RENAL FUNCTION BIOMARKERS

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ARGENTINA

2015

NOVARTIS PHARMACEUTICALS

DISCLOSURES

- Served as a consultant and/or has received lecture honoraria from:

- ALEXION
- BRISTOL MYERS SQUIBB
- GENZYME
- NOVARTIS
- PFIZER

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RENAL FUNCTION BIOMARKERS

WHY DO WE NEED RENAL FUNCTION BIOMARKERS?

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RENAL FUNCTION BIOMARKERS

Biomarkers have been used to detect and monitor disease processes for over a century, although the term 'biomarker' first appeared as a MeSH term (Medical Subject Heading, used for indexing articles and books) only in 1989, when it was defined as a "*measurable and quantifiable biological parameter*."

Vasan, R. S. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation 113, 2335–2362 (2006)

This concept was developed further in an NIH working group report in 2001, which defined a biomarker as

"a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin. Pharmacol. Ther. 69, 89–95 (2001)

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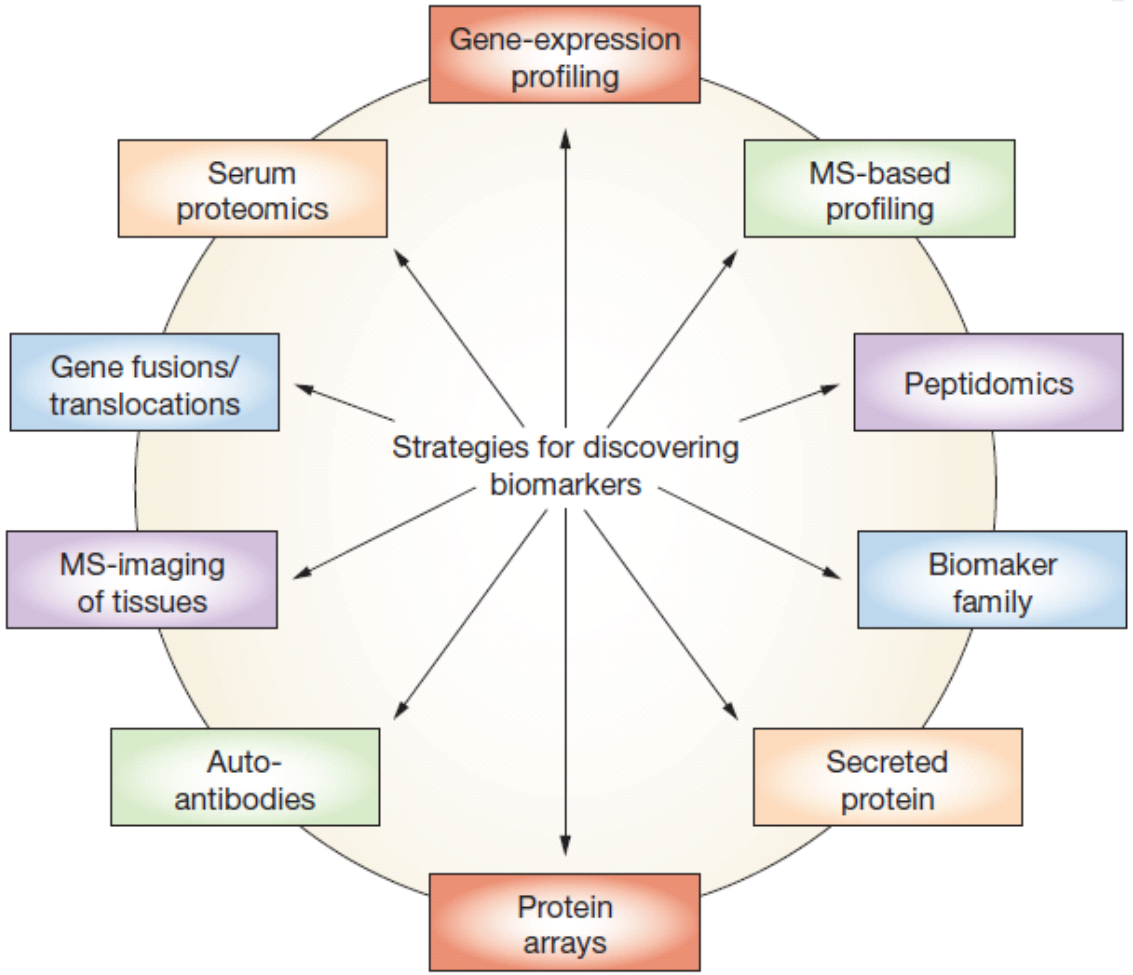
Biomarkers used in nephrology are parameters measured from blood or urine, although they can also be quantifiable measures derived from DNA analysis or biopsy specimens, or from any type of imaging modality.



