs in treatment and management of the disease have not been achieved because of the lack of informative biomarkers ……"

RFA-DK-07-009 Ancillary Studies in the Natural History of Acute Kidney Injury (U01)



PubMed Search for "AKI Biomarkers" or "Acute Kidney Injury Biomarkers"

Early Pharmacological Therapies of AKI

Drug	Biological rationale	Animal experiments	Uncontrolled human data	Small RCT	Large RCT
Loop diuretics	Present	Favorable	Favorable	Negative	N/A
Low-dose dopamine	Present	Favorable	Favorable	Variable	Negative
Mannitol	Present	Favorable	Favorable	N/A	N/A
Ca antagonist	Present	Favorable	Favorable	Variable	N/A
Theophylline	Present	Favorable	Favorable	Variable	N/A
Prostaglandins	Present	Favorable	Favorable	N/A	N/A
ANP	Present	Favorable	Favorable	Variable	N/A
α-receptor antagonist	Present	N/A	N/A	Positive	N/A
Endothelin antagonist	Present	Favorable	N/A	N/A	N/A
Thromboxane antagonist	Present	Favorable	N/A	N/A	N/A
Thyroxine	Present	Favorable	N/A	Negative	N/A
Saline	Present	Favorable	Favorable	Positive	N/A
NAC	Present	Favorable	N/A	Variable	N/A
Non-ionic media	Present	Favorable	Favorable	Positive	positive

NO FDA APPROVED DRUGS FOR AKI

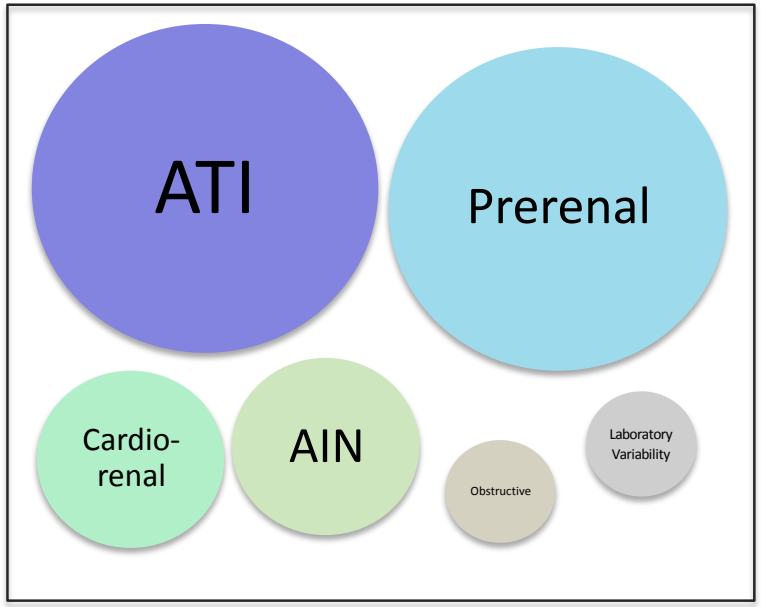
C. Ronco and R.Bellomo Nephron Clinical Practice 93:C13, 2003
ADQI 4th International Consensus Conference 2005

BARRIERS TO SUCCESSFUL CLINICAL TRIALS

- AKI is a multisystem disorder
- The lack of success due to clinical trial design:
  - low statistical power
  - lack of a consensus definition in previous trials
  - improper end points, difficulty in timely administration of the drug
  - adverse effects of the drug, and patient heterogeneity
- Lack of good animal models
- Heterogeneous population of humans with AKI
- Multifactorial insults in AKI
- The lack of in vivo pharmacokinetic and pharmacodynamics properties of drugs for the prevention of AKI in humans.
  - better understanding of the concentration of the drug at the site of action in relation to the on-target biological effect
- The lack of implementation in clinical trials of appropriate damage/biochemical markers and physiological biomarkers to identify patients at an early time point after AKI.

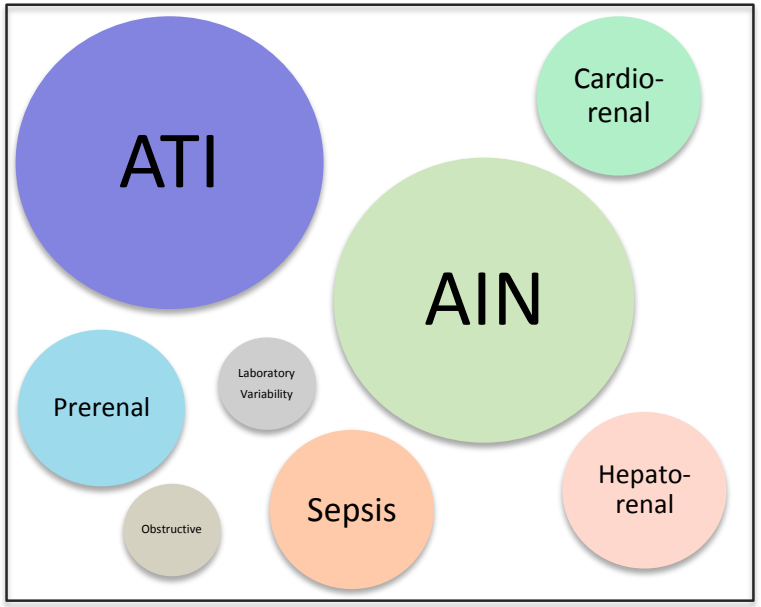
Heterogeneity of AKI by Serum Creatinine Definition

Perioperative



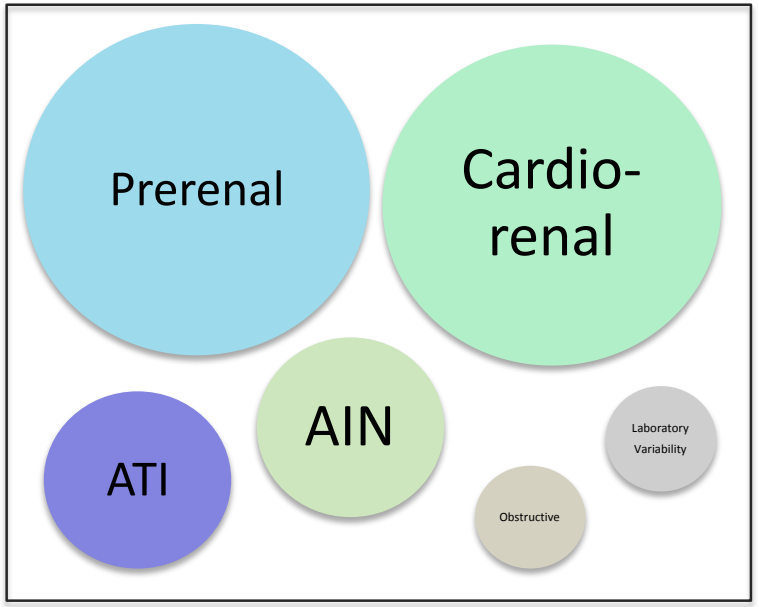
Serum Creatinine 2 mg/dl

Intensive Care Unit



Serum Creatinine 2 mg/dl

Cardiac Care Unit

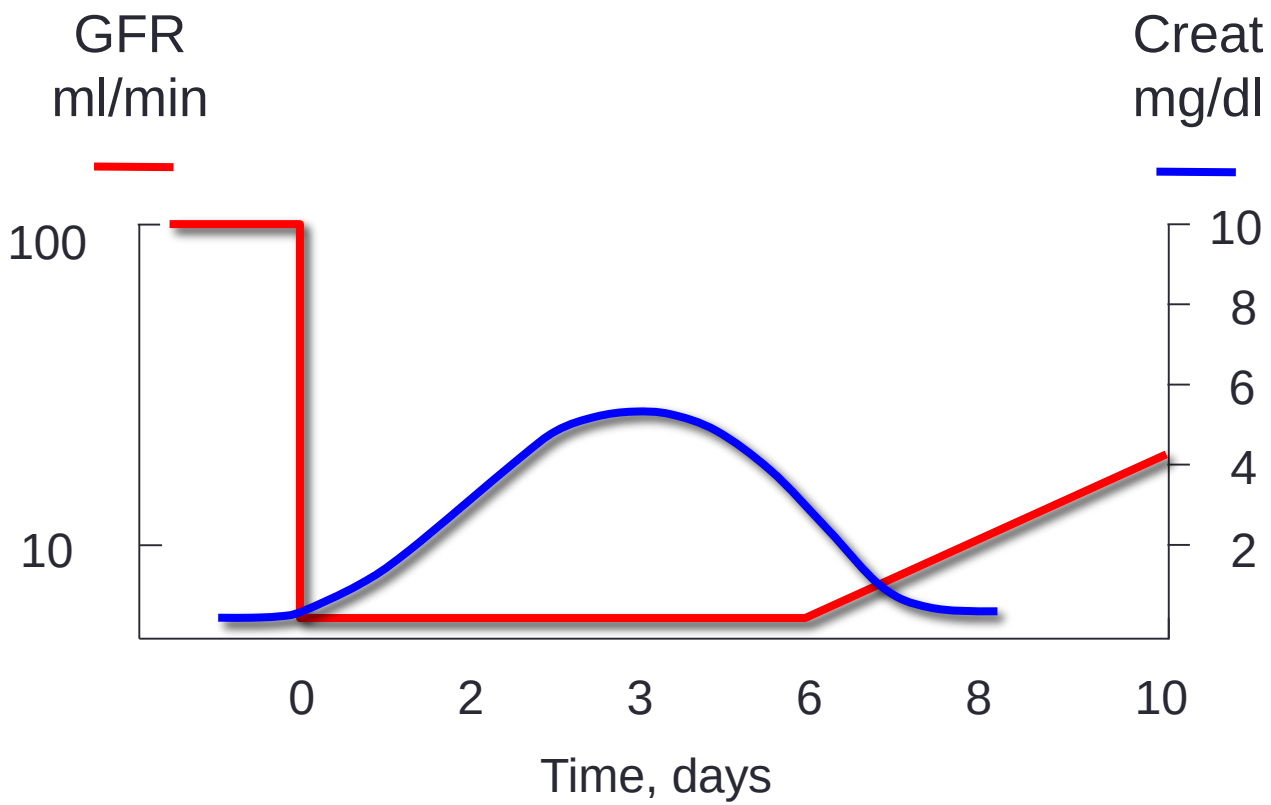


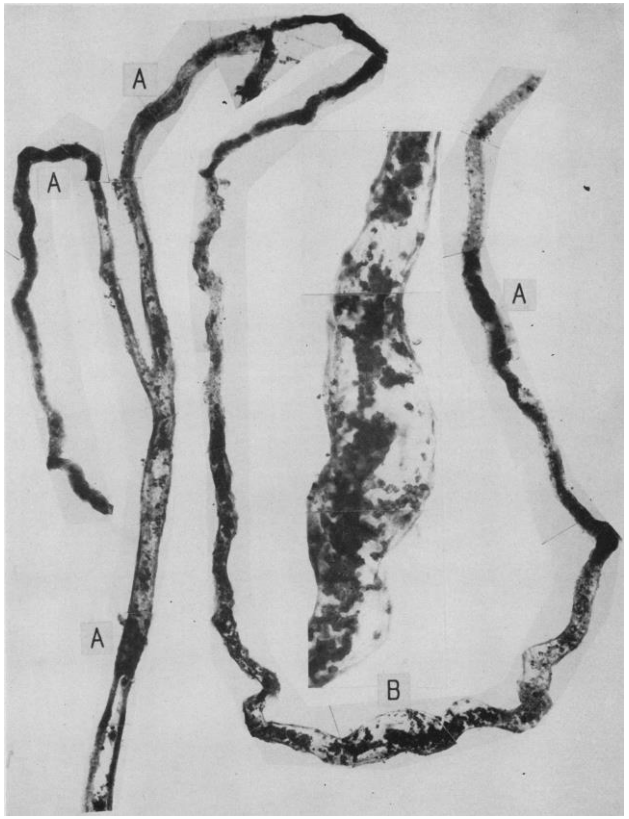
Serum Creatinine 2 mg/dl

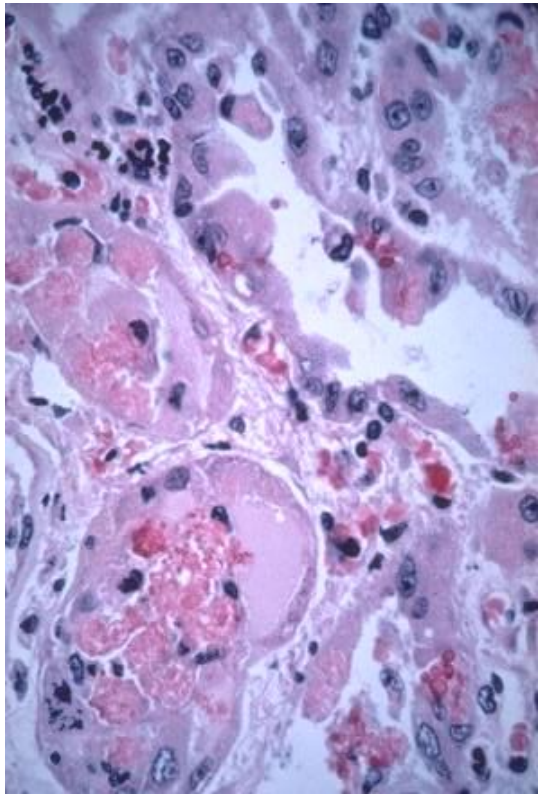
Courtesy of Chirag Parikh, Johns Hopkins U.

