IRRIV
International Renal Research Institute Vicenza

37th Vicenza Course on AKI & CRRT

May 28-30, 2019

ViCC Vicenza Convention Centre

Fiera di Vicenza

Vicenza - Italy

Biomarkers of Progression to CKD

Giuseppe Castellano, MD, PhD

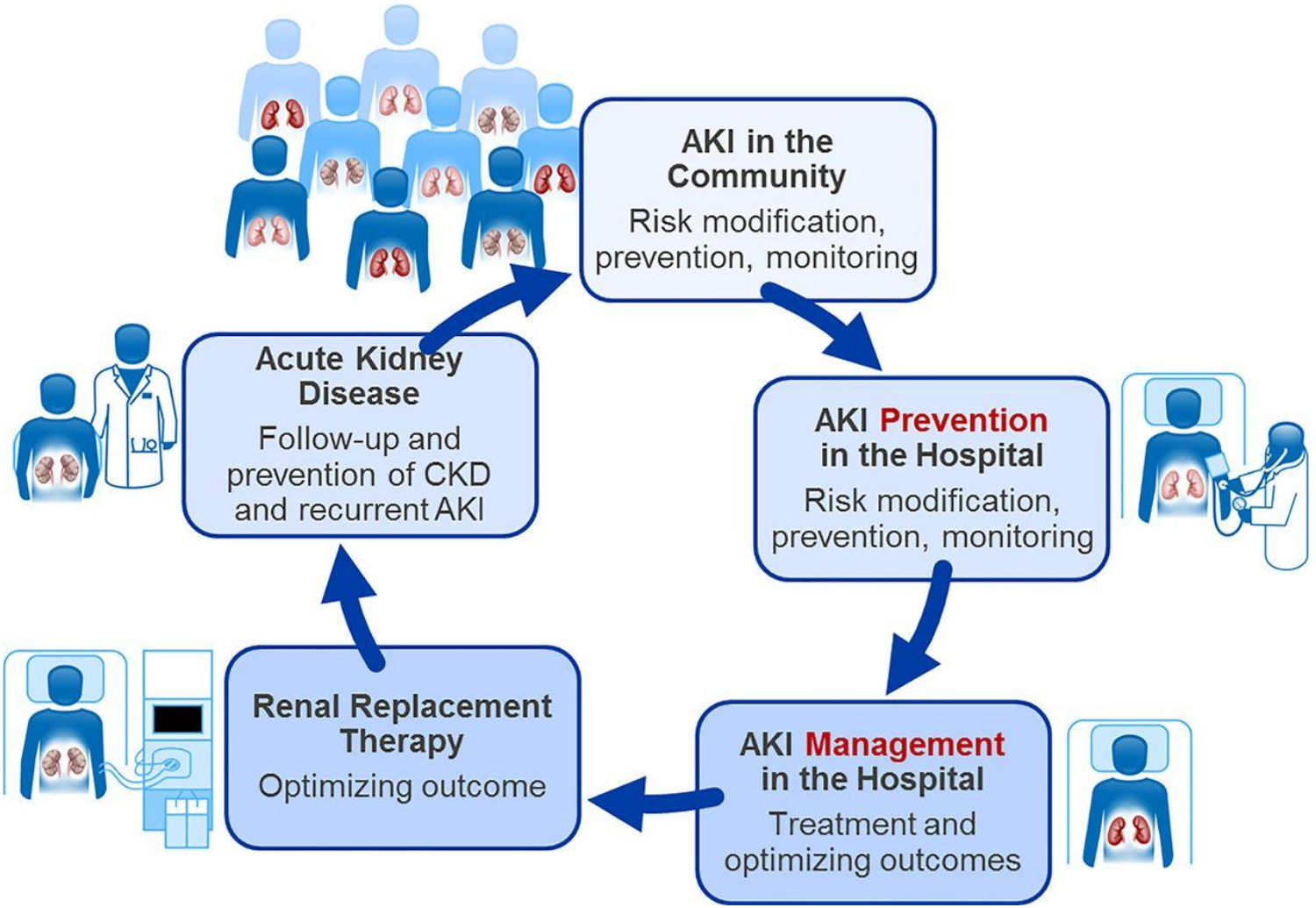
University of Bari,

Dept of Emergency and Organ Transplantation

Università degli Studi Bari

SCUOLA DI SPECIALIZZAZIONE IN NEFROLOGIA

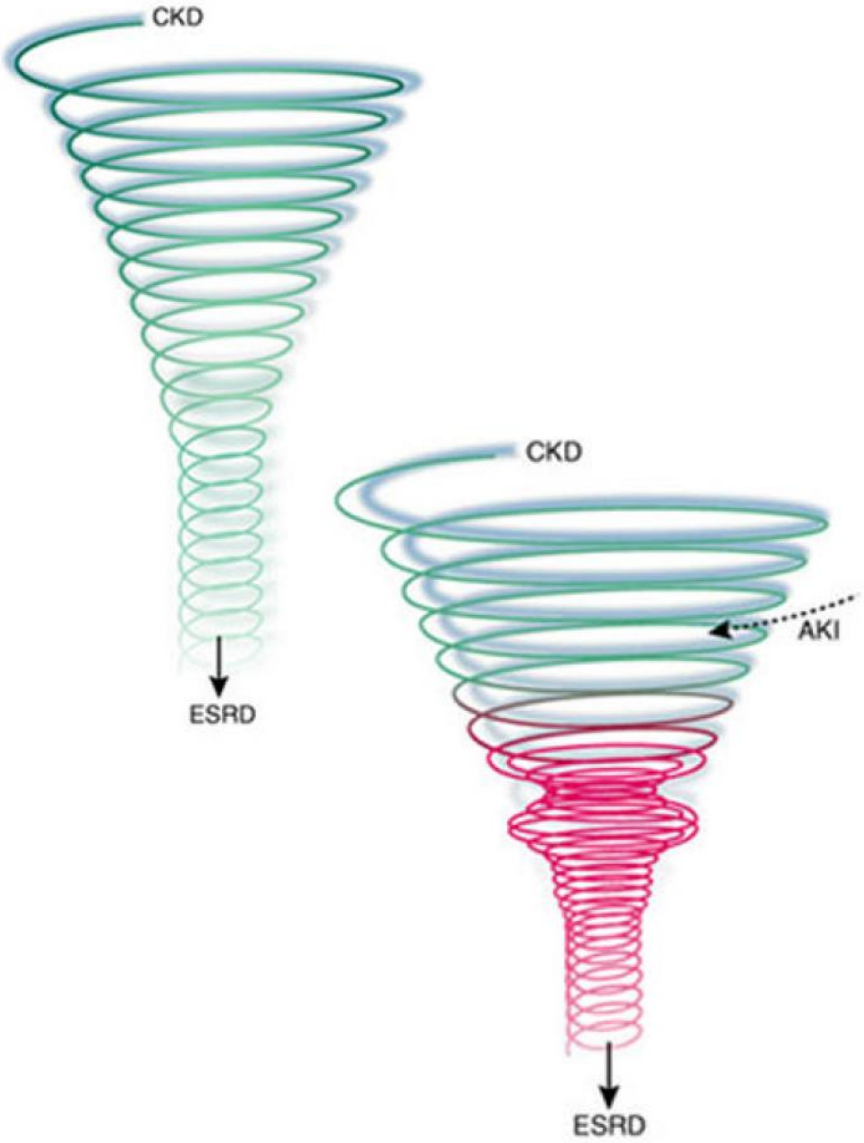
AKI quality care in a continuity



Kianoush Kashani et al. CJASN doi:10.2215/CJN.01250119

CJASN

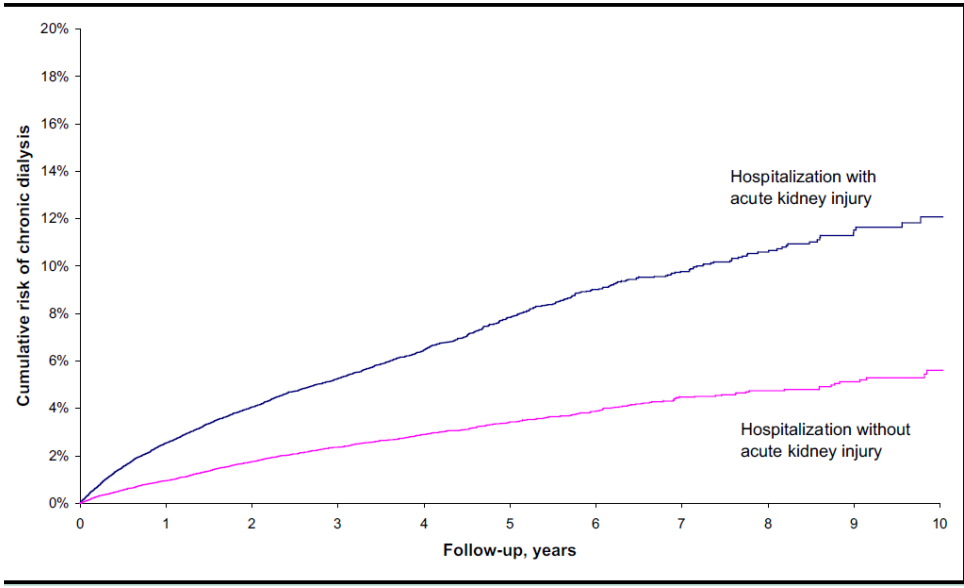
What are the clinical complications of AKI?



Risk of Chronic Dialysis and Death Following Acute Kidney Injury

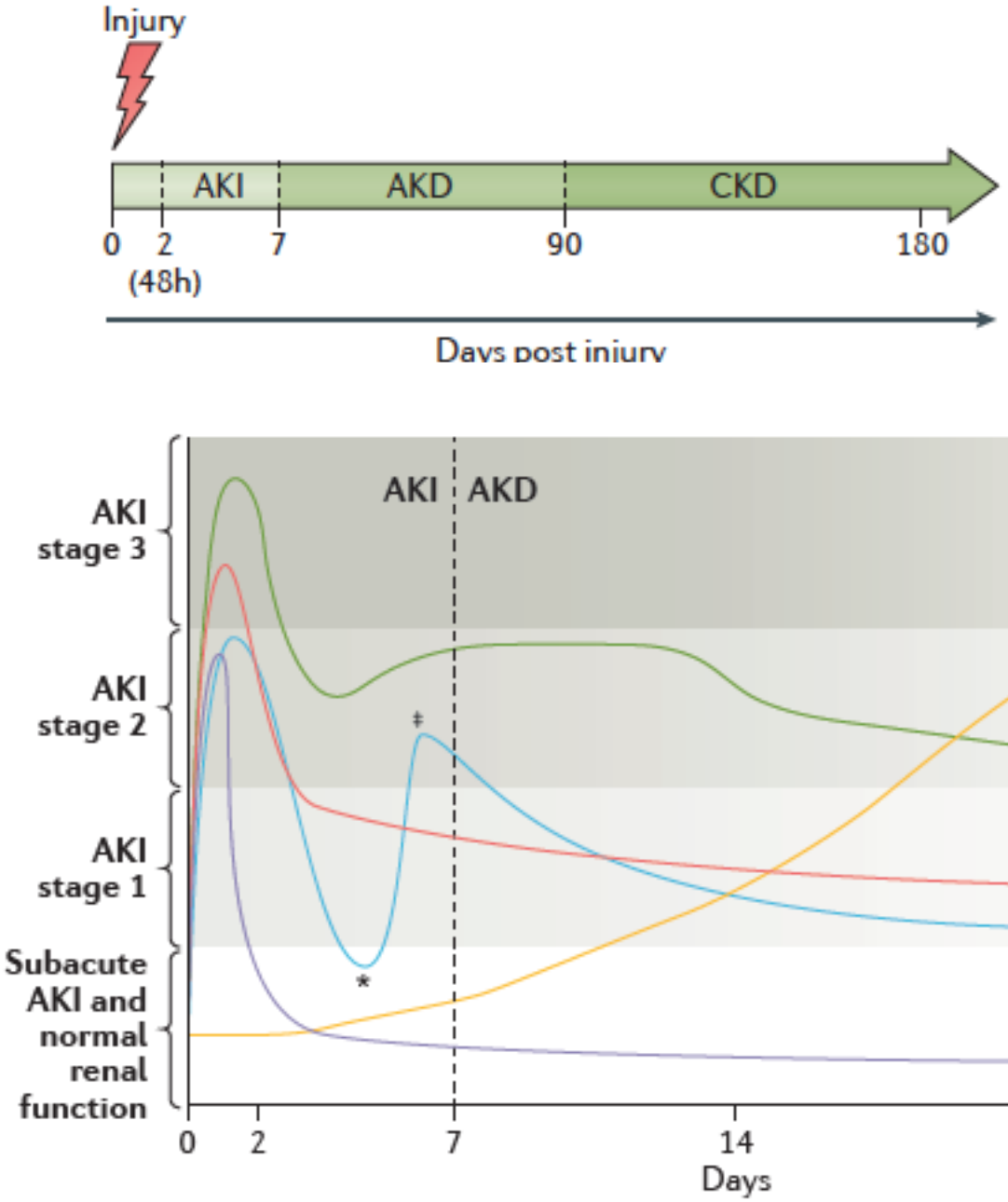
Ron Wald, MDCM,[a,b] Robert R. Quinn, MD,[c] Neill K. Adhikari, MDCM,[d] Karen E. Burns, MD,[b,e] Jan O. Friedrich, MD,[b,e] Amit X. Garg, MD,[f] Ziv Harel, MD,[a] Michelle A. Hladunewich, MD,[d,g] Jin Luo, MD,[h] Muhammad Mamdani, PharmD,[b,i] Jeffrey Perl, MD,[a,b] Joel G. Ray, MD[b,j]; for the University of Toronto Acute Kidney Injury Research Group

In a population based study of hospitalised patients with severe AKI-requiring dialysis, the risk of new ESRD was increased by three-fold.