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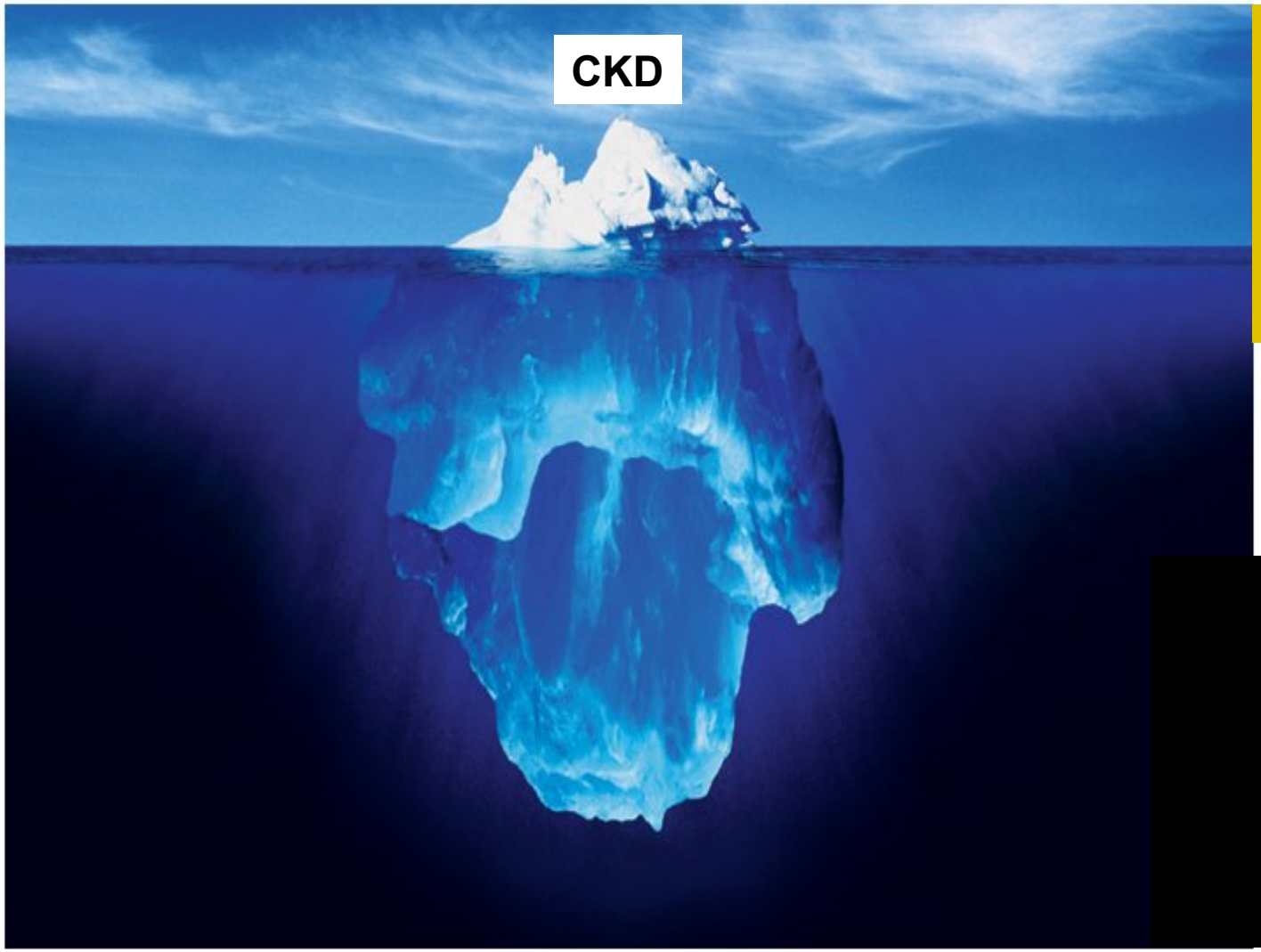
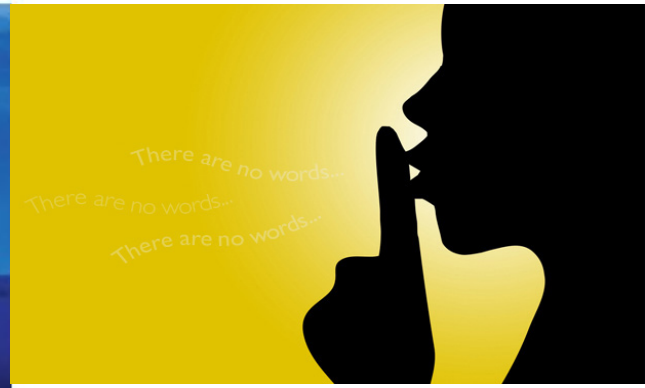
The emphasis on biomarkers for CKD has grown substantially since the first KDOQI guideline on this disease, due to the increasing prevalence of CKD and the availability of automated (eGFR) reporting to detect it.