Biomarkers for AKI

Creatinine is a Late Marker of GFR





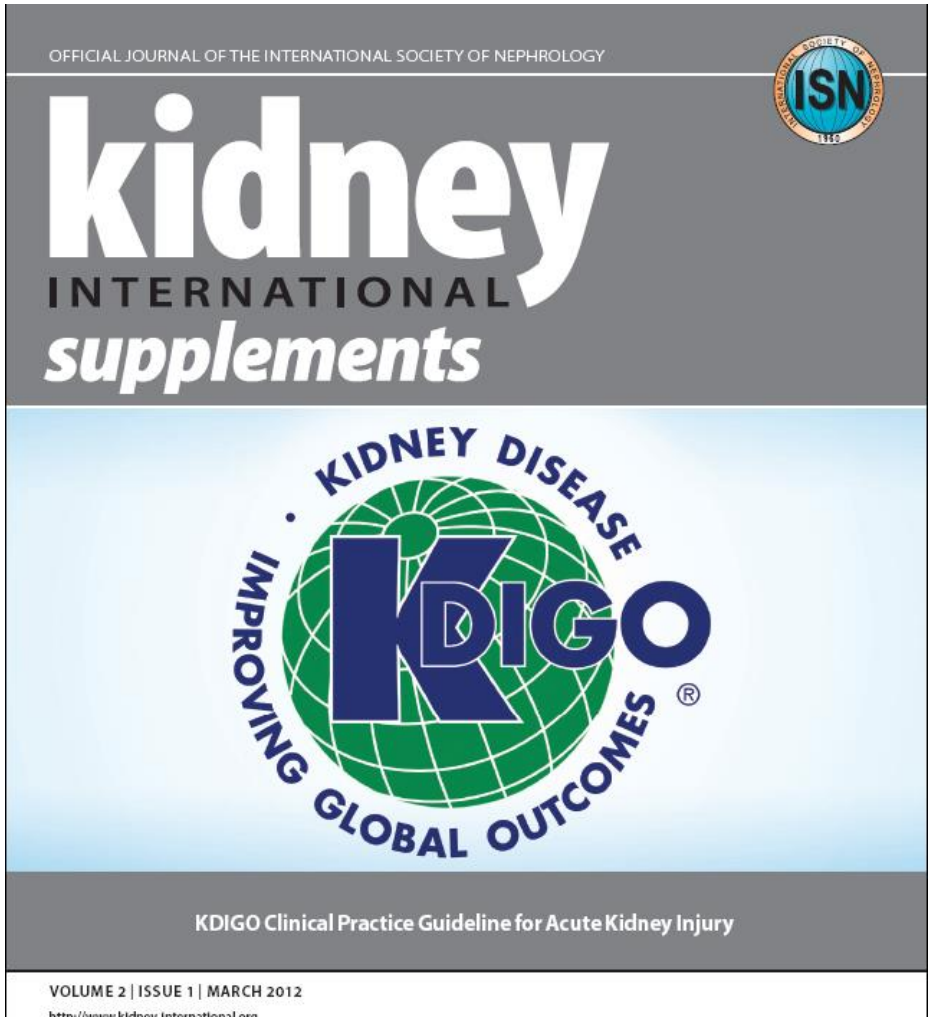


- Serum creatinine -"gold standard" for AKI Dx
- Serum creatinine can take several days to reach a new steady state
- Insensitive: More than 50% of kidney function may be lost before serum creatinine rises

J. Oliver J Clin Invest 30:1307, 1951

Courtesy of MA Venkatachalam

A standardized definition of AKI: *KDIGO*



- Increase in creatinine of ≥ 0.3 mg/dL in 48h

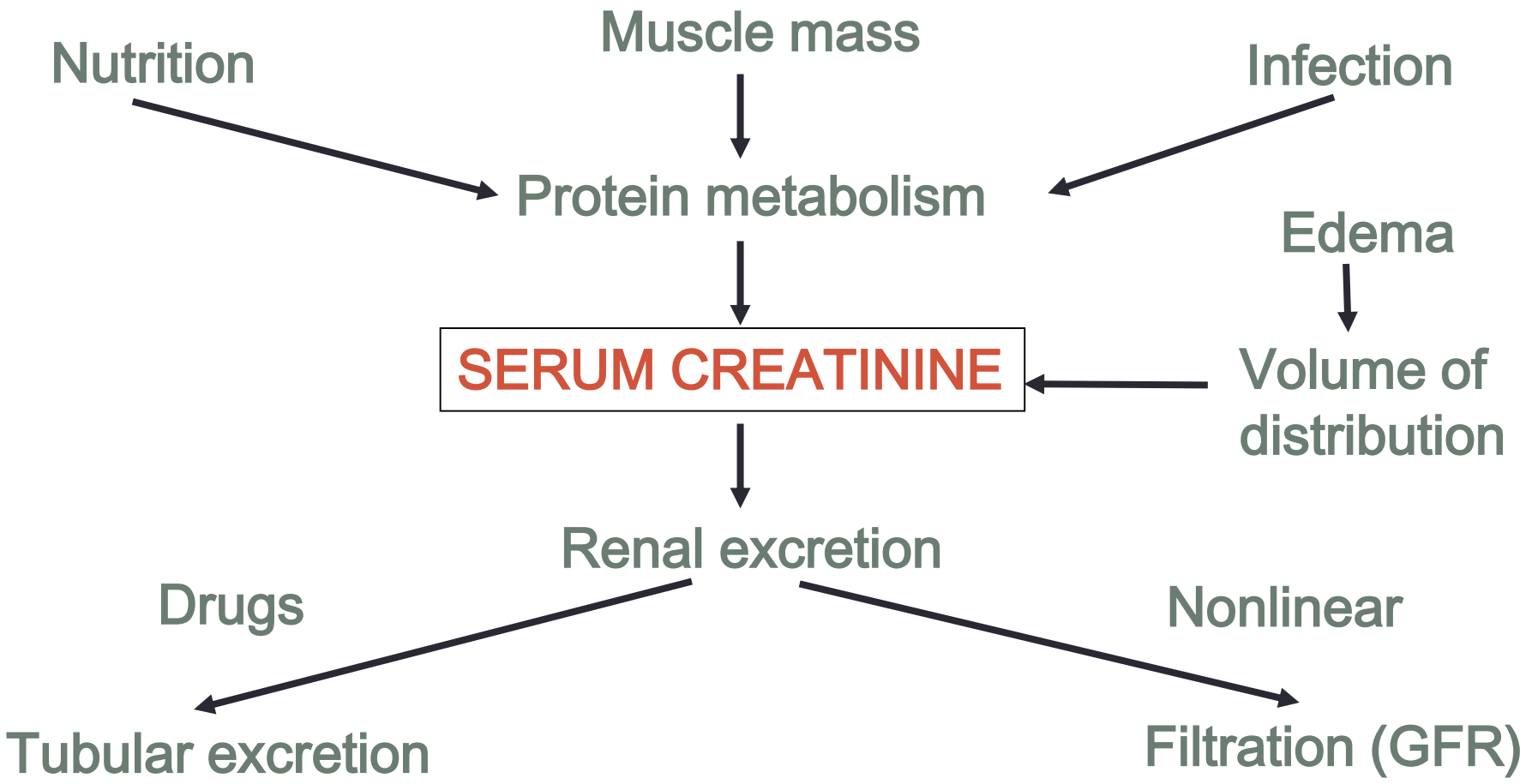
  OR

- 1.5x baseline in 7d

  OR

- Oliguria < 0.5ml/kg/h x 6h

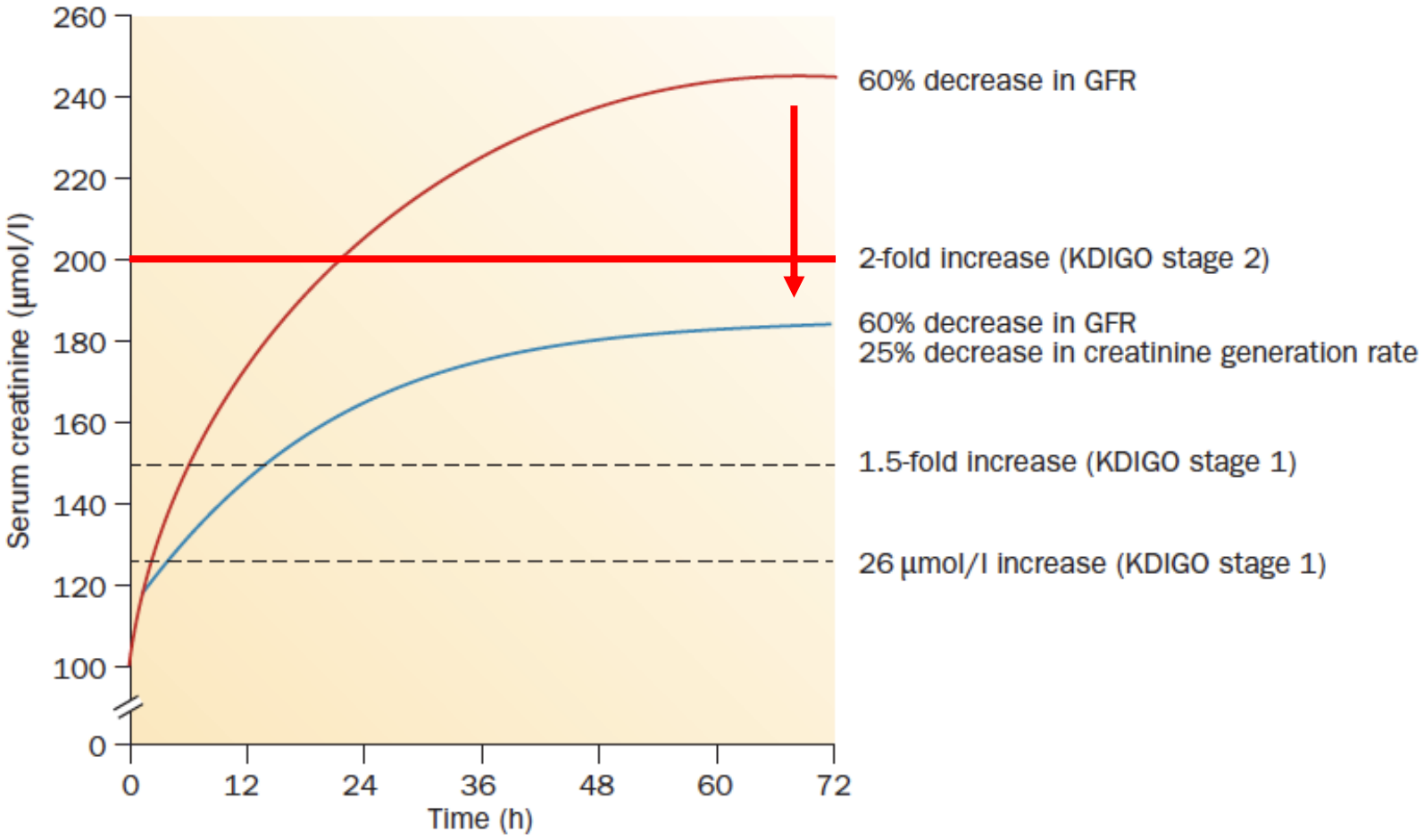
RELATIONSHIP BETWEEN SERUM CREATININE AND GFR IN AKI



Star RA, Kidney Int, 1998

Effect of Creatinine Generation on the Diagnosis of AKI

Predicted effect of a 25% ↓ in creatinine generation.



J. R. Prowle Nat Rev Neprhol , 9: 193, 2013

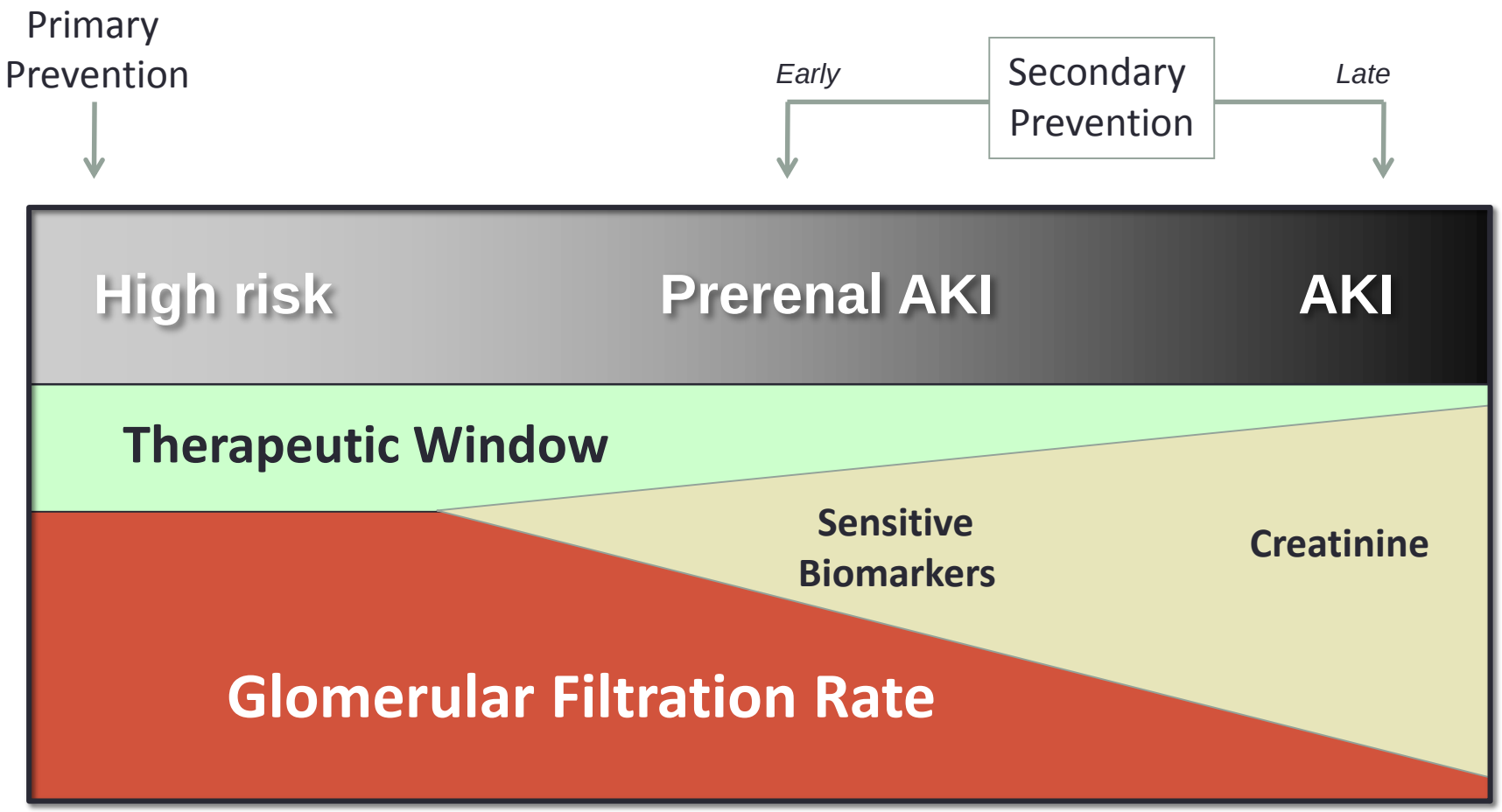
Problem with creatinine – based and urine output assessment of AKI

- Indirect reflection of kidney damage
- Many factors affect serum creatinine levels (fluid overload, age, muscle mass, infection, drugs, decrease Cr generation in AKI etc)
- Retrospective in nature, requiring 6 hrs to several days
- Insensitive (take one kidney and minimal change in creatinine)
- The urine output criteria for AKI diagnosis (<0.5 ml/kg/hr for more than 6 hours) are hindered by
  - Limited sensitivity when diuretics are administered
  - Reduced specificity in the presence of dehydration
  - Lack of practicality in measurement when an indwelling urinary catheter is not present.

Limitations which contribute to the inability to identify individuals at risk, and translate promising pre-clinical therapeutic strategies into successful treatments

AKI Diagnosis

Need for Early Diagnosis and Biomarkers

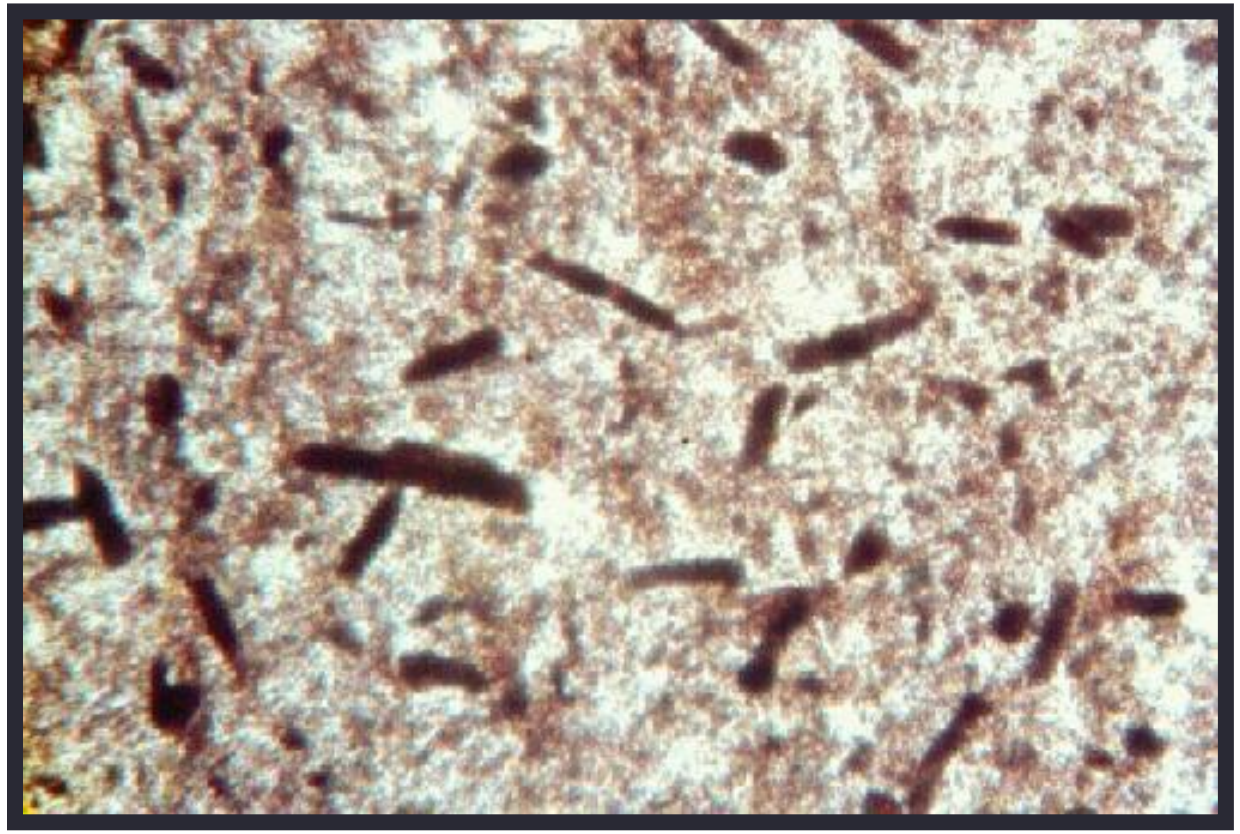


Jo, S.K. M.H. Rosner and M.D. Okusa, Clin. J. Am. Soc. Nephrol. 2: 356 – 365, 2007.