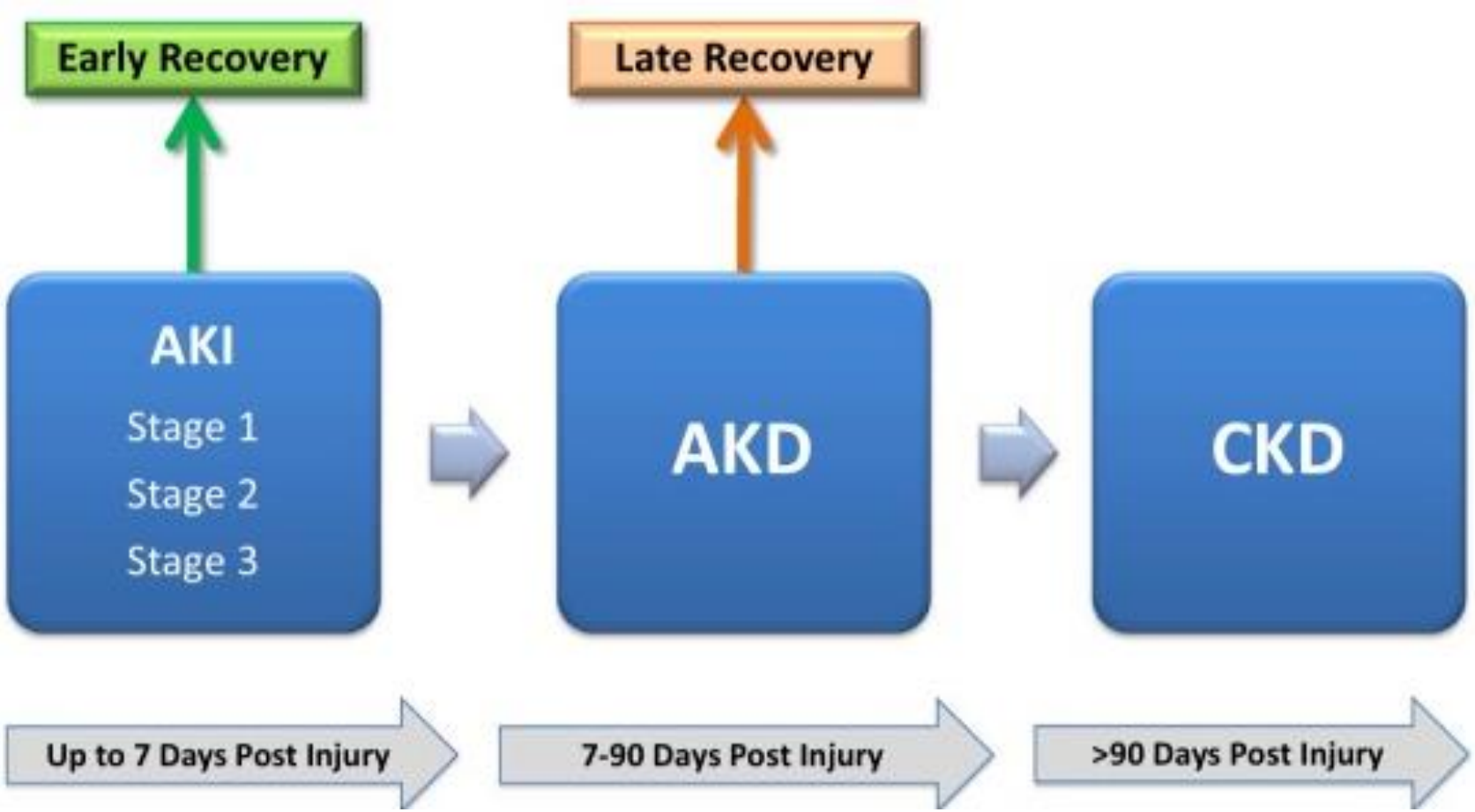


Bellomo R, Intensive Care Med 2017

Wald R, Risk of chronic dialysis and death following acute kidney injury. Am J Med 2012


Injury
AKI
AKD
CKD
0
2
(48h)
7
90
180
Days post injury
AKI
AKD
AKI stage 3
AKI stage 2
AKI stage 1
Subacute AKI and normal renal function
1
2
3
4
5
*
‡
Rapid reversal
0
2
7
14
28
Days

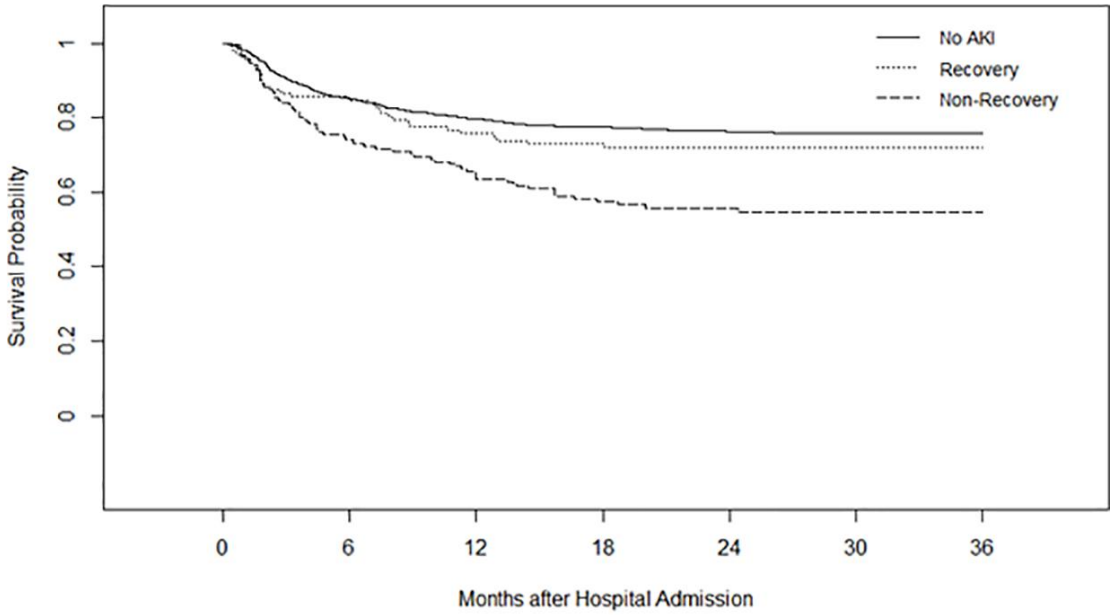
Recovery from AKI



L. G. Forni et al , Intensive Care Med (2017) 43:855–866

Long-term survival in patients with septic acute kidney injury is strongly influenced by renal recovery

Marco Fiorentino[1,2☯], Fadi A. Tohme[1,3,4☯], Shu Wang[1,5], Raghavan Murugan[1,3], Derek C. Angus[3], John A. Kellum[1,3,4]*



Number of subjects at risk							
	0	6	12	18	24	30	36
No AKI	1480 (100%)	1261 (85%)	1180 (79.7%)	979 (66%)	496 (33.5%)	196 (13.2%)	25 (1.6%)
Recovery	111 (100%)	94 (84.6%)	84 (75.6%)	70 (63%)	37 (33.3%)	20 (18%)	6 (5.4%)
Non recovery	151 (100%)	112 (74.2%)	97 (64.2%)	77 (50.9%)	41 (27.1%)	14 (9.2%)	3 (1.9%)

Fig 2. Kaplan-Meier survival curves stratified by recovery status. The three groups are significantly different overall, $p < 0.001$ (Peto-Peto-Prentice test).

Fiorentino et al. PLOS One 2018

see commentary on page 430

Increased risk of death and *de novo* chronic kidney disease following reversible acute kidney injury

Ion D. Bucaloiu[1], H. Lester Kirchner[2], Evan R. Norfolk[1], James E. Hartle II[1] and Robert M. Perkins[1,3]

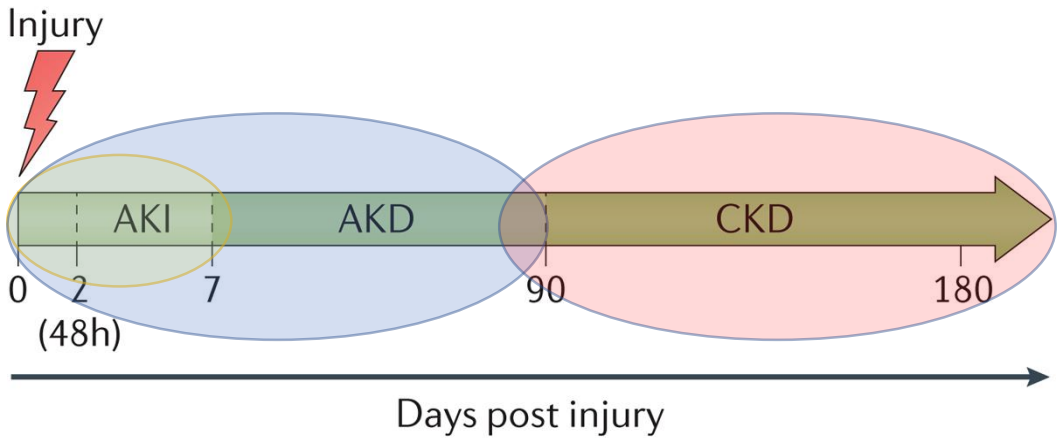
[1]Department of Nephrology, Geisinger Medical Center, Danville, Pennsylvania, USA; [2]Biostatistics and Research Data Core, Center for Health Research, Geisinger Medical Center, Danville, Pennsylvania, USA and [3]Center for Health Research, Geisinger Medical Center, Danville, Pennsylvania, USA

Table 4 | Cox proportional hazard for time to death and *de novo* CKD (recovered AKI vs. controls)

	Death HR (95% CI)	*De novo* CKD HR (95% CI)
Adjusted for index hospital length-of-stay	1.48 (1.19, 1.82) $P=0.0003$	1.91 (1.75, 2.09) $P<0.0001$
Adjusted for index hospital length-of-stay and *de novo* CKD	1.18 (0.95, 1.46) $P=0.13$	NA