Coresh, J. et al. Prevalence of chronic kidney disease in the United States. JAMA 298, 2038–2047 (2007).

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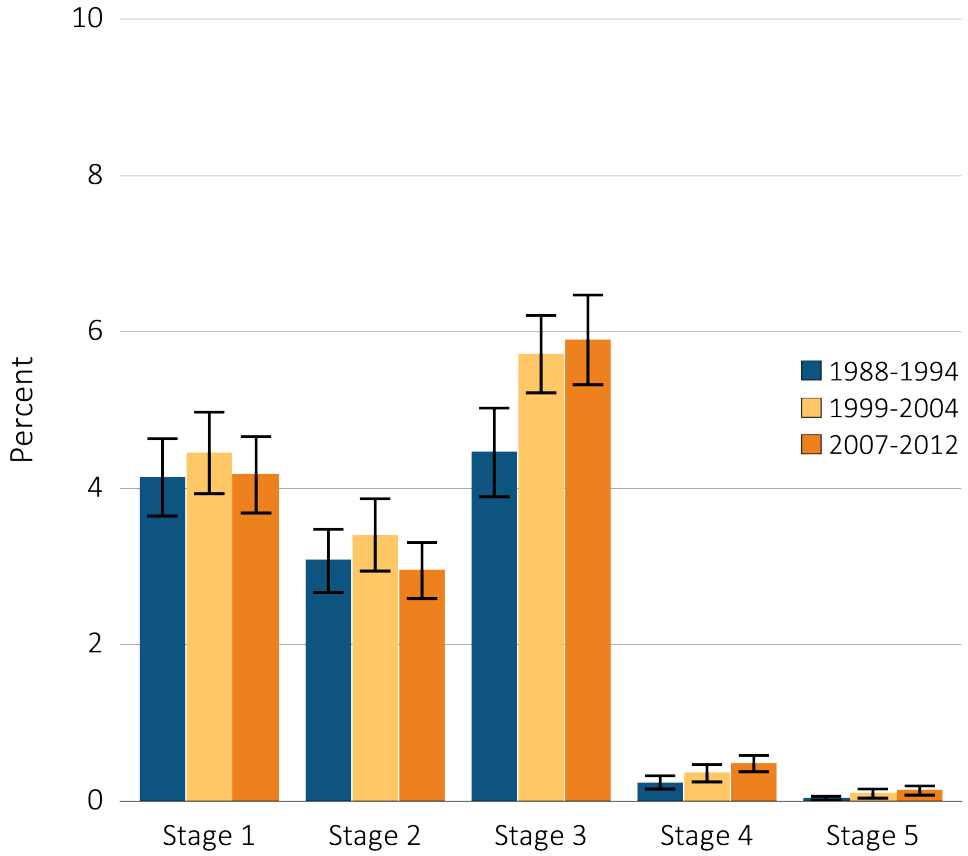
USRDS 2014 Prevalence of CKD by stage among NHANES participants, 1988-2012



Stages of CKD – KDOQI 2002 Definitions

Stage 1: eGFR ≥ 90 ml/min/1.73m^2 and ACR ≥ 30 mg/g

Stage 2: eGFR 60-89 ml/min/1.73m^2 and ACR ≥ 30 mg/g

Stage 3: eGFR 30-59 ml/min/1.73m^2

Stage 4: eGFR 15-29 ml/min/1.73m^2

Stage 5: eGFR < 15 ml/min/1.73m^2

Data Source: National Health and Nutrition Examination Survey (NHANES), 1988–1994, 1999-2004 & 2005–2012 participants age 20 & older. Whisker lines indicate 95% confidence intervals. Abbreviations: CKD, chronic kidney disease.