Characteristics of an Ideal AKI Biomarker

- Predict and diagnose AKI early (before increase in serum creatinine)
- Identify the primary location of injury (proximal tubule, distal tubule, interstitium)
- Pinpoint the type (pre-renal, AKI, CKD), duration and severity of kidney injury
- Identify the etiology of AKI (ischemic, septic, toxic, combination)
- Predict clinical outcomes (dialysis, death, length of stay)
- Monitor response to intervention and treatment
- Expedite the drug development process (safety)

Urine microscopy in early diagnosis of AKI



- 267 hospitalized patients with a diagnosis of AKI.
- Findings on urine microscopy led change in the initial diagnosis of
  - PRA to ATN in 27 patients (23%)
  - ATN to PRA in 15 patients (14%)

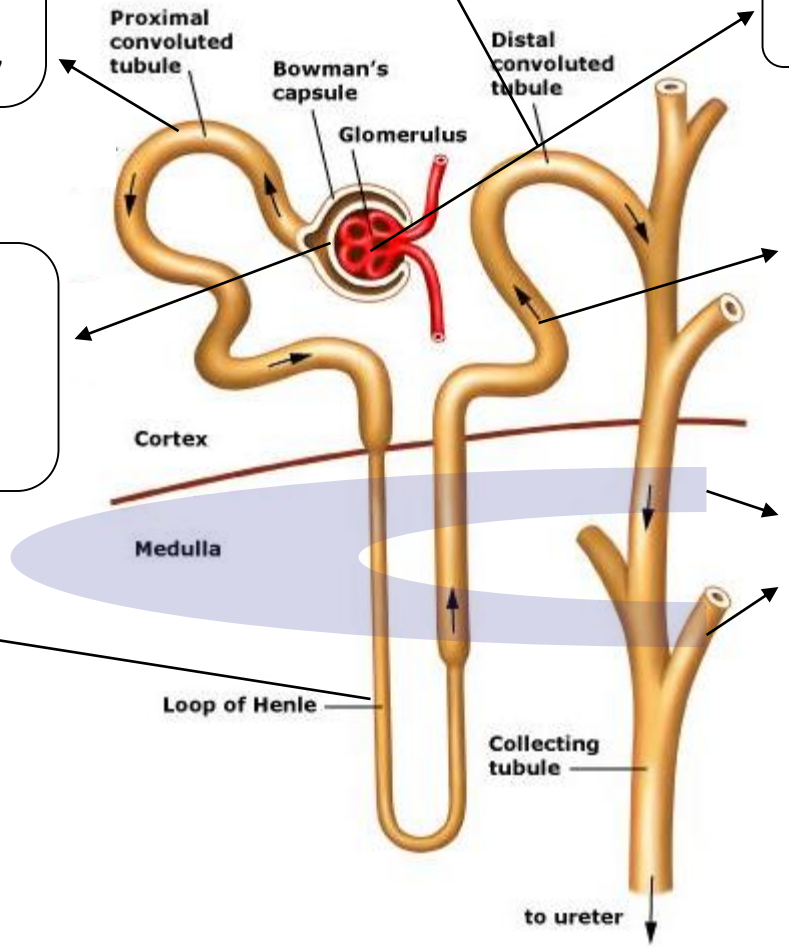
MA Perazella et al. Clin J Am Soc Nephrol 3: 1615-1619, 2008

Protein Biomarkers in Relation to Site of Injury in Nephron

Proximal Tubule Injury
- Urine IL-18
- Urine KIM-1
- Urine L-FABP
- Urine TIMP2/IGFBP7

Distal Tubule
- Urine NGAL

Glomerular Injury
- Urine albumin excretion



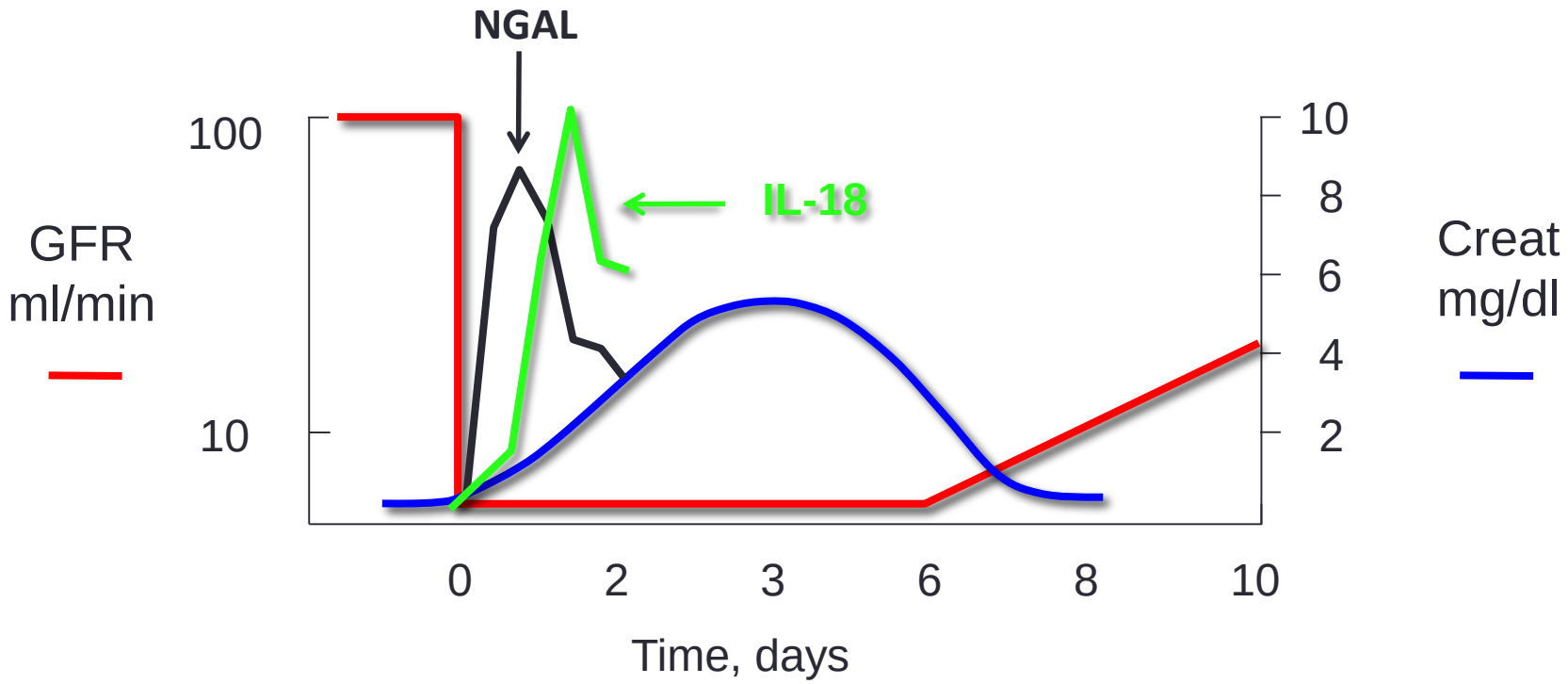
Glomerular Filtration and Proximal Tubule Function
- Serum Creatinine
- Serum Cystatin C
- Urine Cystatin C

Interstitial Fibrosis
- Urine TGF-β_1

Loop of Henle Injury
- Uromodulin

Novel Biomarkers for AKI

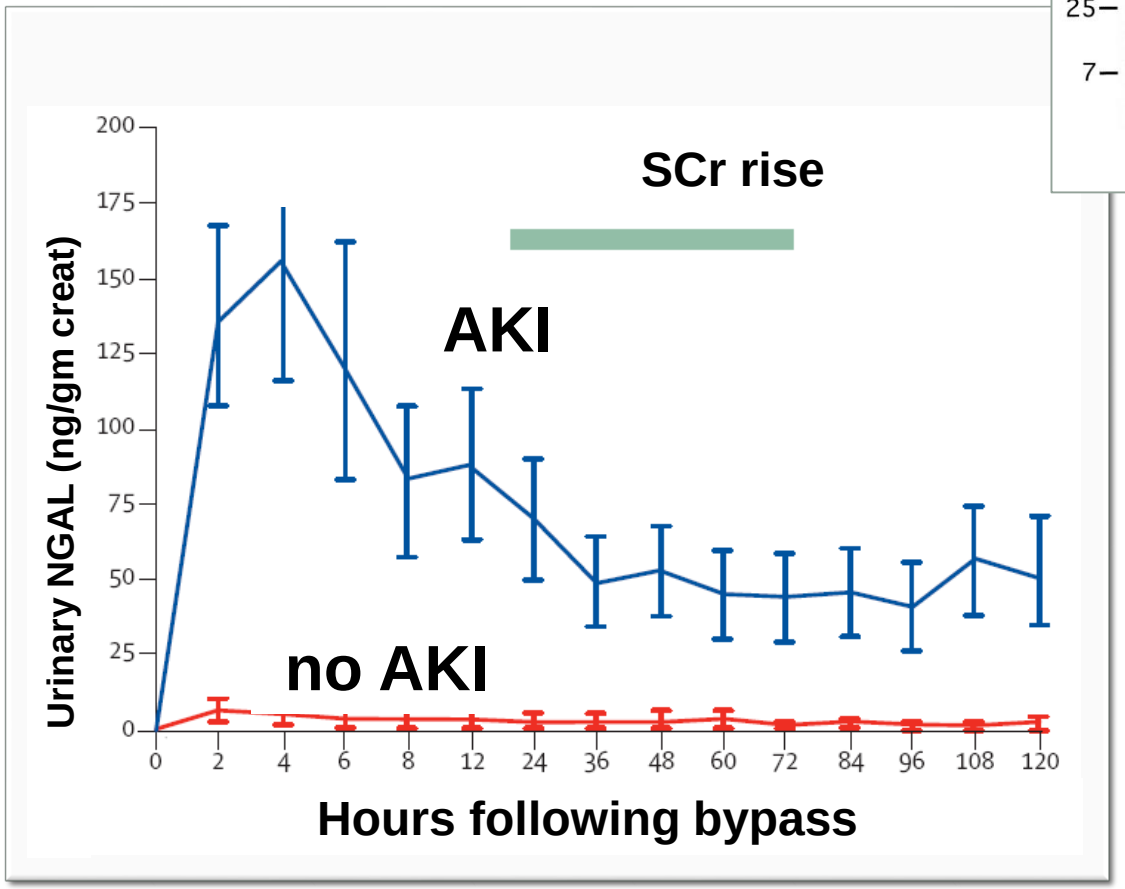
Creatinine is a Poor Marker of GFR

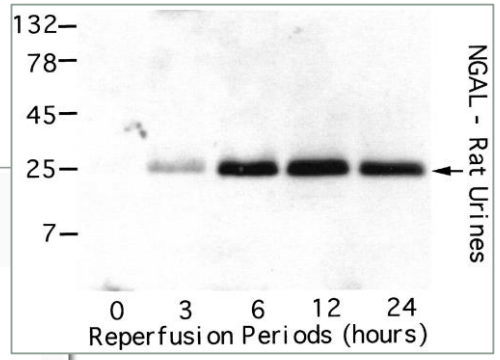


- NGAL (urine and plasma)
- KIM-1 (urine)
- L-FABP (urine)
- IL-18 (urine)
- Cystatin C (urine and plasma)
- Albumin (urine)
- NAG (urine)
- GST α- and –π (urine)
- GGT (urine)
- Beta 2-microglobulin (urine)

NGAL in Pediatric Cardiac Surgery

- 71 children undergoing cardiopulmonary bypass (20 AKI)
- Urinary NGAL at 2hrs ***nearly perfect*** in identifying subsequent AKI (increase in SCr by at least 50%):





Mishra, et al. JASN 2003

Mishra et al. *Lancet* 2005

Most Candidate Markers Being Tested Perform Well in Established AKI

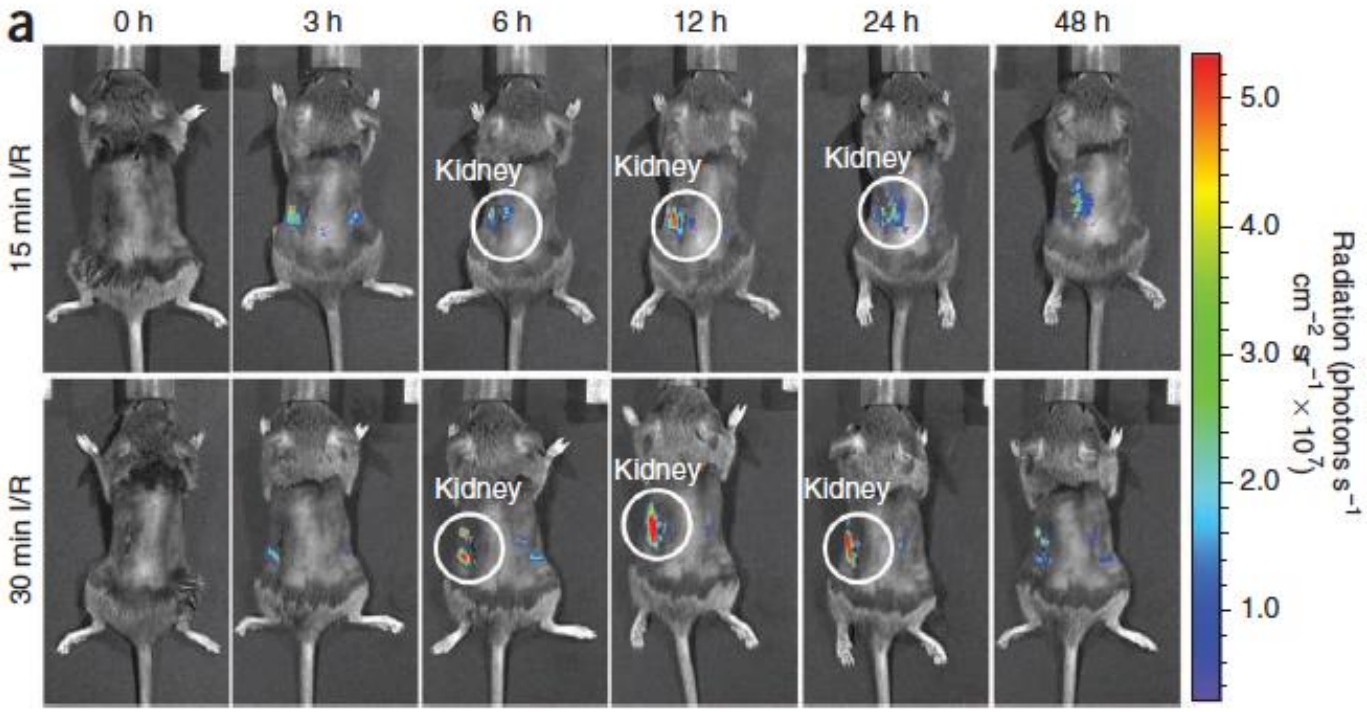
Biomarker	AUC-ROC (95% CI)	Cutoff	Sensitivity (95% CI)	Specificity (95% CI)
L-FABP (ng/mg)	0.93 (0.88–0.97)	47.1	83% (73–90%)	90% (77–97%)
NGAL (ng/mg)	0.92 (0.86–0.96)	186.8	81% (71–89%)	100% (92–100%)
KIM-1 (ng/mg)	0.89 (0.82–0.94)	1.7	77% (67–85%)	100% (92–100%)
NAG (U/mg)	0.89 (0.82–0.94)	0.007	99% (94–100%)	64% (48–78%)
IL-18 (pg/mg)	0.83* (0.76–0.89)	2.2	71% (60–80%)	90% (77–93%)