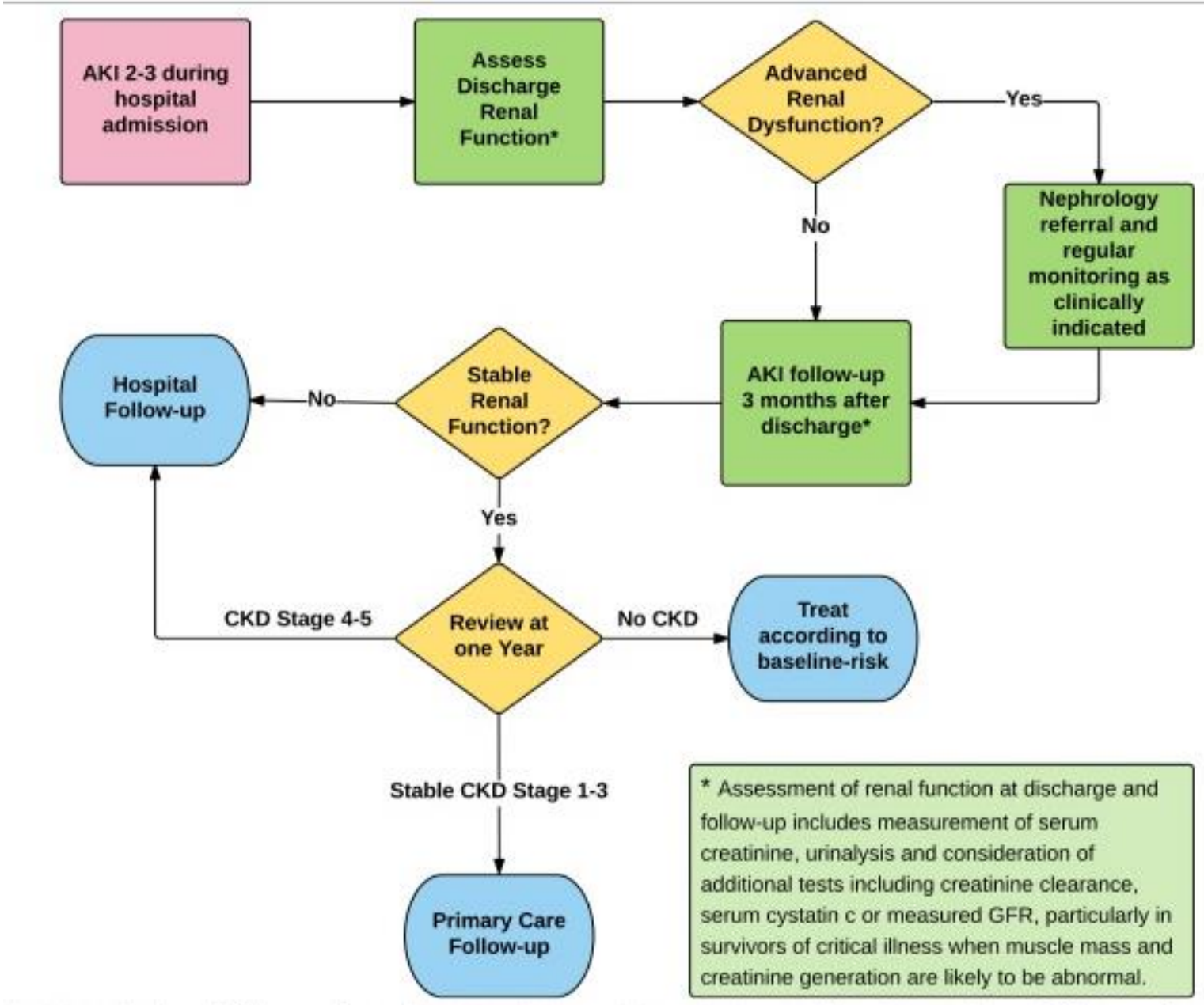
Abbreviations: AKI, hospital-associated acute kidney injury; CI, confidence interval; CKD, chronic kidney disease; HR, hazard ratio.

AKI and CKD are not separate entities: *a continuum of disease*



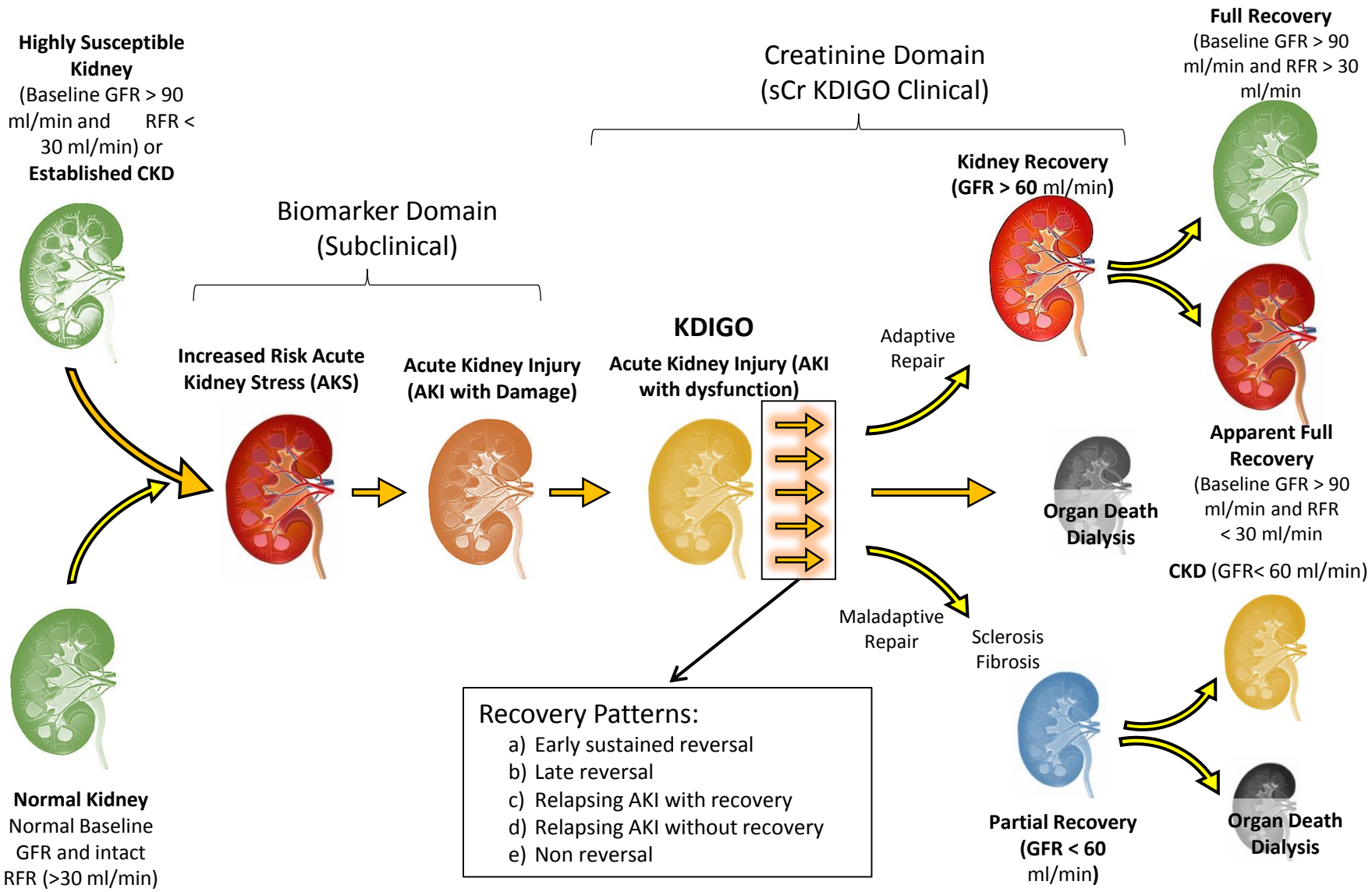
- AKI : an abrupt decrease in kidney function occurring **over 7 days or less**,
- CKD is defined by the persistence of kidney disease for **a period of >90 day**;
- AKD describes acute or subacute damage and/or loss of kidney function for a duration of between **7 and 90 days** after exposure to an AKI initiating event;
- Recovery from AKI within 48h of the initiating event typically heralds rapid reversal of AKI.
- Patients who suffer AKD with pre-existing CKD are probably at high-risk of kidney disease progression

Adapted from Chawla, L. S. et al. Nat. Rev. Nephrol. 2017

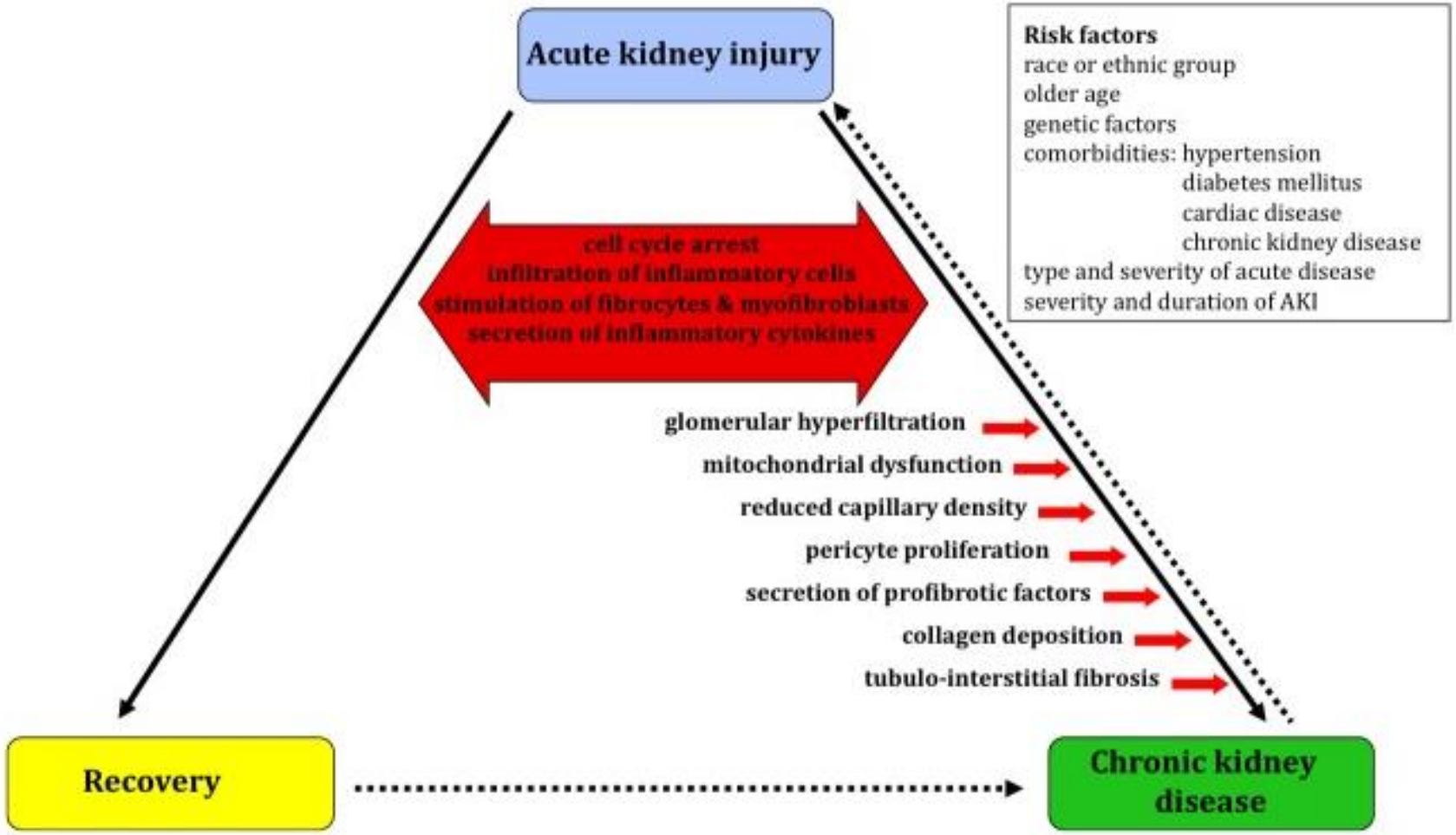


L. G. Forni et al , Intensive Care Med (2017) 43:855–866

Acute Kidney Disease (3 months)



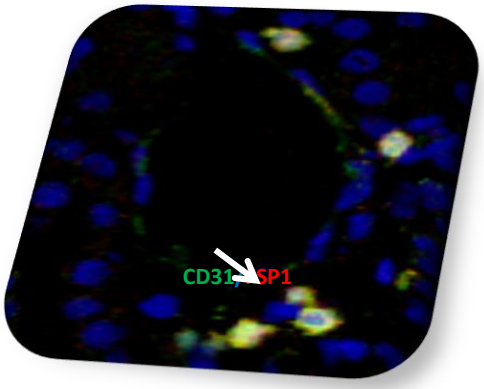
Ronco AJRCCM 2017



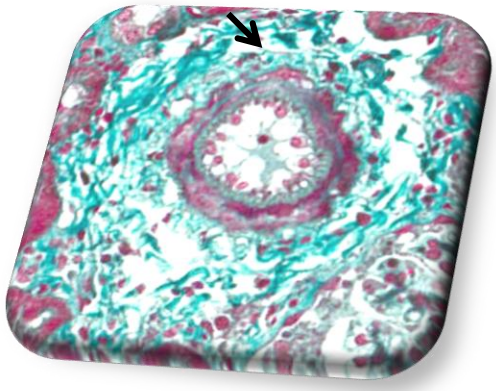
L. G. Forni et al , Intensive Care Med (2017) 43:855–866

Complement-mediated endothelial dysfunction in I/R injury

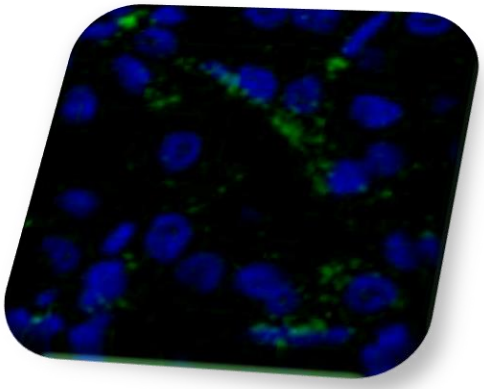
T24h ctr



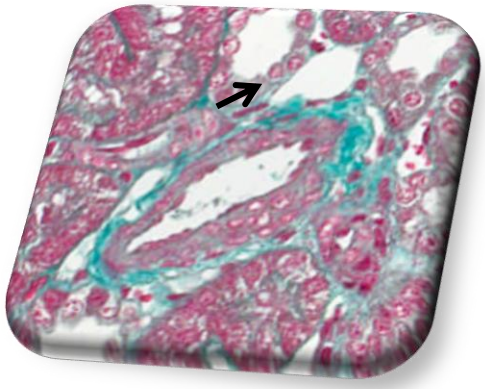
T24h ctr



T24h C1-INH

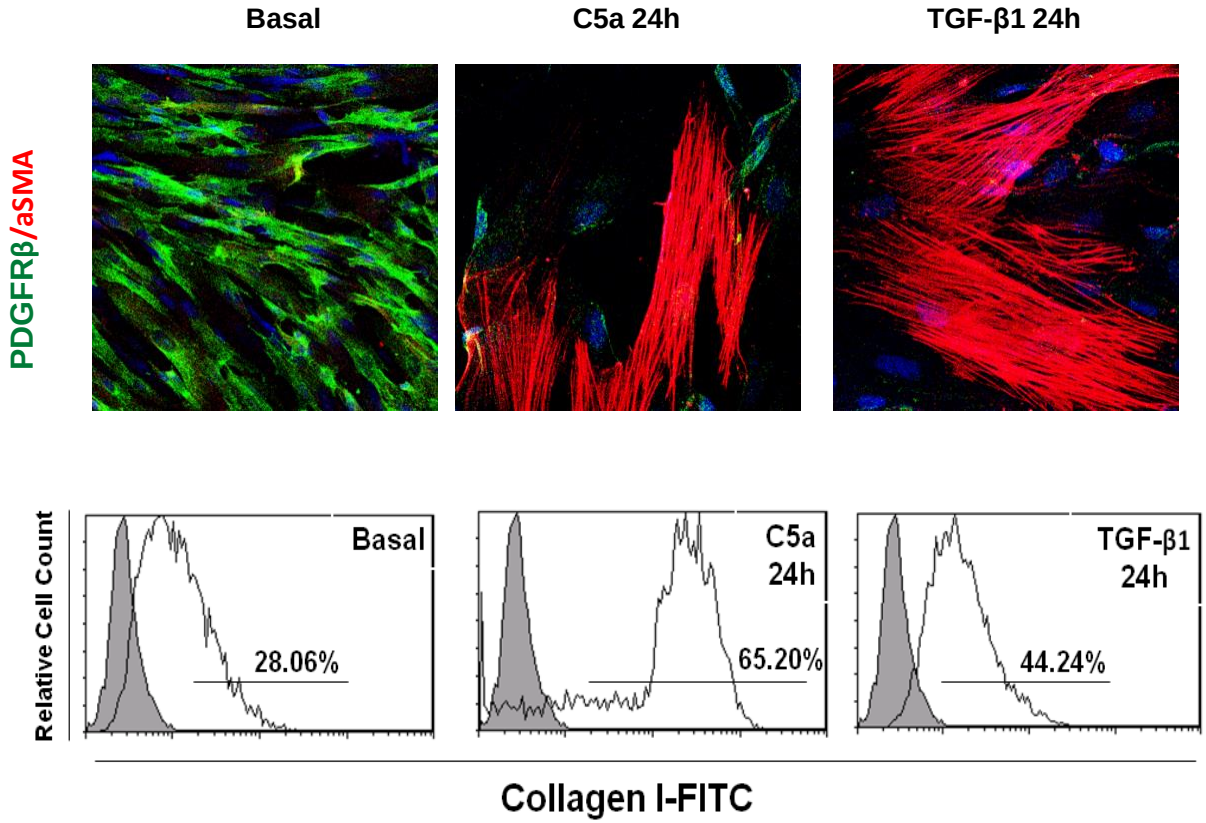


T24h C1-INH



Curci C.,Castellano G. Nephrol Dial Transplant. 2014

C5a drives Perycite to Myofibroblast Transdifferentiation in AKI



*n=3, * *p<0,001, *p<0.05, t-test*

Castellano G, Franzin R et al, Frontiers in Immunology 2018

CKD

The prevalence of CKD is increasing worldwide and consequently the risks for renal replacement therapies, such as dialysis and transplantation and the incidence of cardiovascular events are also increasing.

Therefore there is an urgent need to identify novel and efficient **biomarkers** for predicting the decline of glomerular filtration rate (GFR) and the progression of renal disease, as well as to provide tools for more efficient **treatments** for patients with CKD.

[The United States Renal Data System. 2016 USRDS Annual Data Report: Epidemiology of Kidney Disease 2016]

[Jha V, Lancet 2013]

What is a biomarker?

" a characteristics that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process, or pharmacologic response to a therapeutic intervention".

Test	Biomarker
Height	Growth
Urinary dipsticks for nitrites	UTI
Proteinuria	Disease severity in IgA nephropathy.
Anti-GBM Ab	Good pastures syndrome (anti-glomerular basement membrane disease)

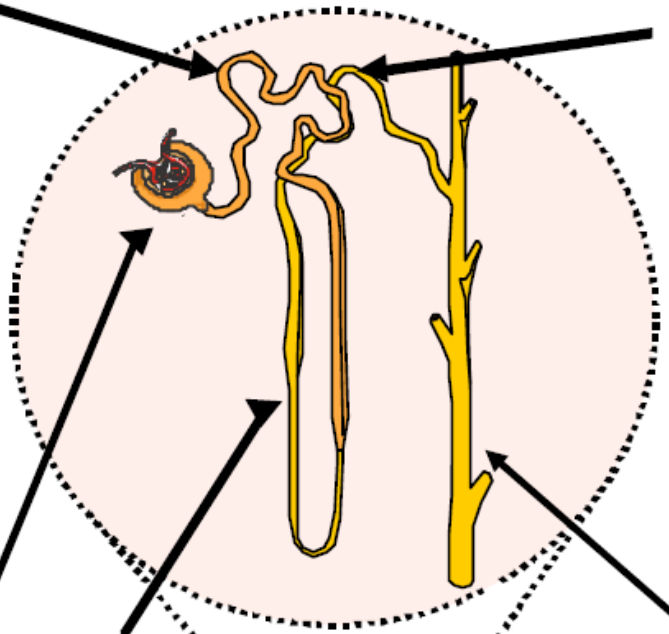
Ideal Biomarker

- Non – invasive
- Easily obtainable
- Measurable using standardized assays
- Fast results
- Cost

Kidney Biomarkers

- > 20 protein biomarkers have been intensively studied:
  - Urinary biomarkers are: non invasive, easy to measure, easily obtainable and provide earlier detection.
- Urinary biomarkers many be classified as those of **structural** injury or **functional** injury.

Candidate Biomarkers for Kidney Injury



Proximal Tubules

- KIM-1
- L-FABP
- Albumin
- Clusterin
- Lipocalin (NGAL)
- GSTα
- β2-microglobulin
- α1-microglobulin
- NAG
- Osteopontin
- Cystatin C
- Netrin-1
- IL-18
- Retinol binding prot
- HGF, NHE-3
- Cyr61
- Exosomal Fetuin-A
- TIMP2
- IGFBP7

Distal Tubules

- Osteopontin
- Clusterin
- Lipocalin (NGAL)
- GST-μ/π
- NAG
- EGF
- Osteopontin
- Cystatin C
- IL-18
- Calbindin-D28

Loop of Henle

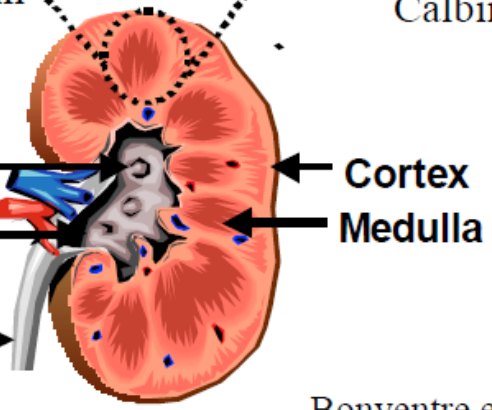
osteopontin

Collecting Duct

Calbindin- D28

Glomerulus

- Creatinine
- Cystatin C
- Protein, albumin
- Podocalyxin
- β2-microglobulin
- WT-1 in exosomes



CKD

Bonventre et al. Nature Biotech 2010

Biomarkers of functional injury

Cystatin C*	Glomerular injury; in urine indicates proximal tubule injury
Total protein β2-microglobulin albumin	Glomerular and tubular dysfunction
Brush border antigens	
Adenosine deaminase binding protein	
Carbonic anhydrase	Proximal tubule injury
Other tubular antigens	
Urinary enzymes	
N-acetyl-β-D-glucosaminidase	
Alanine aminopeptidase	
Cathepsin B	Proximal tubule injury
γ-glutamyltransferase	
α-glutathione-S-transferase	
β-glucosidase	Proximal tubule > distal tubule injury
Alkaline phosphatase	
Lactate dehydrogenase	Distal tubule > proximal tubule injury