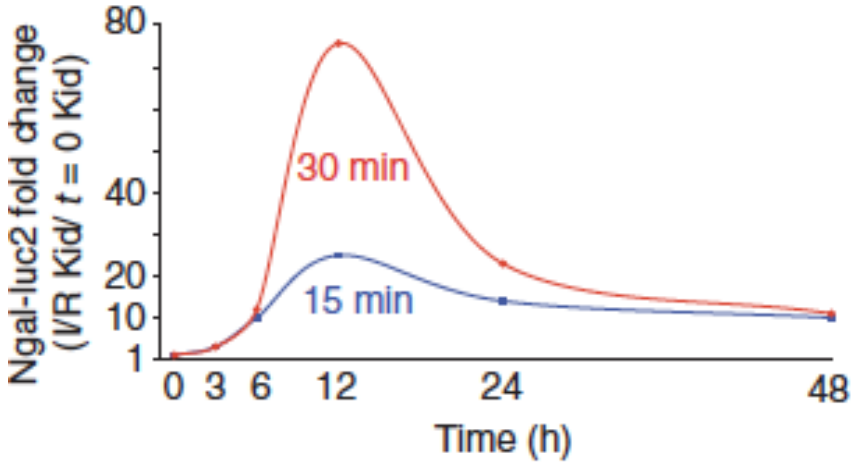
Adapted from Ferguson *Kid Intern* 77, 2010

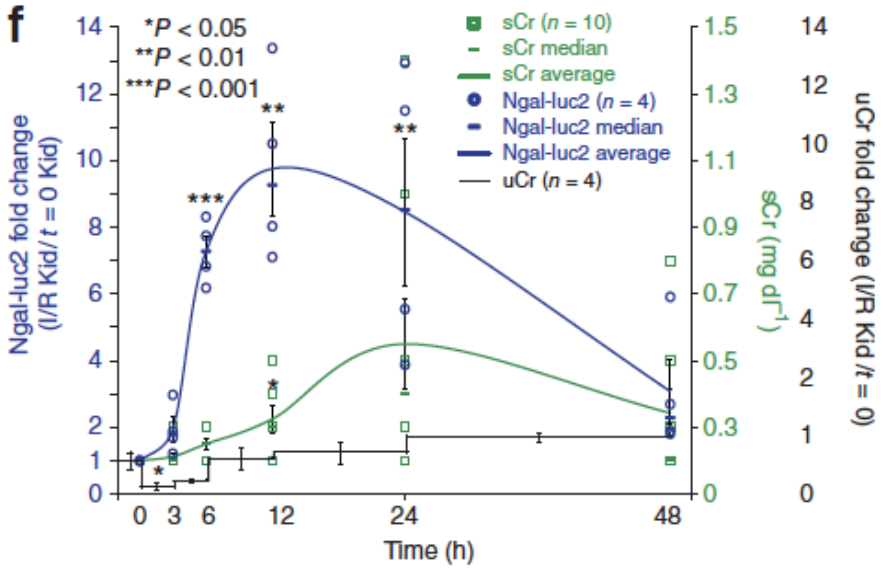
Ngal-Luc2-mC visualized kidney damage in real time *in vivo*

Mild

Severe

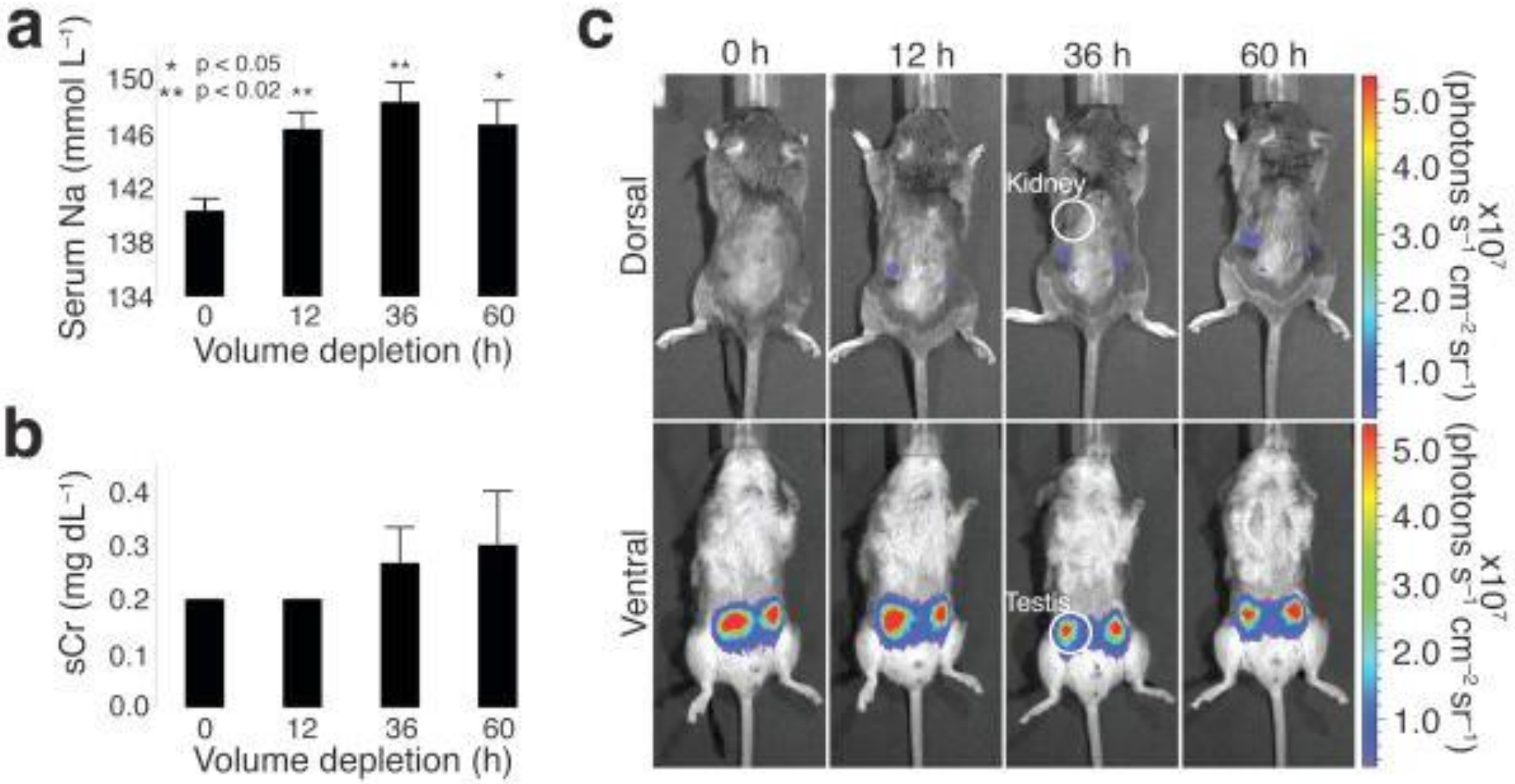






N. Paragas…J. Barasch, Nat Med. 2011 February; 17(2): 216–222

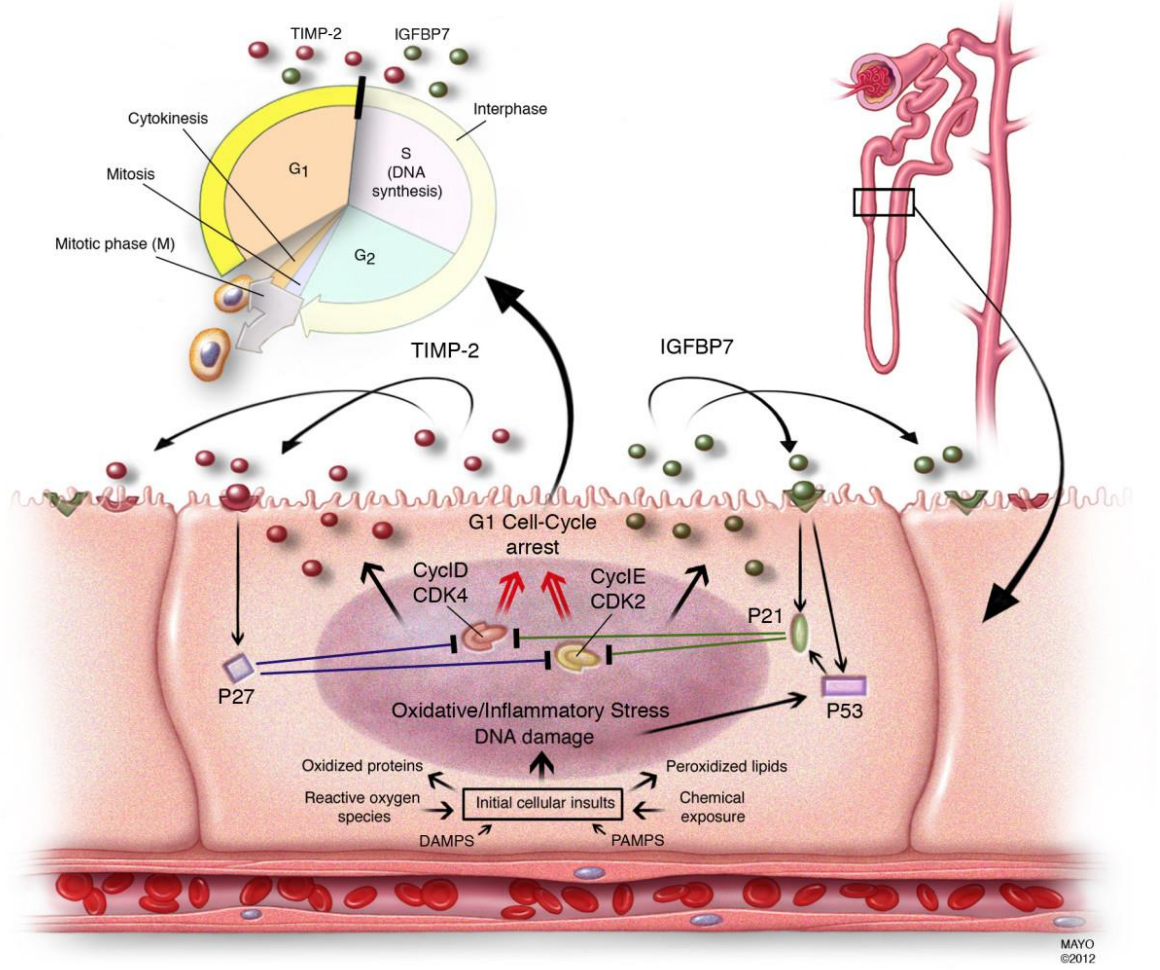
Volume depletion failed to activate Ngal-reporter expression



N. Paragas…J. Barasch, Nat Med. 2011 February; 17(2): 216–222

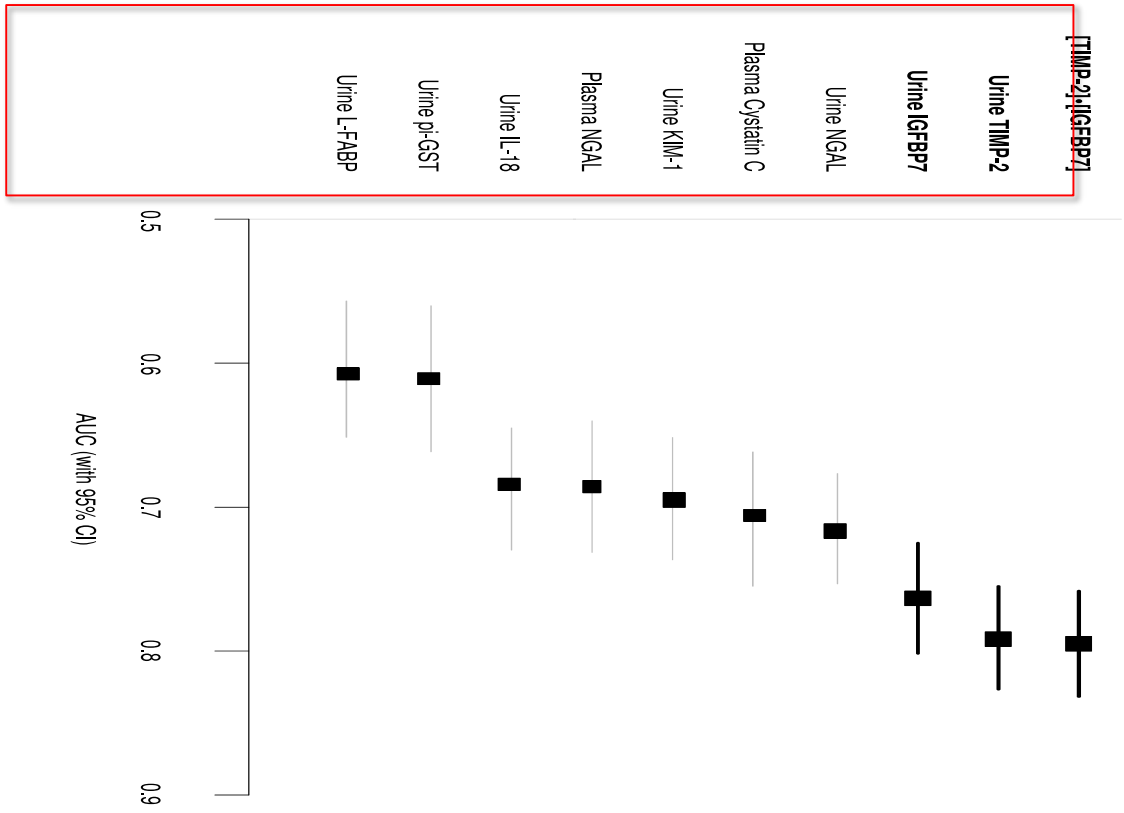
Cell Cycle Biomarkers

- Renal tubular cells enter a period of G1 cell-cycle arrest after inducing ischemia or sepsis.
- IGFBP7 and TIMP-2 - involved in G1 cell cycle arrest during the early phase of cell injury.
- The G1 cell cycle arrest may prevent division of cells with damaged DNA until the DNA damage is repaired.
- Sapphire study: AUC values to predict the development of AKI (AKIN stage 2 or 3) in critically ill patients within 12 hours were 0.76 for IGFBP7 and 0.79 for TIMP-2.
- [TIMP-2]*[IGFBP7] - higher AUC (0.80) and was significantly superior to all previously described markers of AKI.
- [TIMP-2]*[IGFBP7] significantly improved risk prediction when added to clinical scoring systems.