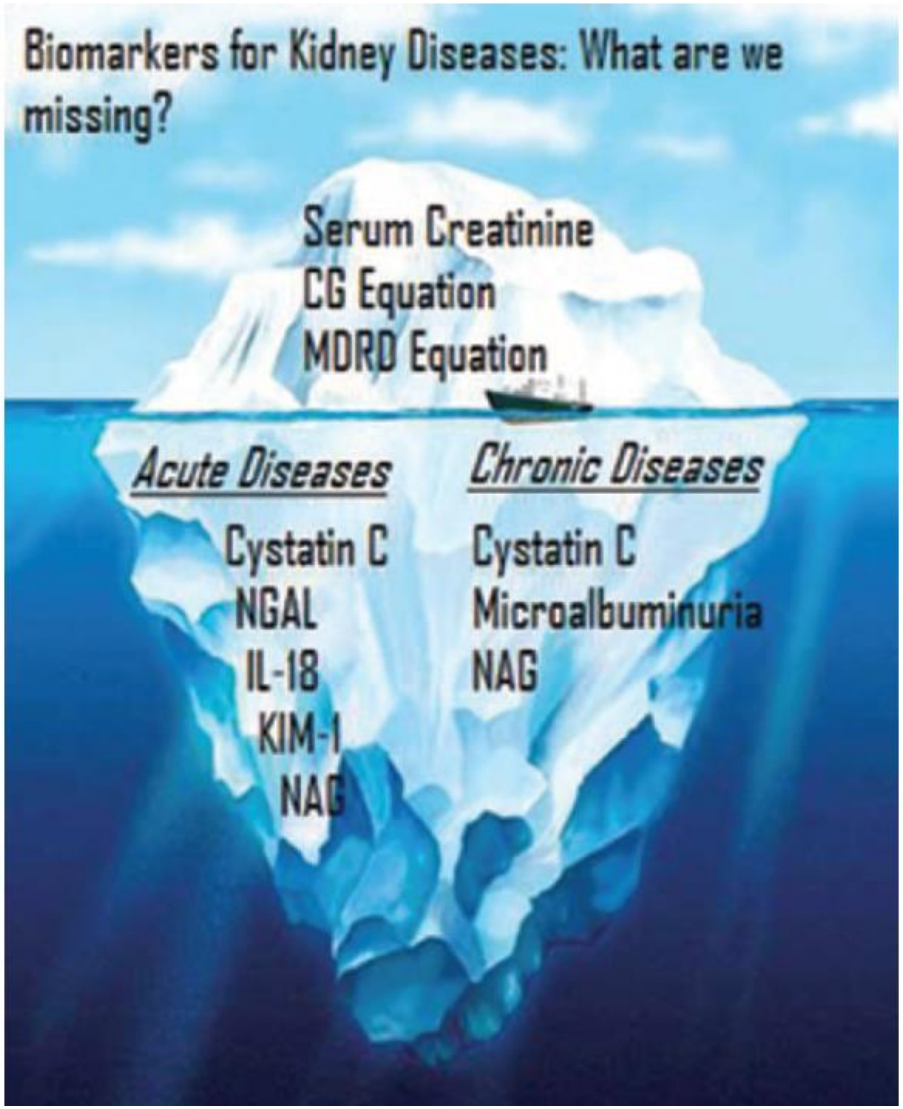
Biomarkers of structural injury

Type of biomarkers	Selective sites and associated types of injury
Kidney injury molecule-1 (KIM-1)*	Proximal tubule injury (Ischemic AKI, nephrotoxins, RCC)
n-acetyl glucosaminadase (NAG)	Proximal tubule injury
Neutrophil gelatinase-associated lipocalin (NGAL)*	Tubule and collecting duct injury (Ischemic AKI, nephrotoxins, delay allograft renal function)
Interleukin (IL)-18*	Tubule injury (AKI, delayed allograft renal function)
Clusterin	Tubule injury

DIAGNOSIS OF CKD

Currently, the diagnosis of CKD is made usually on the levels of blood urea and serum creatinine (sCr).

Limitations of serum creatinine



Ian Wu, Clin JASN 2008

Limitations of serum creatinine

Disease	Limitations of serum creatinine
AKI and CKD	Nonspecific to disease etiology
	Delayed marker of kidney damage
	Dependent on clinical characteristics (age, sex, muscle mass, etc.)
	Insensitive to small changes in GFR
AKI	Dependent on hemodynamic steady state
	Altered in hospitalized patients (i.e., by diuretics, IV fluids)
	Assay-related interference (i.e., by bilirubin)
CKD	Unchanged despite kidney damage in tubulointerstitial and vascular disease
	May be falsely low with significant proteinuria
	Provides imprecise eGFR estimations
	Requires special considerations for eGFR equations with changing muscle mass (i.e., in children, cirrhotics)

William R. Zhang, Annual Review of Physiology 2019

Albuminuria

Prognosis of CKD by GFR and albuminuria category

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

GFR categories (ml/min/1.73 m²) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

- Albuminuria is the classic sensitive marker of early renal dysfunction but excludes many patients that will only have proteinuria when GFR is already significantly decreased.
- Albuminuria is also utilized as a marker of risk for CKD progression.
- However, strategies that decrease albuminuria do not always prevent the progression of kidney disease

KDIGO 2013 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease

New emerging biomarker in the diagnosis of CKD and in the prediction of outcome

- KIM-1
- NGAL
- TIMP2-IGFBP7
- ASYMMETRIC DIMETHYLARGININE (ADMA)
- Symmetric Dimethylarginine (SDMA)
- Uromodulin
- β2M beta-2 microglobulin
- miRNA, ncRNA, lncRNA
- Metabolic biomarkers

Table 2 Biomarkers of short-term acute kidney injury (AKI) recovery versus persistence to acute kidney disease (AKD)

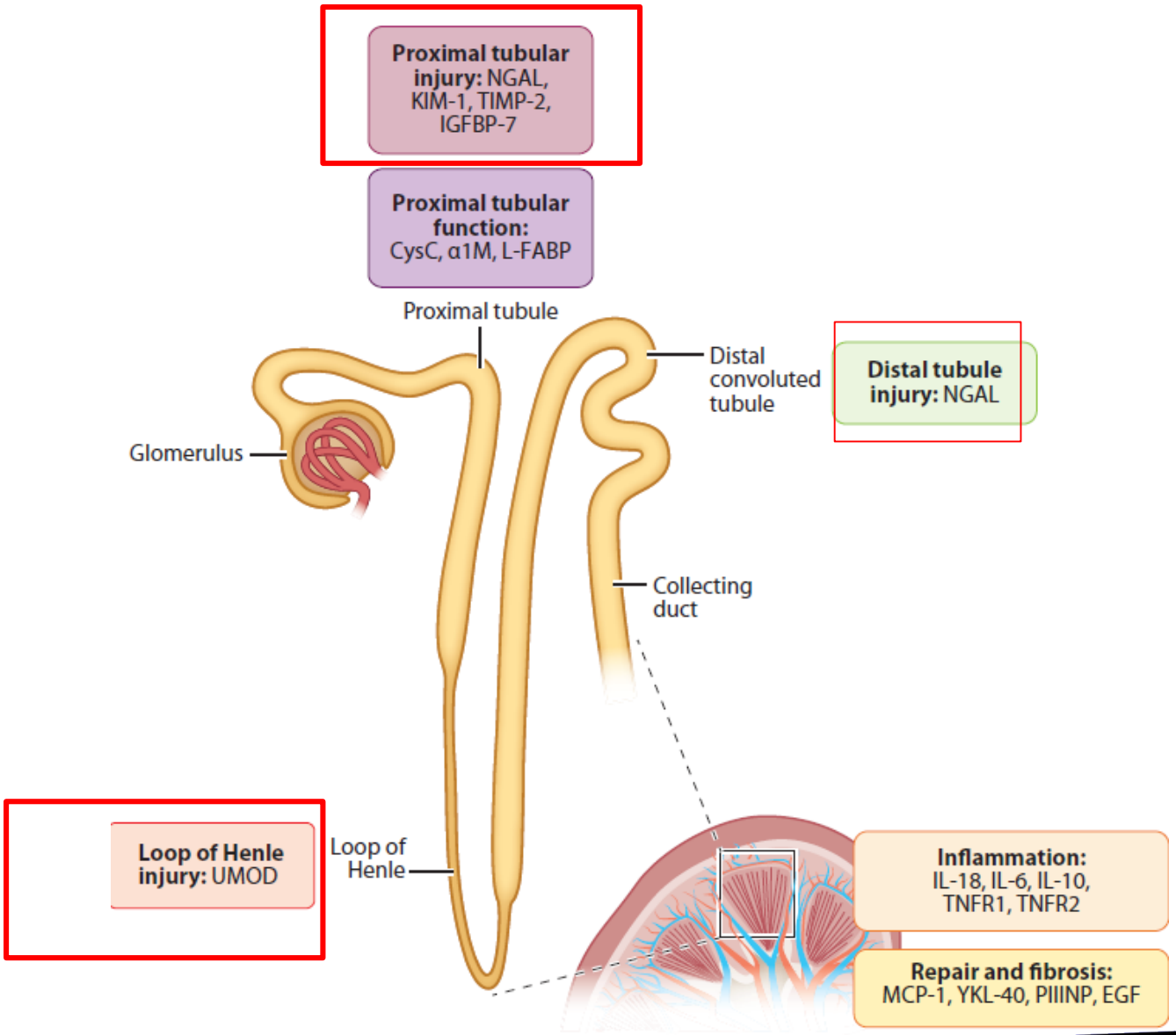
AKI biomarker	Characteristics	Clinical setting	Outcome
Angiotensinogen	453 amino acid protein; precursor of angiotensin I	Acute CRS Cardiac surgery ICU	AKI progression
Cystatin C	13 kDa cysteine protease inhibitor produced by all nucleated human cells; undergoes glomerular filtration	ICU	RRT
Hepatocyte growth factor	Antifibrotic cytokine produced by mesenchymal cells and involved in tubular cell regeneration after AKI	ICU	RRT
IGFBP7 TIMP-2	29 kDa and 21 kDa proteins involved in cell cycle arrest; released into urine after tubular cell stress	ICU Cardiac surgery	RRT
IL-18	18 kDa pro-inflammatory cytokine; regulates innate and adaptive immunity; released into urine after proximal tubular cell injury	ICU Acute CRS Cardiac surgery Renal transplantation	AKI progression RRT DGF
KIM-1	39 kDa transmembrane glycoprotein involved in tubular regeneration; released into urine following ischaemic or nephrotoxic tubular cell damage	ICU Hospitalised patients Renal transplantation	AKI progression Need for RRT DGF
L-FABP	14 kDa intracellular lipid chaperone produced in proximal tubular cells; aids in regulation of fatty acid uptake and intracellular transport; excretion into urine after tubular injury	ICU Cardiac surgery	AKI progression RRT
MicroRNA	Endogenous single-stranded molecules of non-coding nucleotides; upregulated following tubular cell injury and cell proliferation; detectable in plasma and urine	ICU Cardiac surgery	AKI progression RRT
NAG	>130 kDa lysosomal enzyme; produced in proximal and distal tubular cells; released into urine after tubular cell injury	Hospitalised patients	RRT
NGAL	At least three different types: Monomeric 25 kDa glycoprotein produced by neutrophils and epithelial cells, including renal tubules Homodimeric 45 kDa protein produced by neutrophils Heterodimeric 135 kDa protein produced by renal tubular cells released into urine following systemic production or tubular injury	ICU Cardiac surgery Acute CRS Renal transplantation	AKI progression RRT DGF

Relevant references are mentioned in the text

AKI acute kidney injury, *CRS* cardiorenal syndrome, *DGF* delayed graft function, *ICU* intensive care unit, *IGFBP-7* insulin-like growth factor binding protein 7, *IL-18* interleukin 18, *L-FABP* liver-type fatty acid-binding protein, *KIM-1* kidney injury molecule-1, *NAG* N-acetyl-β-D-glucosaminidase, *NGAL* neutrophil gelatinase-associated lipocalin, *TIMP-2* tissue metalloproteinase 2, *RRT* renal replacement therapy, *kDa* kilodalton

L. G. Forni et al , Intensive Care Med (2017) 43:855–866

Tubular localization of CKD biomarkers



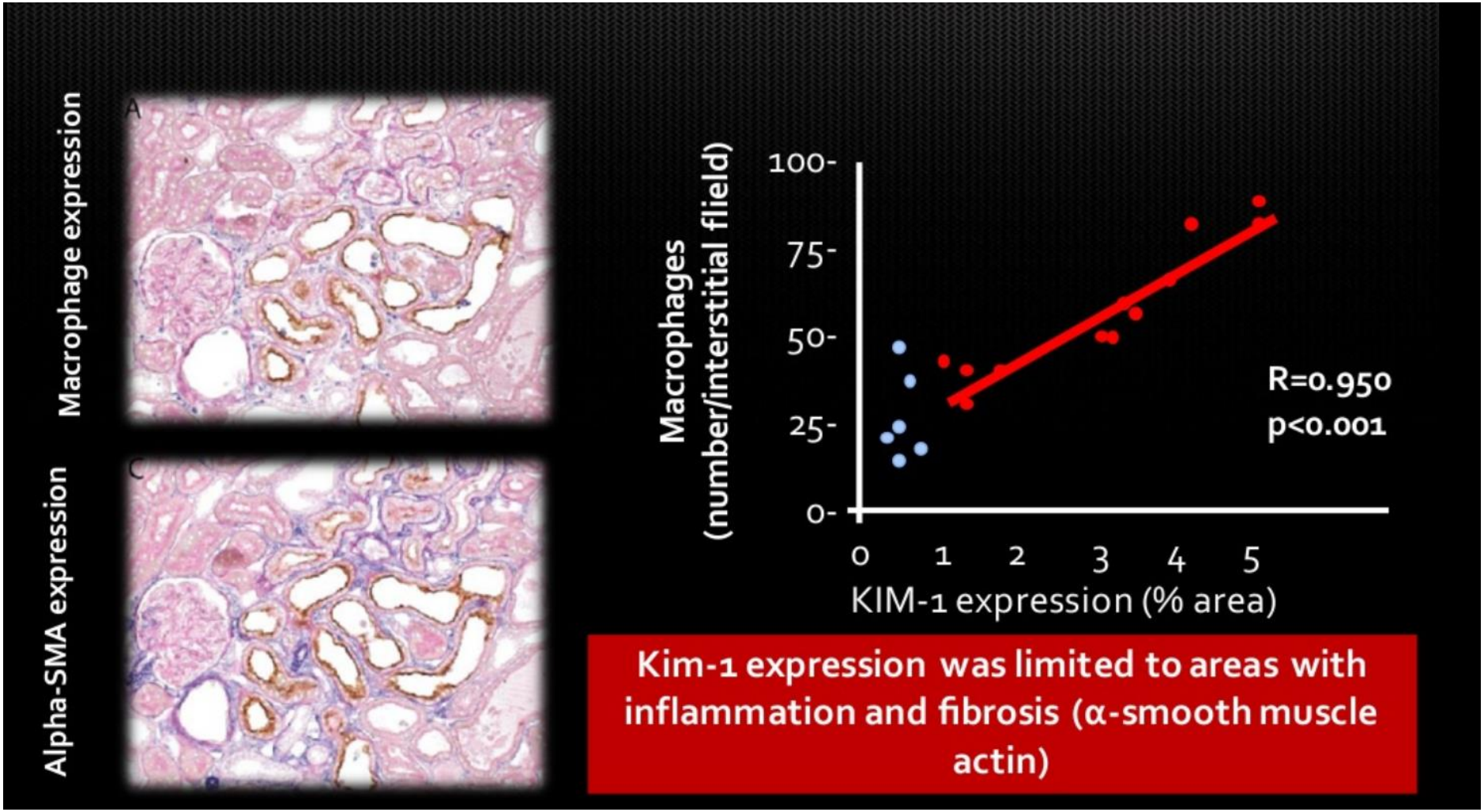
Kidney Injury Molecule-1 (KIM-1)

- Preclinical data have also demonstrated that KIM-1 is **upregulated in the later phases of AKI** and is believed to play an important role in renal repair.

- **Higher urinary KIM-1 associated with worse outcome in established AKI.**

IchimuraT, J.Biol.Chem. 273:4135–42 21.
Hubank M, Schatz DG. 1994
ProzialeckWC, Kidney Int. 2008

KIM-1 and tubule interstitial damage



Van Timmerman et al, Am Journal Physiol Renal Physiol Kashani k, Critical CARE 2013

Neutrophil gelatinase – associated lipocalin (NGAL)

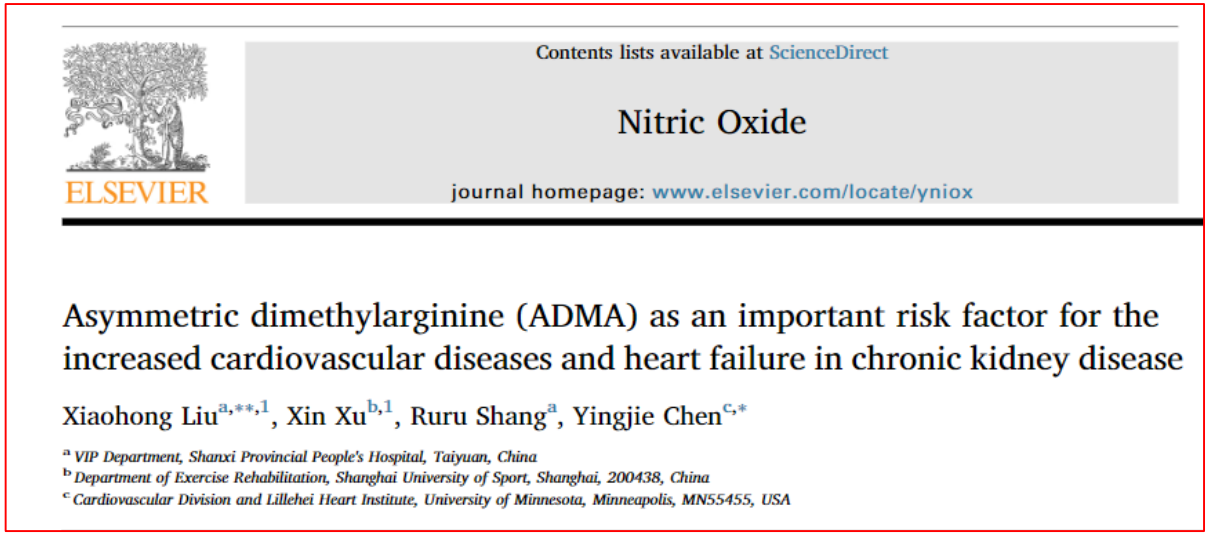
- Predominantly found in proliferating nuclear antigen–positive proximal tubule cells.

- Most consistent biomarkers found during AKI to CKD transition.

- Also predicts development of DGF.

ASYMMETRIC DIMETHYLARGININE (ADMA)

- ADMA is considered to be the **"missing link" between cardiovascular disease and CKD**
- Is an endogenous inhibitors of nitric oxide synthases.
- Higher ADMA levels hamper the generation of **endothelial nitric oxide** and promote the development of **endothelial dysfunction and hypertension in CKD.**
- ADMA impairs endothelial function by diminishing arterial endothelial nitric oxide synthase (eNOS) phosphorylation via the inhibition of Ca/calmodulin-dependent protein kinase CaMKII.



Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

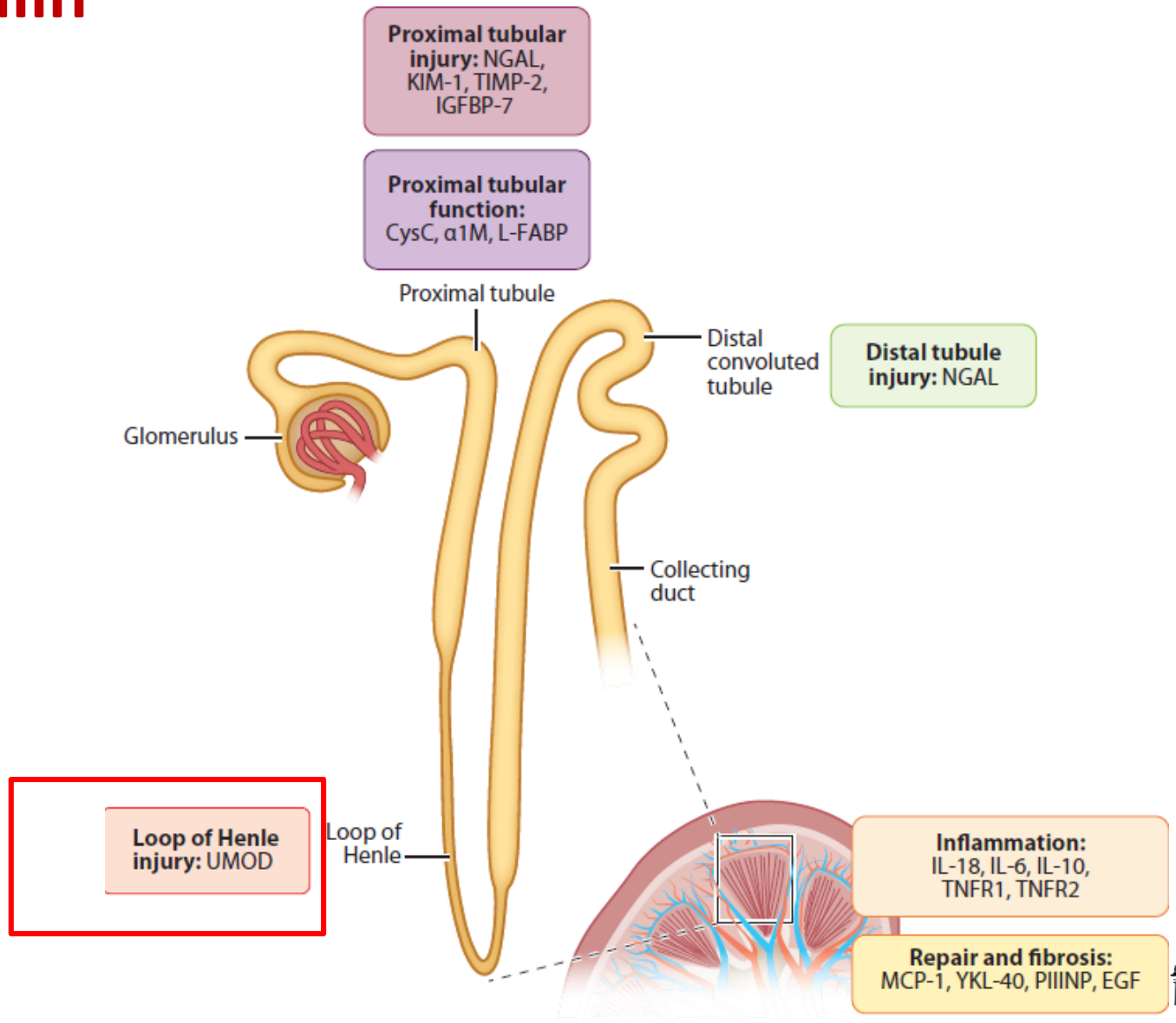
Asymmetric dimethylarginine (ADMA) as an important risk factor for the increased cardiovascular diseases and heart failure in chronic kidney disease

Xiaohong Liu[a,**,1], Xin Xu[b,1], Ruru Shang[a], Yingjie Chen[c,*]

[a] VIP Department, Shanxi Provincial People's Hospital, Taiyuan, China
[b] Department of Exercise Rehabilitation, Shanghai University of Sport, Shanghai, 200438, China
[c] Cardiovascular Division and Lillehei Heart Institute, University of Minnesota, Minneapolis, MN55455, USA

Ueda, S. et al Nephrology **2007**

Uromodulin


Proximal tubular injury: NGAL, KIM-1, TIMP-2, IGFBP-7
Proximal tubular function: CysC, α1M, L-FABP
Proximal tubule
Distal convoluted tubule
Distal tubule injury: NGAL
Glomerulus
Collecting duct
Loop of Henle injury: UMOD
Loop of Henle
Inflammation: IL-18, IL-6, IL-10, TNFR1, TNFR2
Repair and fibrosis: MCP-1, YKL-40, PIIINP, EGF

Uromodulin

- Uromodulin (UMOD) is an 85-kDa glycoprotein exclusively produced by cells of the thick ascending limb of Henle.

- Studies in animal models and clinical settings have demonstrated its ability to serve as a **biomarker for tubular mass** and **function** and accordingly, UMOD has been shown to be inversely associated with many kidney disease states:

  - a marker for the number of remaining functional tubules.