TIMP-2 Tissue Inhibitor of Metalloproteinase 2
IGFBP7 Insulin like growth factor binding protein 7

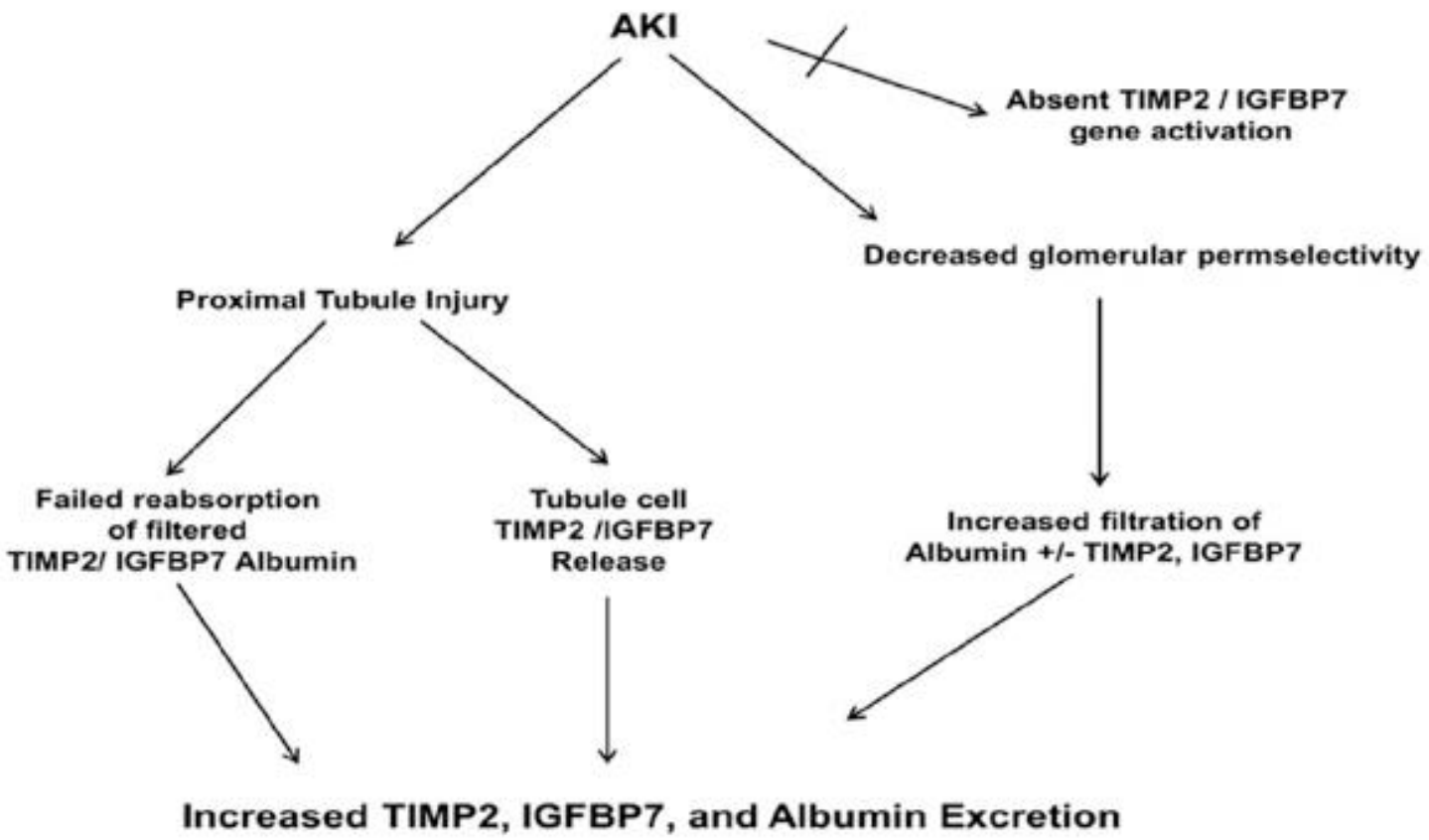
[TIMP-2]*[IGFBP] Performance



AUC from Sapphire study - endpoint (KDIGO stage 2 or 3 within 12 hours of sample collection). Samples were collected within 18 hours of enrollment

Kashani et al. Critical Care 2013 17:R25 doi:10.1186/cc12503

Mechanisms for increased TIMP2/IGFBP7 Excretion in AKI



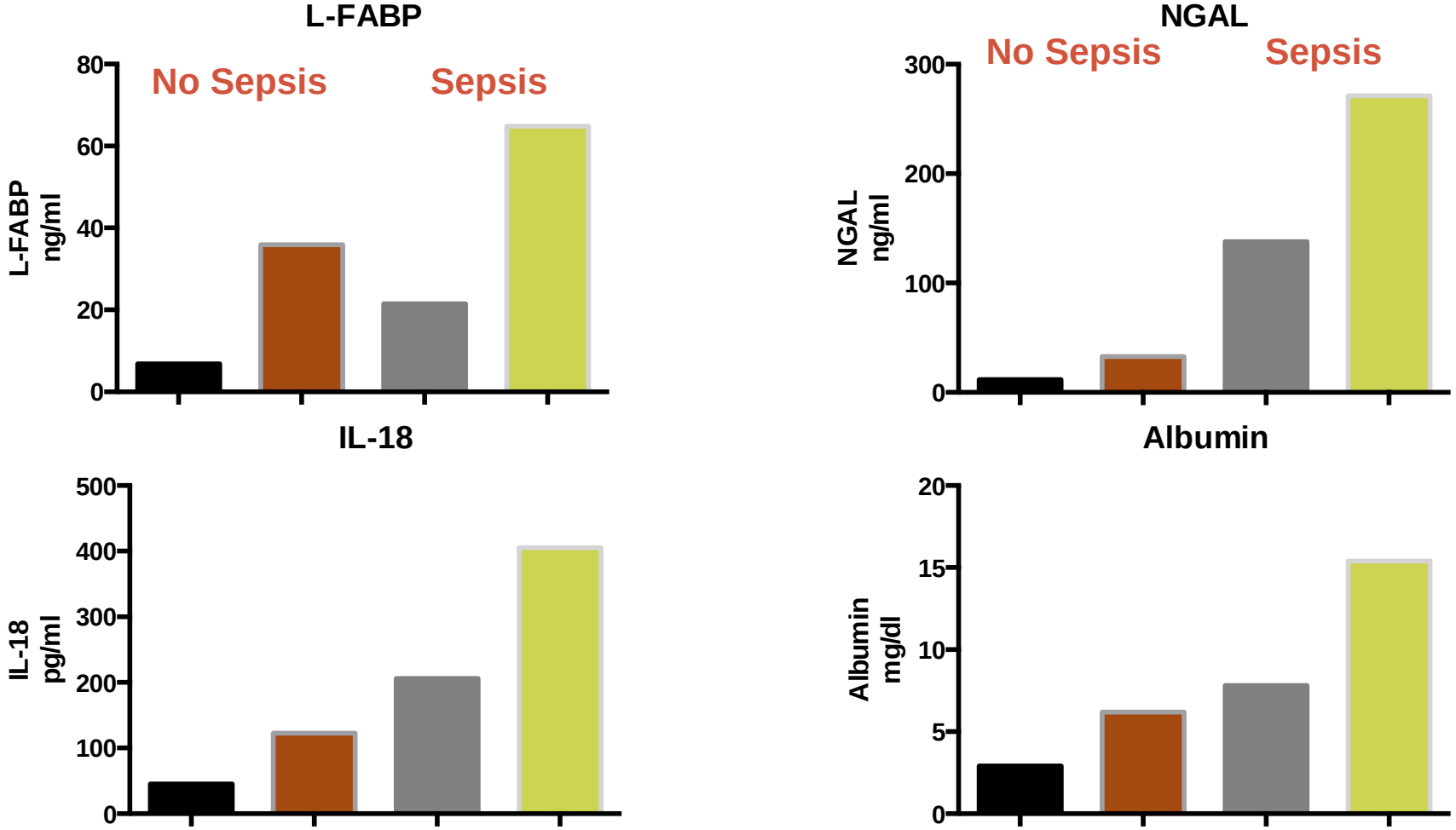
ACM Johnson and RA Zager, J Am Soc Nephrol 29: 2157–2167, 2018

Why biomarkers have not been as robust as previously demonstrated?

- Early data from single center studies
- AKI was on the basis of elevations in serum creatinine, raises the issue of a flawed outcome variable (i.e. creatinine) to analyze the performance of novel biomarkers.
- Relatively homogenous population – cardiac surgery.
- Young, healthy pediatric population vs older population with comorbidities
- Context specific cut points-thresholds vary depending on condition (septic vs nonseptic AKI)

Urinary Biomarkers and Sepsis

Sepsis Affects Threshold



Non-sepsis No AKI

Sepsis No AKI

Non-sepsis AKI

Sepsis AKI

K. Doi, E. Noiri et al
Crit Care Med 39:2464, 2011

Future of Biomarkers

Emerging diagnostic and staging criteria for AKI

	CURRENT AKIN CRITERIA	EMERGING CRITERIA
STAGE 1	Increased serum creatinine ≥ 0.3 mg/dl or 150% ≤48 hours or urine output <0.5 ml/kg/h for > 6 hours, or decrease in mGFR by 33%	+
STAGE 2	Increased serum creatinine by 200% or urine output <0.5 ml/kg/h for > 12 hours, or decrease in mGFR by 50%	++ Biomar positive
STAGE 3	Increased serum creatinine by 300% (or ≥ 4.0 mg/dl with an acute increase of ≥0.5 mg/dl) or urine output <0.3 ml/kg/h for > 24 hours or anuria for > 12 h or acute RRT, or decrease in mGFR by 66%	+++

Persistent and severe renal injury in whom rapid spontaneous recovery of renal function is improbable

Spectrum of AKI

	NO STRUCTURAL DAMAGE	STRUCTURAL DAMAGE
NO FUNCTIONAL CHANGE	Crea (-) Biomarker (-)	Crea (-) Biomarker (+)
FUNCTIONAL CHANGE	Crea (+) Biomarker (-)	Crea (+) Biomarker (+)

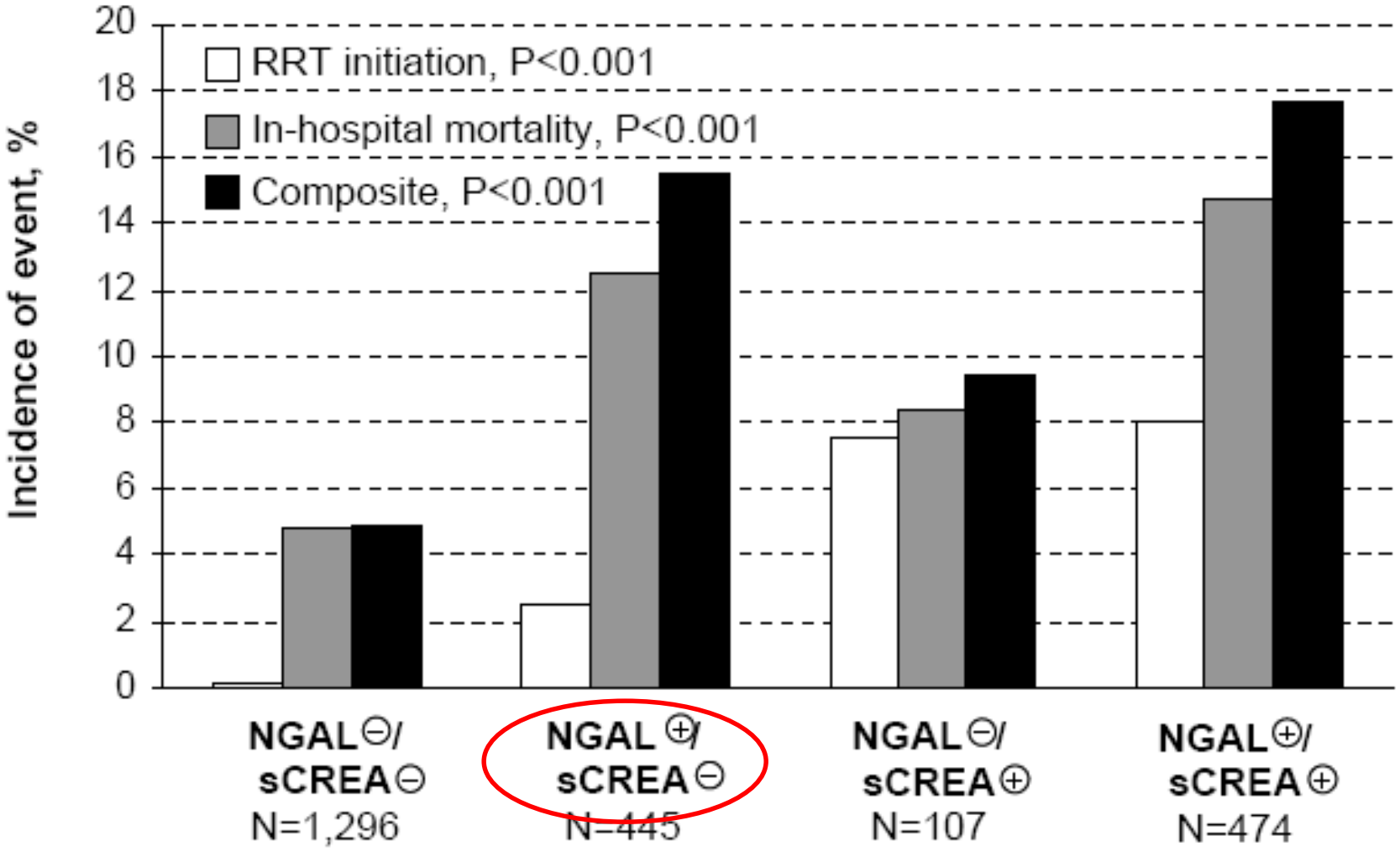
Subclinical AKI

ADQI

- Dublin, Ireland, August 30th-September 1st, 2011

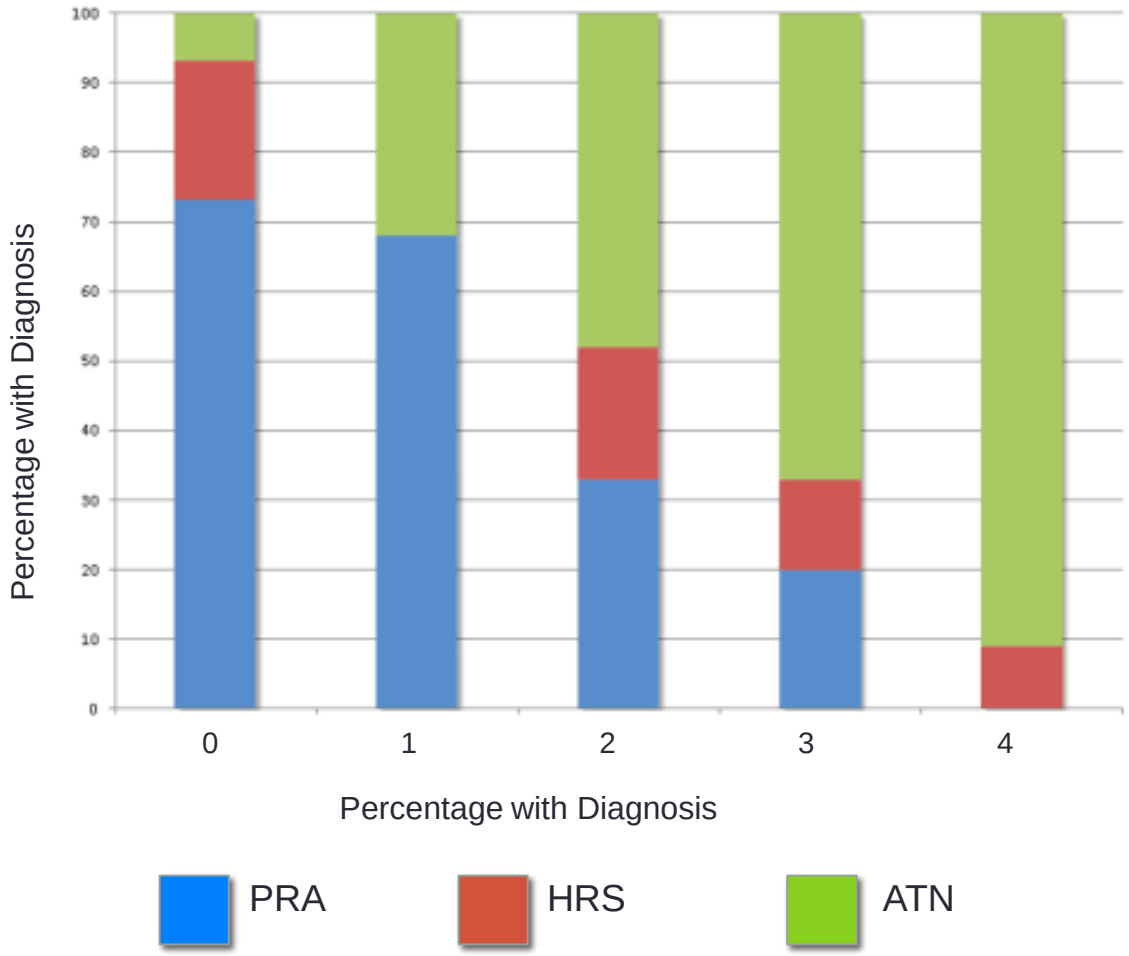
NGAL: Subclinical AKI & Clinical Outcomes