SteublD, et al. Medicine 95,2016. :e3011

New emerging biomarkers in the diagnosis of CKD and in the prediction of outcome

- KIM-1
- NGAL
- TIMP2-IGFBP7
- ASYMMETRIC DIMETHYLARGININE (ADMA)
- Symmetric Dimethylarginine (SDMA)
- Uromodulin
- β2M beta-2 microglobulin
- **miRNA, ncRNA, lncRNA**
- **Metabolomic biomarkers**

Proteomic and Metabolomic Biomarkers

- 287 disease-specific biomarkers for focal segmental glomerulosclerosis (FSGS)
- 291 for minimal change disease (MCD)
- 311 for membranous nephropathy (MN)
- 172 for lupus nephritis (LN)
- 509 for renal vasculitis
- 116 for IgA nephropathy (IgAN)
- 619 diabetic nephropathy and nephrosclerosis (DN&N)-specific biomarker

Jacek Rysz, Int. J. Mol. Sci. 2017, 18, 1702;

Proteomic and Metabolomic Biomarkers

- In a majority of CKD types, altered abundance of collagen fragments has been observed

- Urinary collagen fragments are early biomarkers for CKD (in patients with GFR > 60 mL/min/1.73 m2)

- However, they are not good markers in more advanced stages

Jacek Rysz, Int. J. Mol. Sci. 2017, 18, 1702;

Cell cycle Regulators and Wnt-beta Catenin signalling in AKI

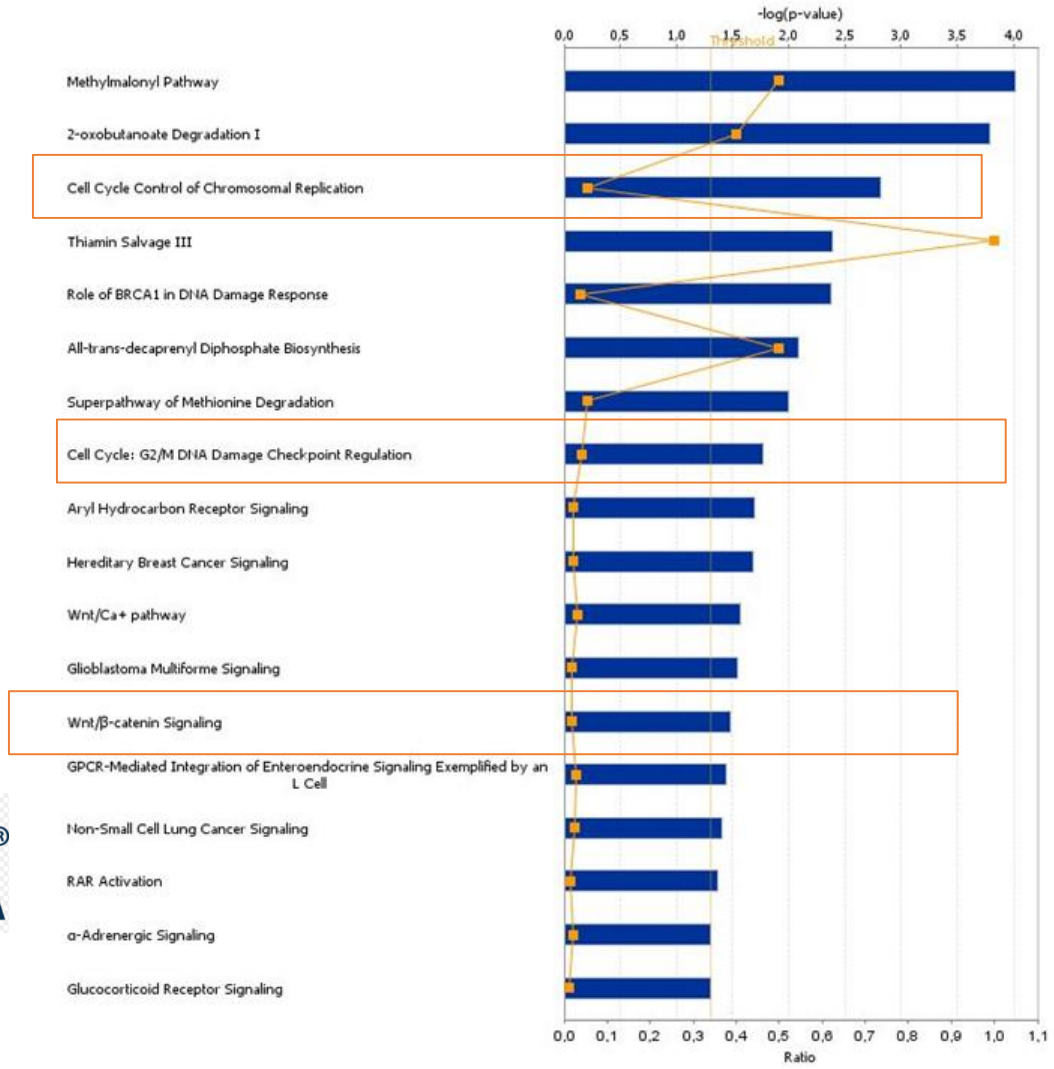


Fig.S1

IPA®

Castellano G, Franzin et al, Under Review

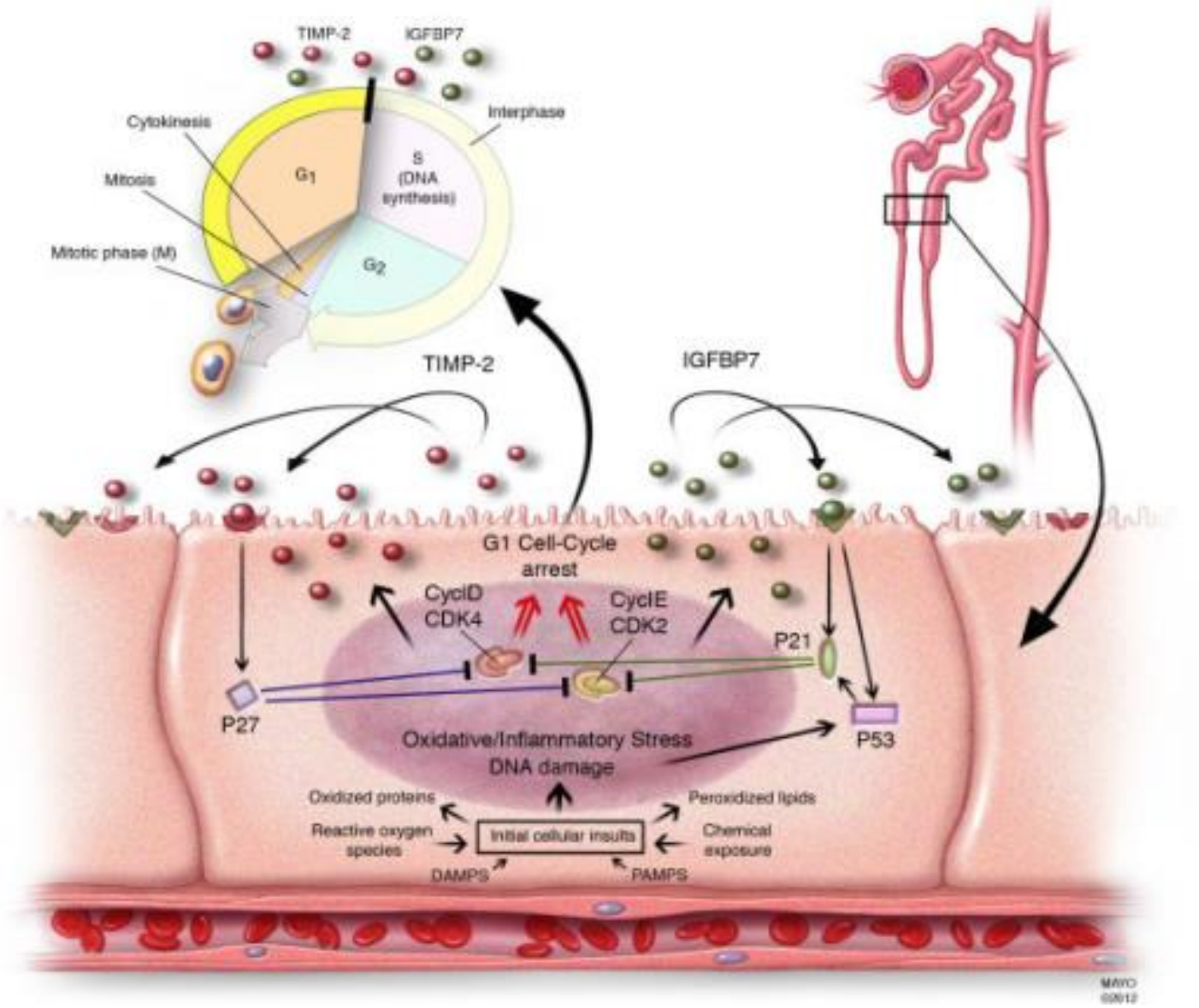
TIMP-2 /IGFBP-7

- **Both IGFBP-7 (through p523 and p21) and TIMP-2 (through p27) block the effect of cyclin-dependent protein kinase complexes and cause short periods of G1 cell-cycle arrest .**

- These biomarkers were originally discovered in the clinical setting of critical illness and have been approved by the FDA for use in conjunction with clinical evaluation in intensive care unit patients who have acute cardiovascular and/or respiratory compromise.

Price PM, Kidney Int 2009.
Boonstra J,Post JA. Gene 2004

TIMP-2 /IGFBP-7



Sharfuddin, A. A. & Molitoris, B. A. Nat. Rev. Nephrol. 7, 189–200 (2011)
Kashani k, Critical CARE 2013

AKI

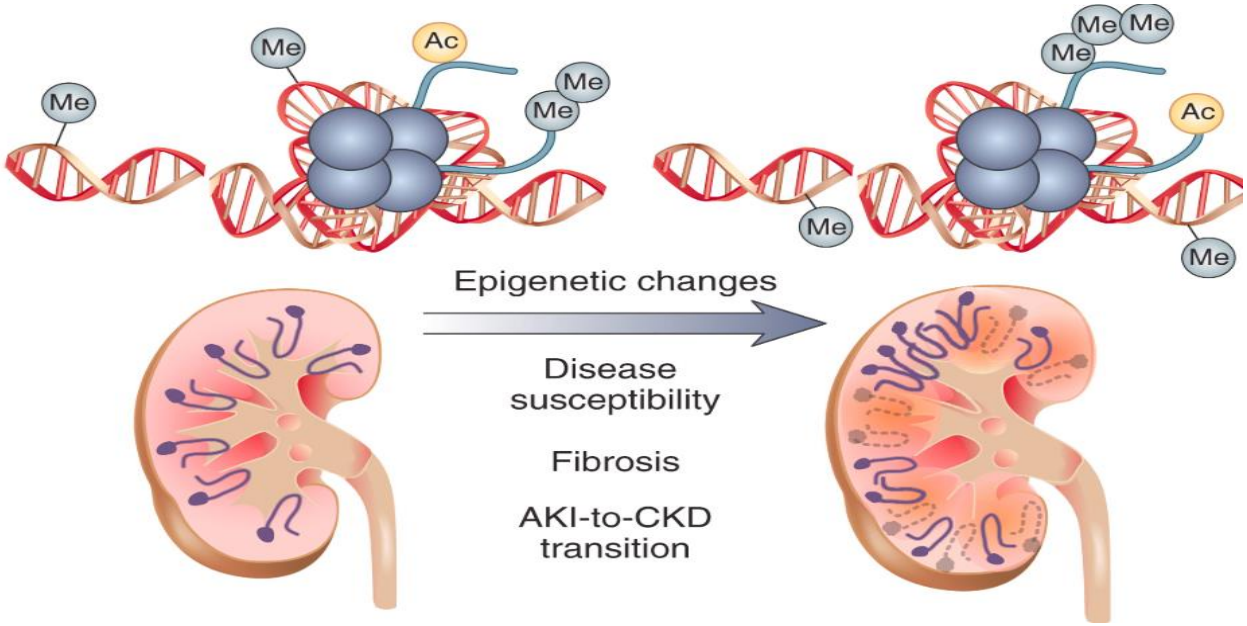
- **Endothelial dysfunction and microvascular rarefaction**
  - renal hypoxia
  - ↓ nitric oxide production
  - EndMT
  - Pericyte-myofibroblast transition
- **Incomplete regeneration of tubular cells**
  - EMT
  - G2/M cell cycle arrest and ↑ production of pro-fibrotic factors (TGF-ß, CTGF)
- **Persistent chronic inflammation**
  - switch M1→ M2 macrophage phenotype
  - ↑ complement cascade
  - Adaptive immune system response
- **Mitochondrial dysfunction**
- **Epigenetic modifications**
  - DNA methylation
  - histone changes
  - expression of specific microRNA
- **RAS activation**
  - ↑ urinary angiotensinogen as AKI-to-CKD biomarker
- **Cell and tissue senescence**
  - activation of pro-senescent molecules (p21, p16*INK*4α)
  - ↓ α-Klotho levels