2,322 critically ill pts from 10 prospective observational NGAL studies



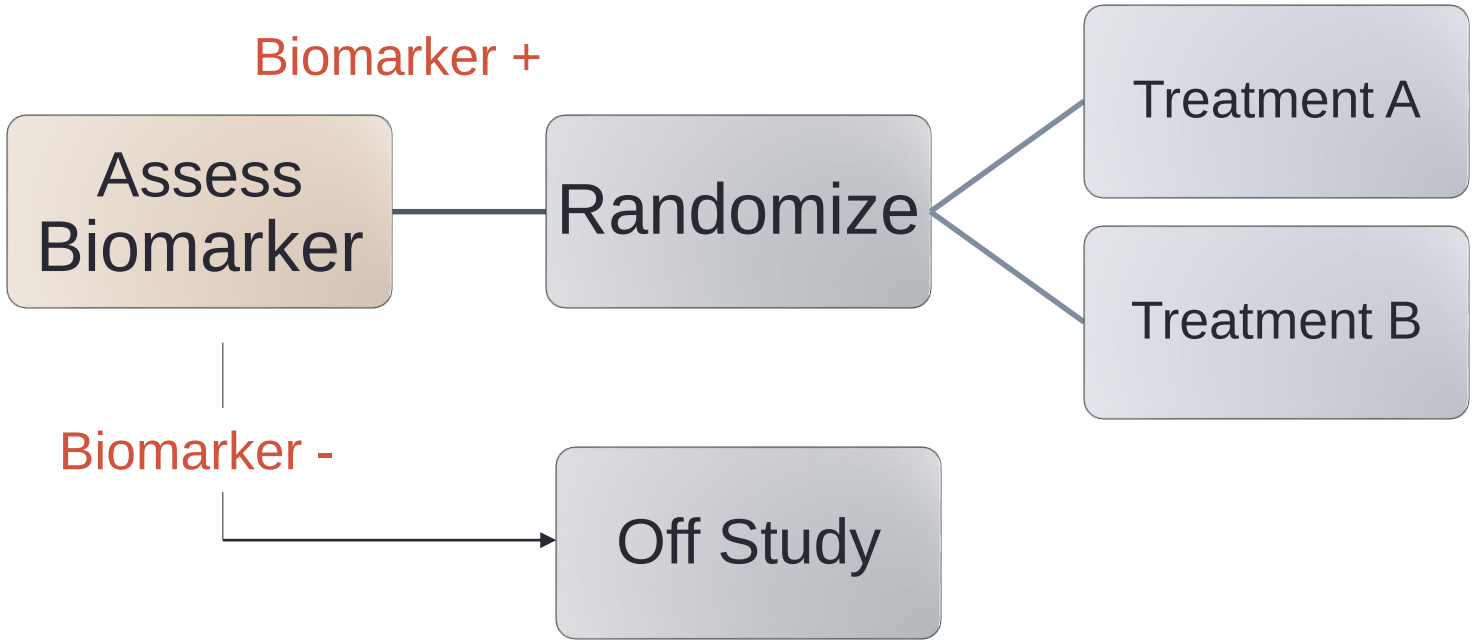
Haase. JACC 2011;57:1752-1761

Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury



J. Belcher, C. Parikh, et al. Hepatology, 60: 622-632, 26 JUN 2014

Biomarkers in Therapeutic Trial Design

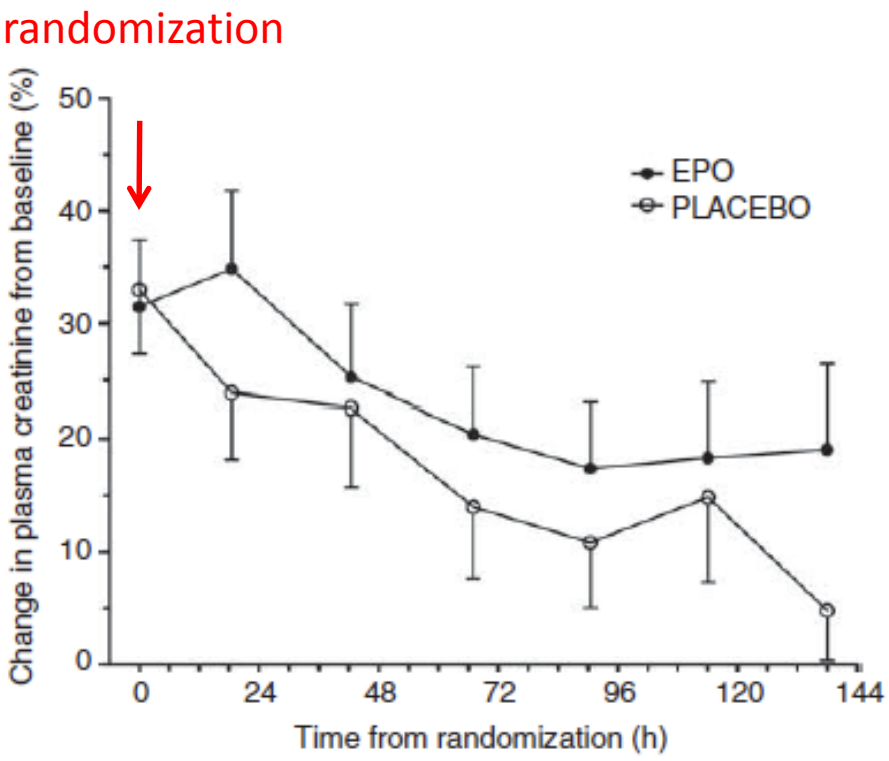


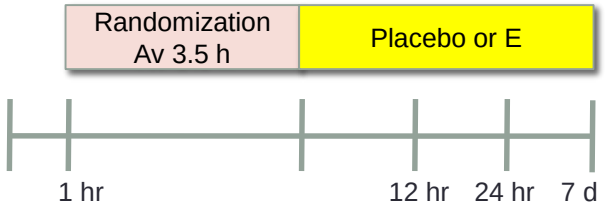
The biomarker is evaluated on all patients, but random assignment is restricted to patients with specific biomarker values

Timing in Secondary Prevention

EarlyARF Study

- Double-blind placebo-controlled trial -early treatment with erythropoietin (E) could prevent the development of AKI in ICU setting.
- Urinary levels of two biomarkers, γ-GT and AP (46.3) triggered randomization to either placebo or two doses of E
- Primary outcome of relative average plasma creatinine increase from baseline over 4 to 7 days.
- Of 529 patients, 162 were randomized within an 3.5 h of a positive sample.
- Early intervention with high-dose erythropoietin did not alter the outcome.

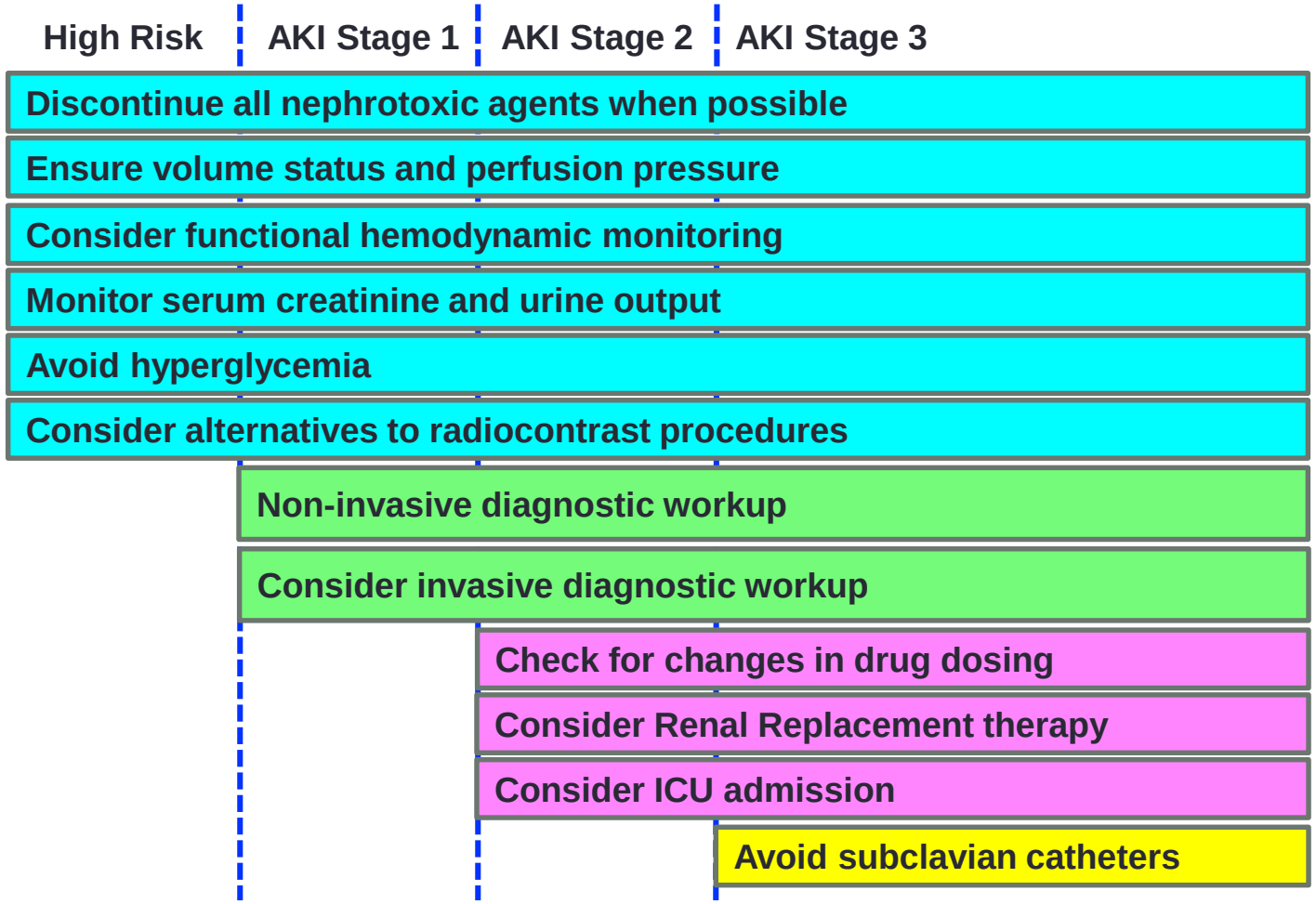




ZH Endre, et al,Kidney Int. (2010) 77, 1020–1030

Kidney Disease: Improving Global Outcomes (KDIGO) guidelines for AKI

Stage Based Management of AKI

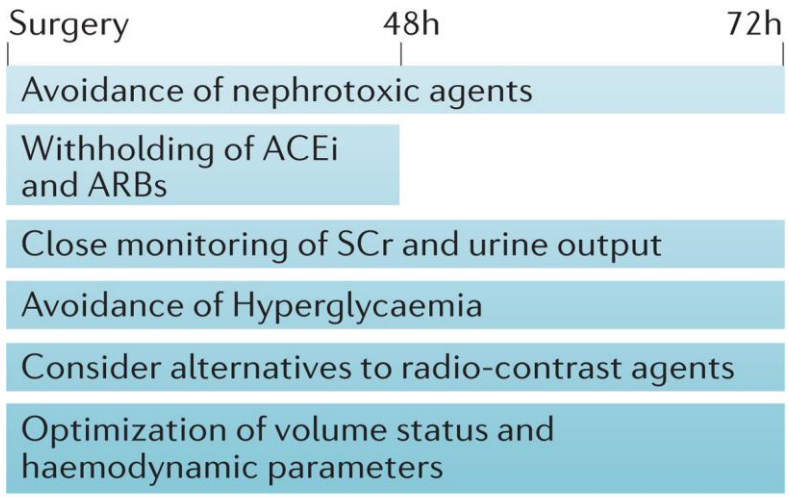


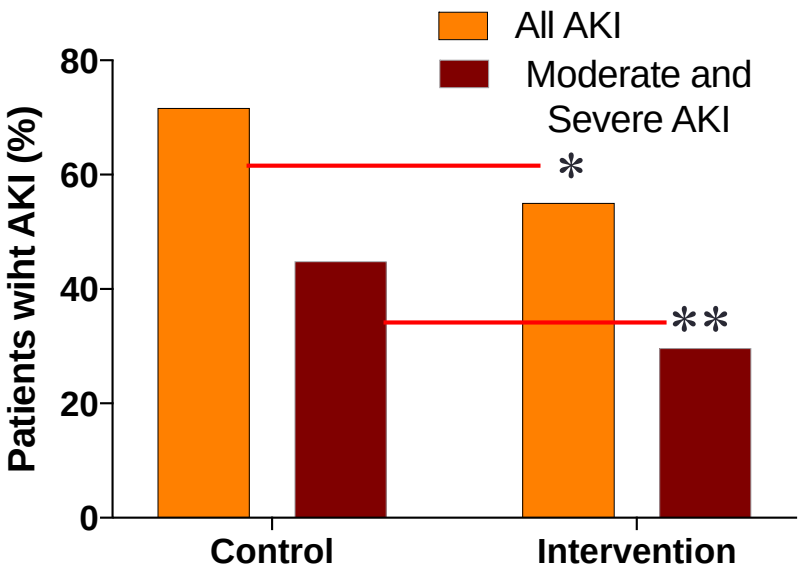
Kidney International Supplements (2012) 2, 19–36

Prevention of AKI in Cardiac Surgery in Biomarker +/ high risk patients

- Single center trial patients undergoing cardiac surgery
- randomized 276 patients identified as high risk by urinary [TIMP-2]·[IGFBP7] > 0.3
- Implementing "KDIGO bundle" resulted in
  - Hyperglycemia ↓33%
  - ACEI/ARB use ↓64%
  - Urine TIMP-2/IFGBP7 ↓31%
- Implementation of KDIGO guidelines in high risk patients compared with standard care reduced the frequency and severity of AKI.