CKD

Fiorentino et al. Contrib Nephrol 2018

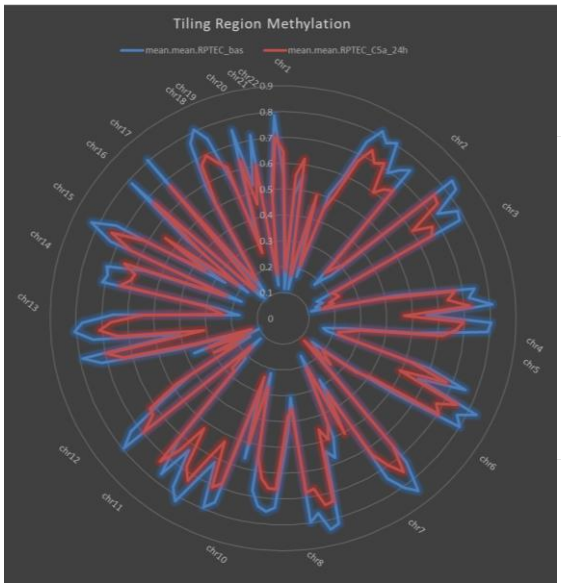
Epigenetic mechanisms are implicated in the AKI-to-CKD progression

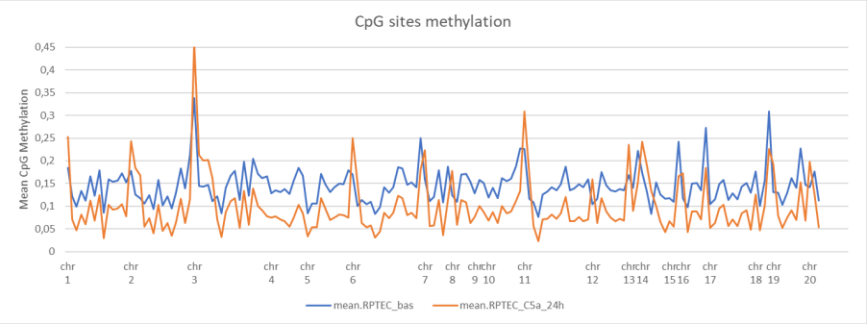
Epigenetics: The heritable mechanisms that control gene expression without changes in DNA sequence.



Christina M. Wyatt, KI 2018

C5a induces significant alterations in methylation profile

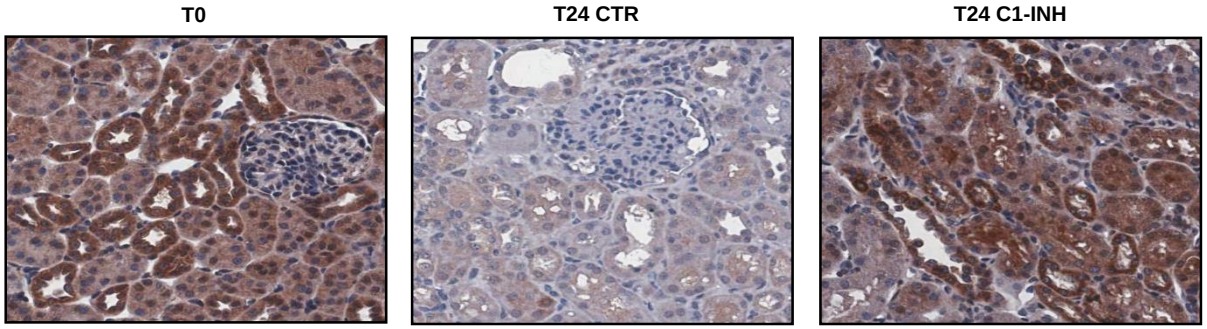




C5a induced a whole chromosomes hypomethylation

Castellano G, Franzin et al, Under Review

Klotho modulation during experimental AKI and DGF

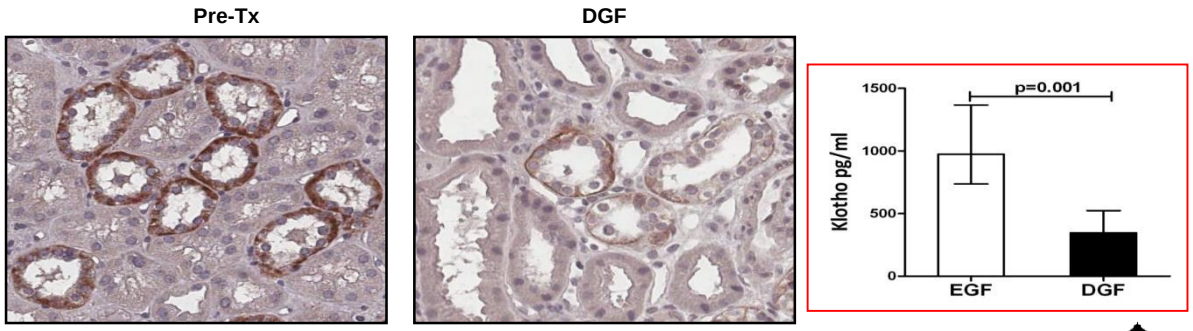


Swine model of I/R injury

I/R injury causes a reduction in renal Klotho expression in a Complement-dependent manner

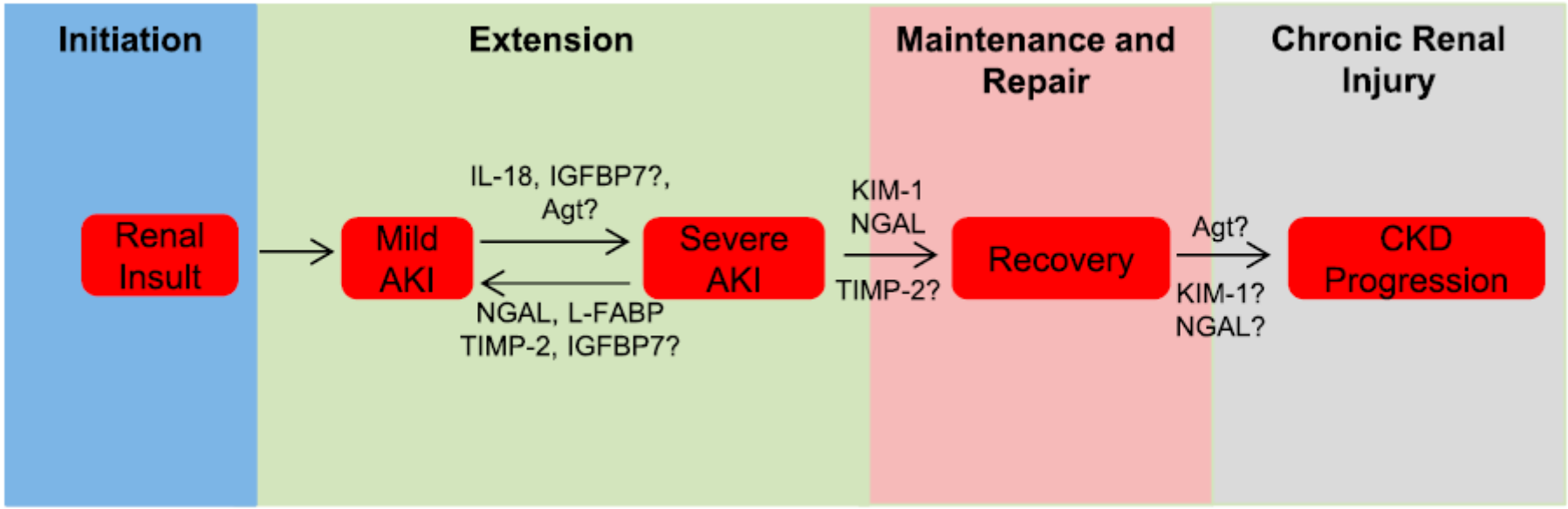
Transplant Patients

Patients with DGF develop a **persistent** deficiency in local and systemic production of soluble Klotho



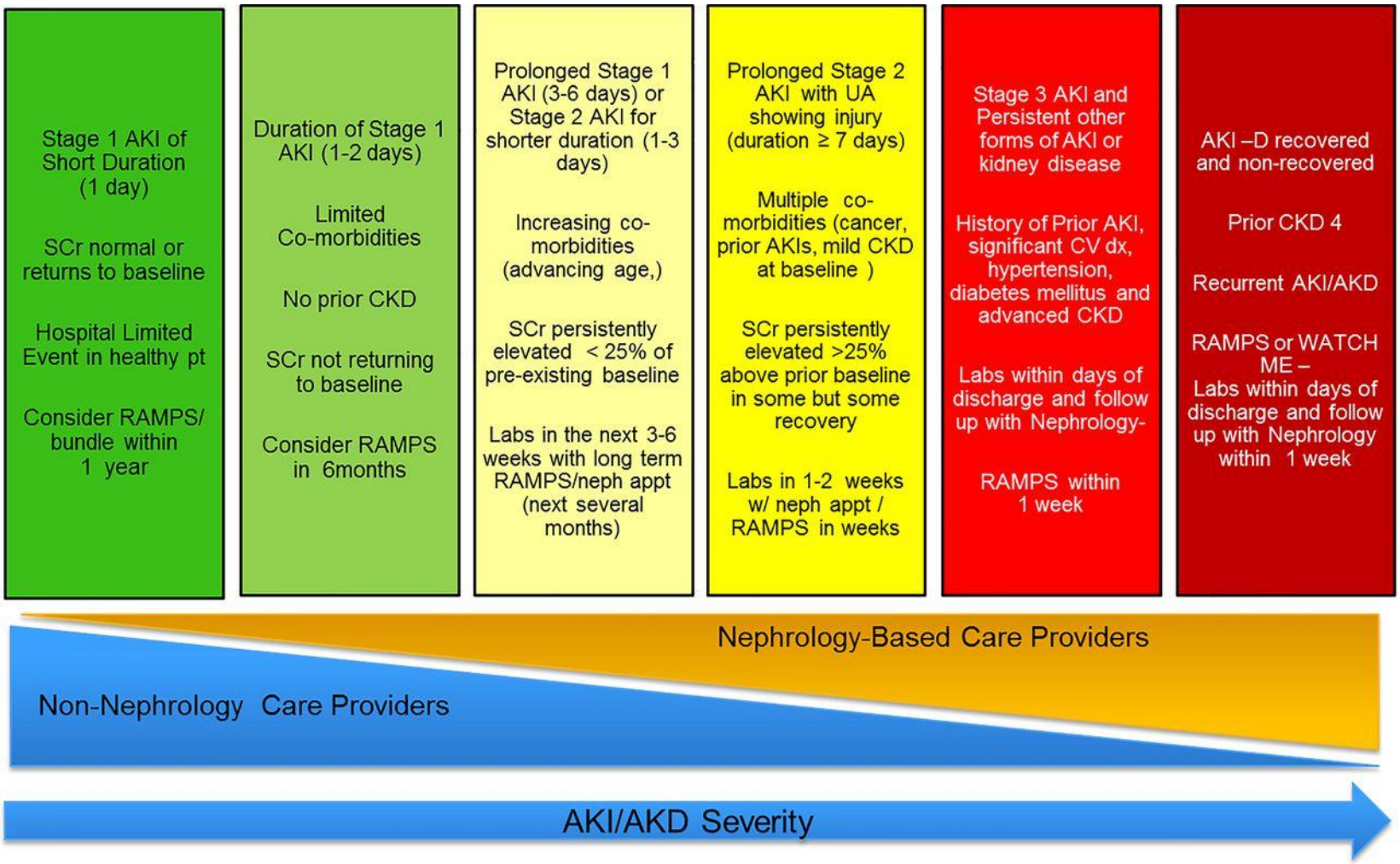
Castellano G, Intini A et al. Am J Transplant. 2016

Biomarker integrated model of AKI



Clin J Am Soc Nephrol 10: 147–155, January, 2015

Schematic for AKI/AKD follow-up.



Kianoush Kashani et al. CJASN doi:10.2215/CJN.01250119

CJASN

After an episode of AKI?

- Serial follow-up measurements of:
  - sCreatinine, Creatinine clearance, sCystatin C
  - Proteinuria:
    - strongly associated with cardiovascular risk and progression of CKD at all levels of GFR
  - Biomarkers of Tubulo-Interstitial:
    - Inflammation
    - Senescence
    - Fibrosis

→ **New Therapies**

Art is I, Science is We

THANK YOU

Giuseppe Castellano, MD, PhD

University of Bari, Dept of Emergency and Organ Transplantation