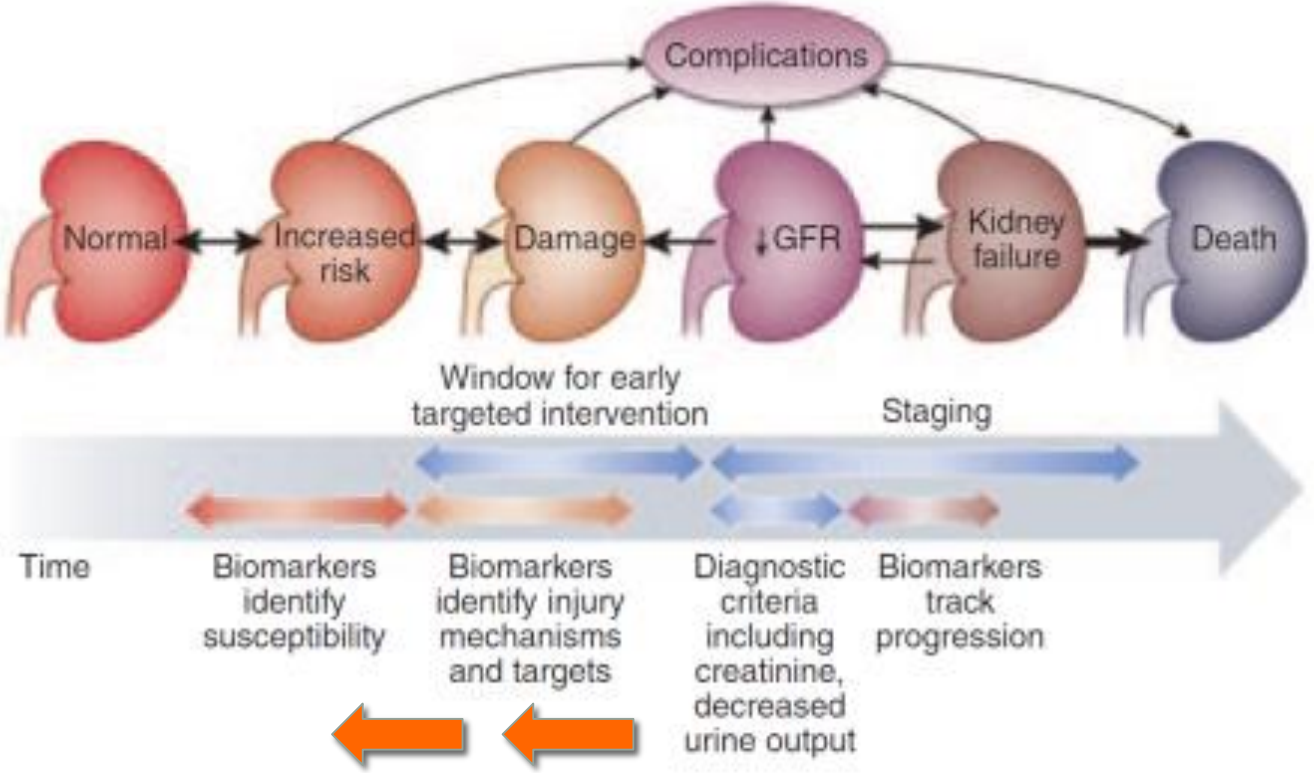
KDIGO "Bundle"





M. Meersch…A. Zarbock et al. Intens Care Med 43: 1551–1561, 2017
Kellum, J. A. *Nat. Rev. Nephrol.* Nature Reviews Nephrology 13, 140–141, 2017

Physiological Biomarkers of AKI

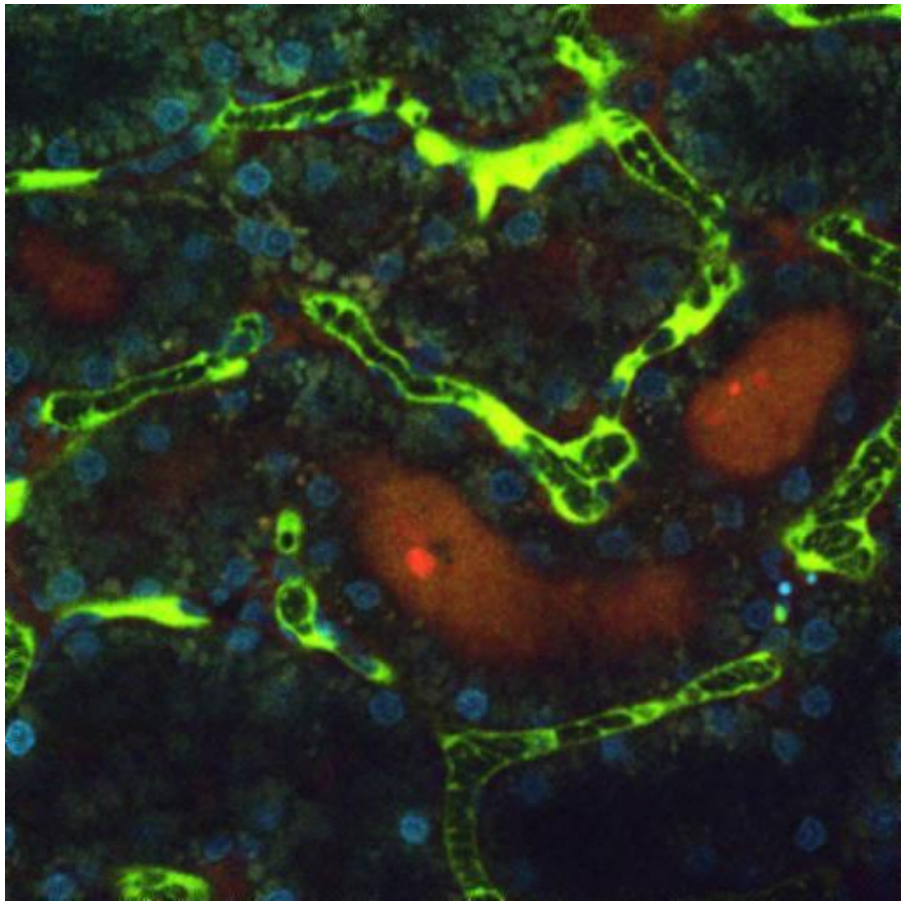


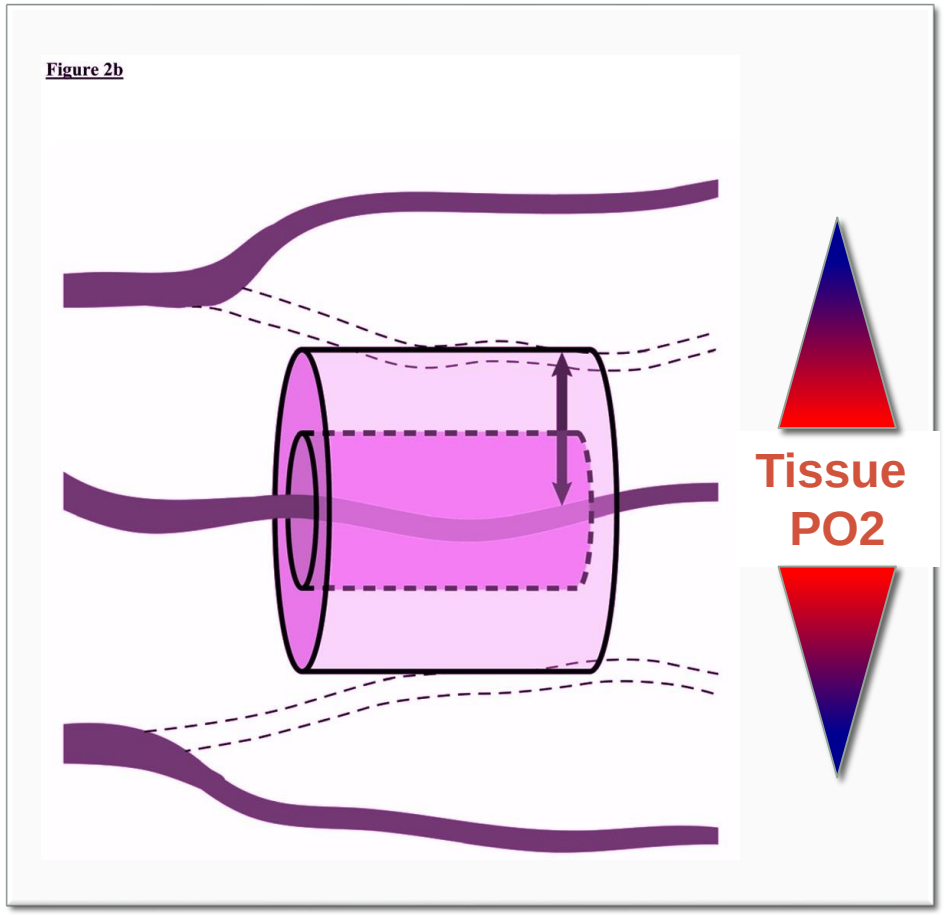
Prior to cellular injury, alterations in the renal microcirculation and tissue oxygenation occur, rendering epithelial cells susceptible to injury. This represents an earlier phase of AKI that might help identify patients at risk, provide a window for therapeutic intervention to prevent AKI before cellular injury.

Acute Dialysis Quality Initiative (ADQI) Consensus Conference on
"Acute Kidney Injury Biomarkers" held in Dublin, Ireland, in August 2011

Hemodynamics

Microvascular Blood Flow





Courtesy of B. Molitoris

Trzeciak S et al. Acad Emerg Med 2008; 15:399-413

Realtime GFR

- In vivo rapid detection and instantaneous calculation of GFR
- Portable analyzer developed for this purpose allowed point-of-care determination of GFR
- Ratiometric emission at 490nm and 590 nm. 2 compartment model. Within 60 min of administration of the fluorescent conjugates,
- Minimal adverse effects and excellent agreement with the 6-h iohexol-based GFR technique

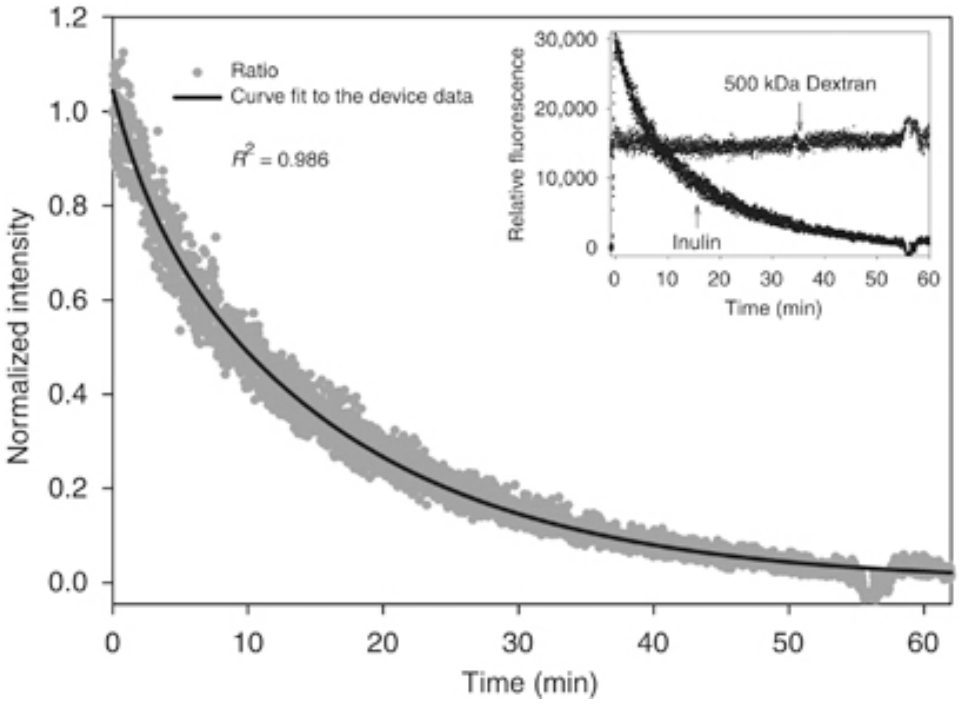


Table 1 | Normal and CKD GFR of dogs

Dog ID	GFR (ml/min/kg) by spectroscopy	GFR (ml/min/kg) by the device	GFR (ml/min/kg) by iohexol	Condition	Weight (kg)	Plasma volume (ml)
1	4.06	4.25	4.4	Normal	22.5	1344
2	3.76	4.8	4.5	Normal	33	1838
3	3.77	3.35	4.07	Normal	27.3	1352
4	1.27	1.19	1.45	CKD	29.8	1482

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.
GFR values obtained by three different techniques along with plasma volumes from three normal dogs and one CKD dog are shown. During the tests dogs 1 and 2 were with mild sedation with 0.1 mg/kg intravenously administered acepromazine; dogs 3 and 4 were anesthetized using isofluorane.

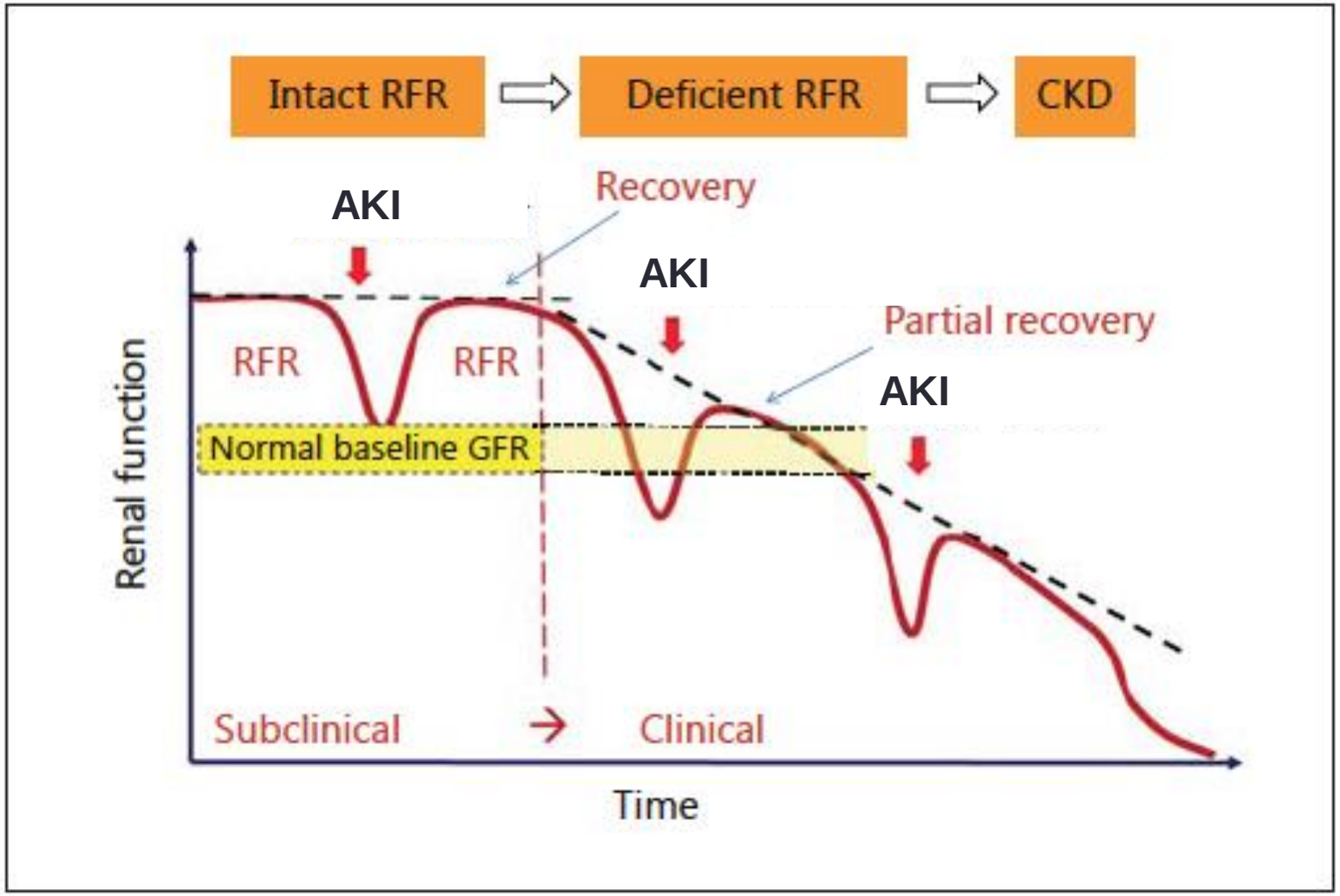
E. Wang…G.J. Schwartz and B.A. Molitoris et al. Kidney int 81: 112-117, 2012

Utility of Physiological Markers in AKI

Physiological Marker	Risk Assessment	Early Diagnosis	Differential Diagnosis	Prognosis	Therapy Guidance
Urine indices	-	✓	✓	-	✓
Serial Creatinine	✓	✓	✓	✓	✓
Real time measured GFR	✓	✓	✓	✓	✓
Continuous Urine flow	✓	✓	✓	✓	?
Doppler US	✓	✓	✓	?	✓
Contrast enhanced US	✓	✓	✓	?	✓
Urine pO2	✓	✓	?	?	✓
Bladder pO2	✓	✓	✓	?	✓
BOLD MRI	?	?	✓	✓	?
PET	?	?	✓	✓	?
Near infrared spectroscopy	?	?	✓	✓	?
Bioimpedance	-	✓	-	✓	✓

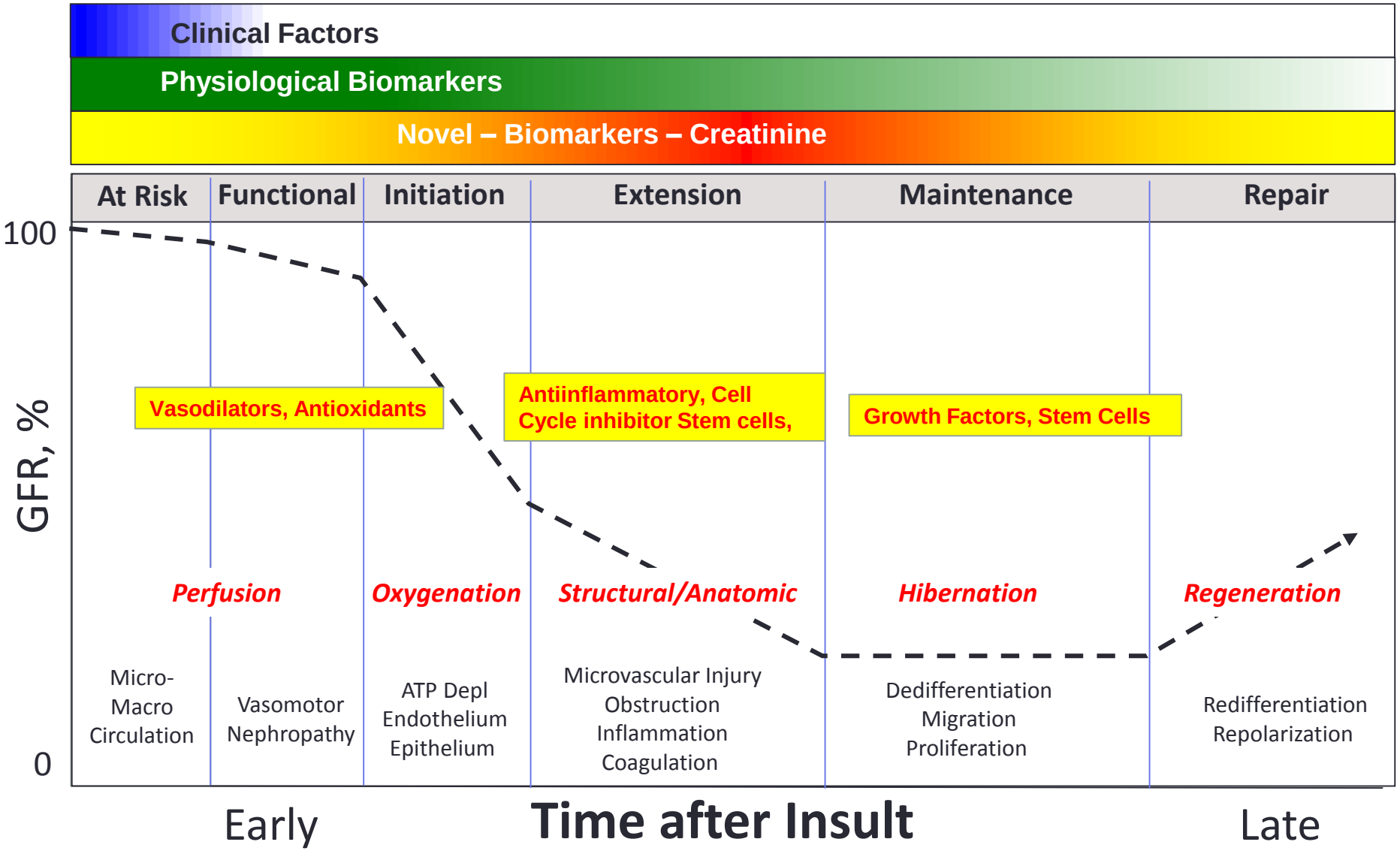
M. D. Okusa et al. Contrib Nephrol. 2013;182:65-81.

AKI and Renal Functional Reserve



A. Sharma et al. Nephron Clin Pract 2014;127:94–100

AKI Biomarkers & Personalized Therapy

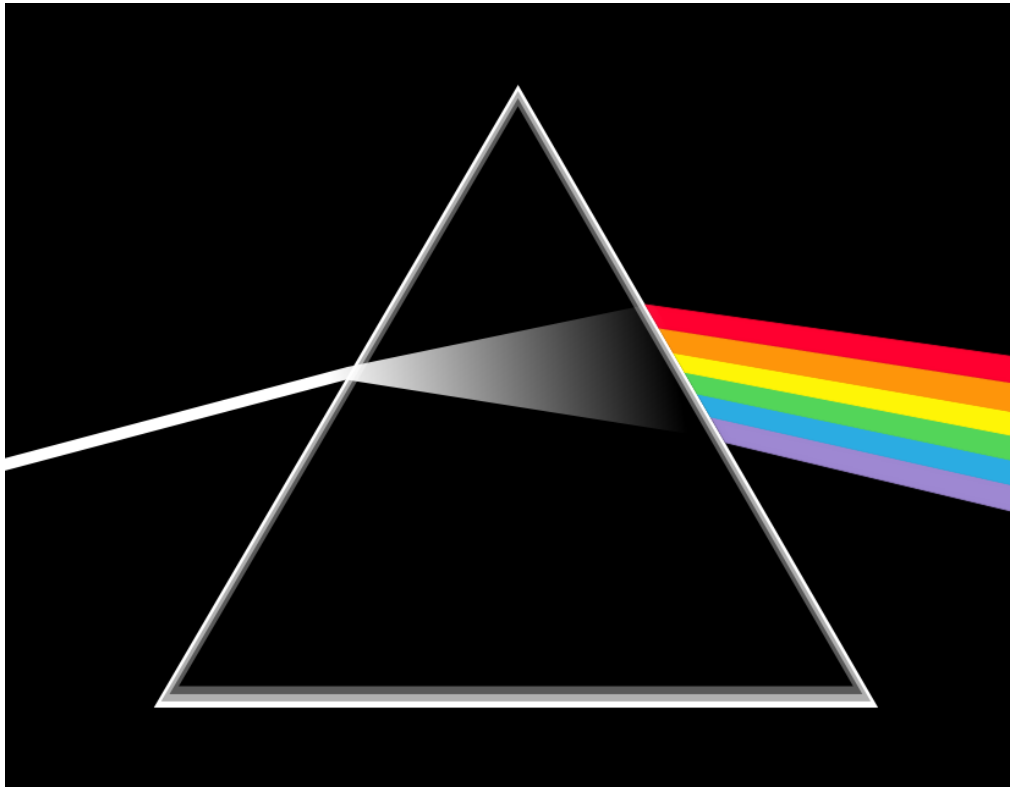


MD Okusa, B Jaber, P Doran J Duranteau, L Yang, C Ince

The Acute Dialysis Quality Initiative (ADQI) Consensus Conference on “Acute Kidney Injury Biomarkers” held in Dublin, Ireland, in August 2011

AKI "Phenotypes"

Acute Kidney Injury



Phenotype 1 → Therapy 1

Phenotype 2 → Therapy 2

Phenotype 3 → Therapy 3

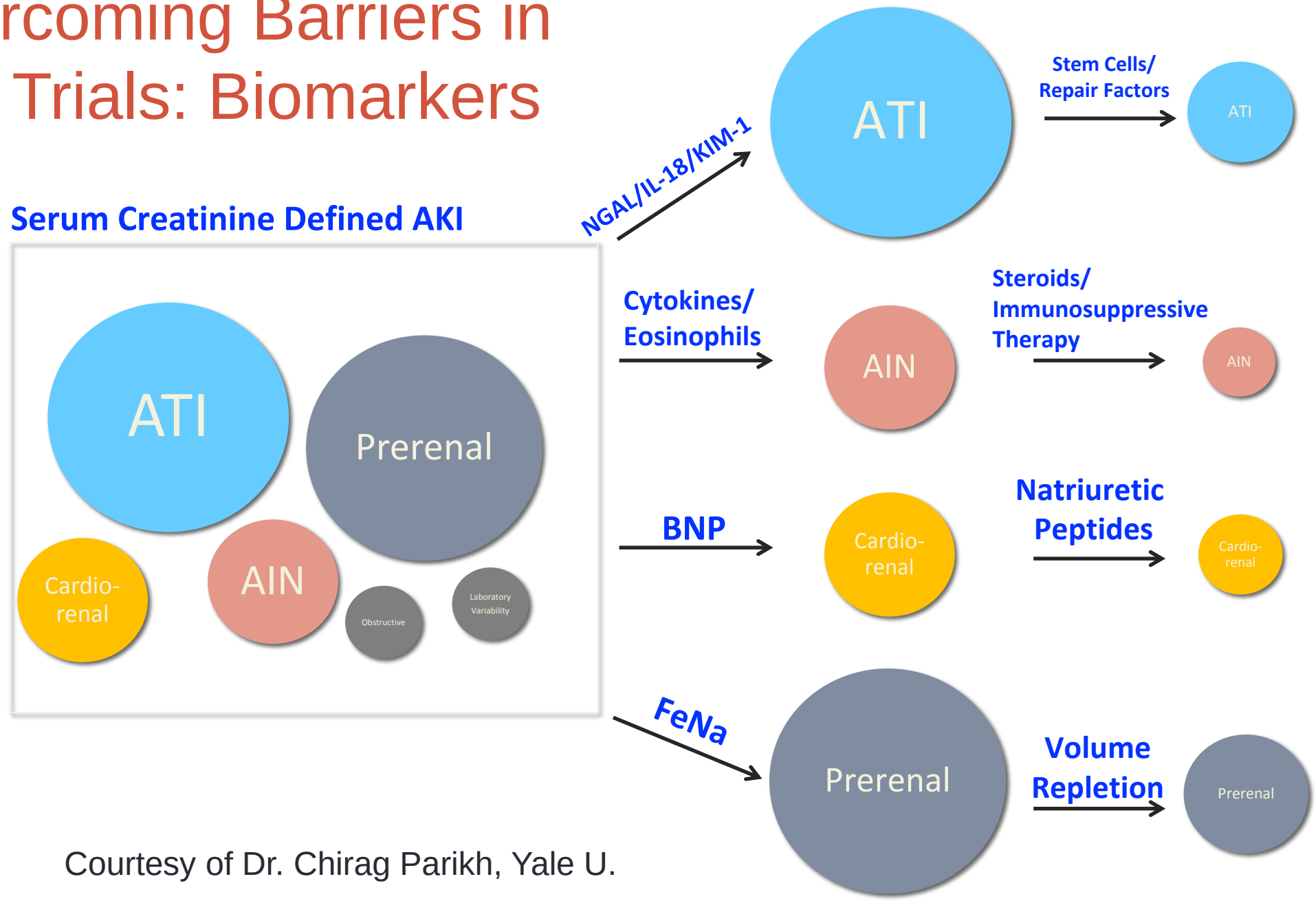
Phenotype 4 → Therapy 4

Phenotype 5 → Therapy 5

- **AKI Phenotypes based on etiology, molecular pathways, and therapy**

Courtesy Chirag Parikh, Johns Hopkins U.

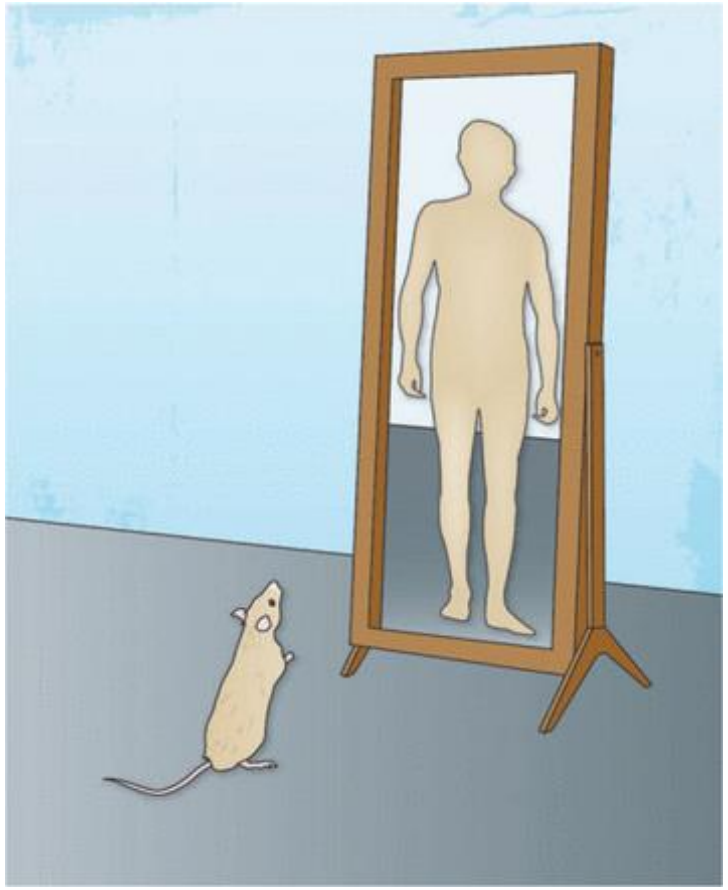
Overcoming Barriers in AKI Trials: Biomarkers



Courtesy of Dr. Chirag Parikh, Yale U.

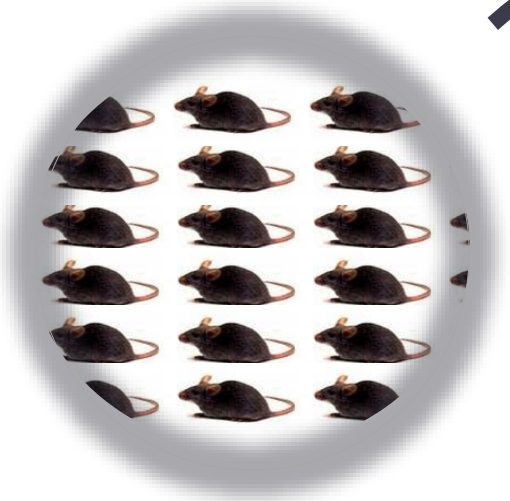
Overcoming Barriers in AKI Trials

Mouse to Humans



M.J. Justice, P. Dhillon Disease Models & Mechanisms (2016) 9, 101-103

Biomarkers
Functional Studies
Biopsy Samples
Genetic Samples



Better Clinical Trial Design

Kidney Precision Medicine Project (KPMP)

Physiologically Relevant Targets
Good Animal Models
Rigorously Conducted Preclinical Studies

NIH National Institute of Diabetes and Digestive and Kidney Diseases

The NEW ENGLAND JOURNAL of MEDICINE

A New Initiative on Precision Medicine

Perspective

Francis S. Collins, M.D., Ph.D., and Harold Varmus, M.D.

FEBRUARY 26, 2015

- One size does not fit all
- Use all current medical and biological knowledge to treat individuals as individuals
- Develop better biomarkers to guide medical care and therapy