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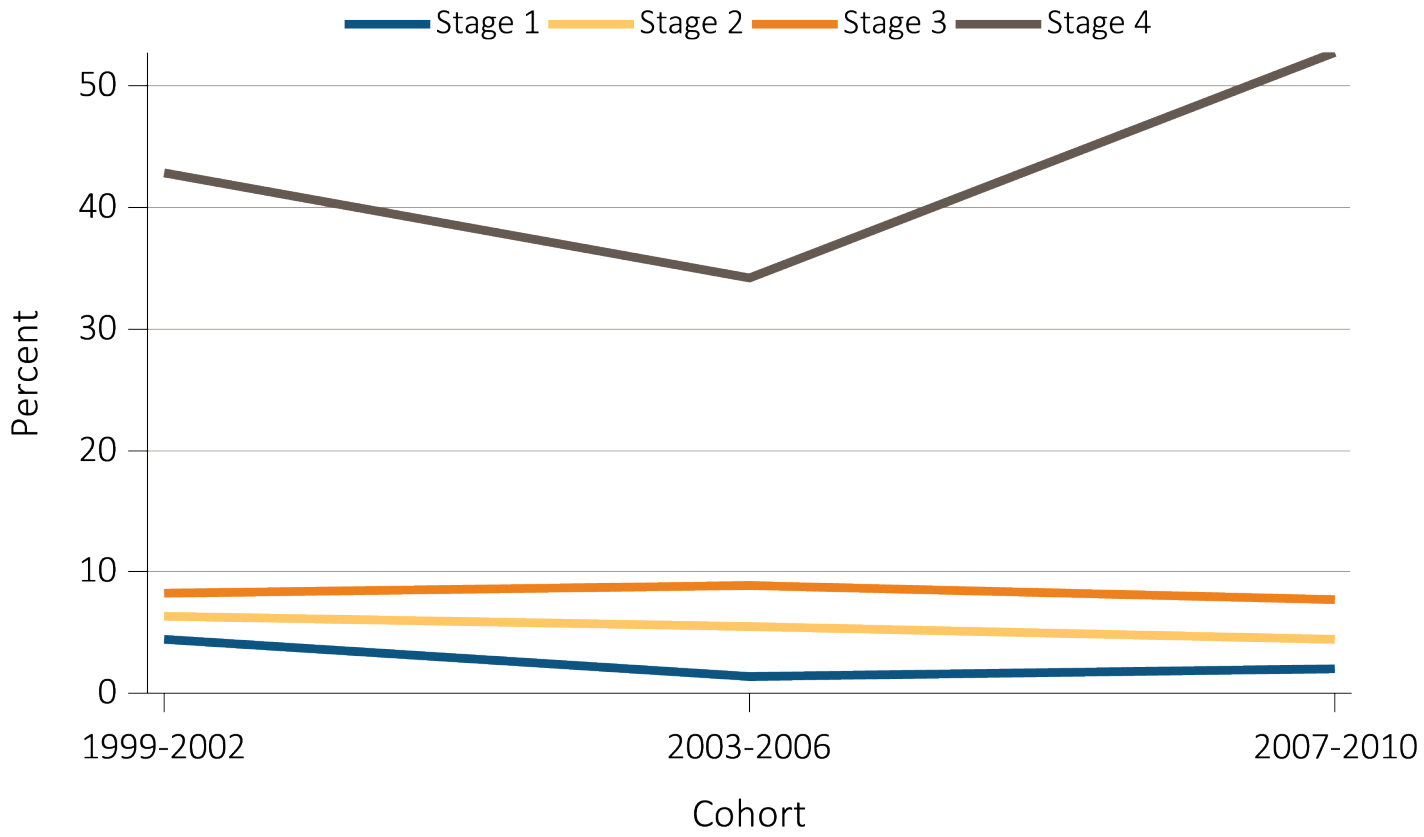
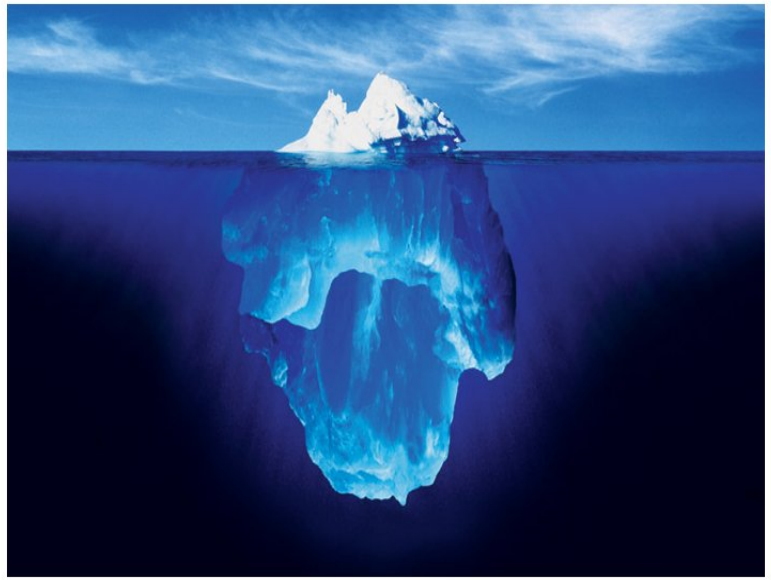
USRDS NHANES participants with CKD aware of their kidney disease, 1999-2010





Data Source: National Health and Nutrition Examination Survey (NHANES), 1988–1994, 1999-2004 & 2007–2012 participants age 20 & older. Abbreviations: CKD, chronic kidney disease.

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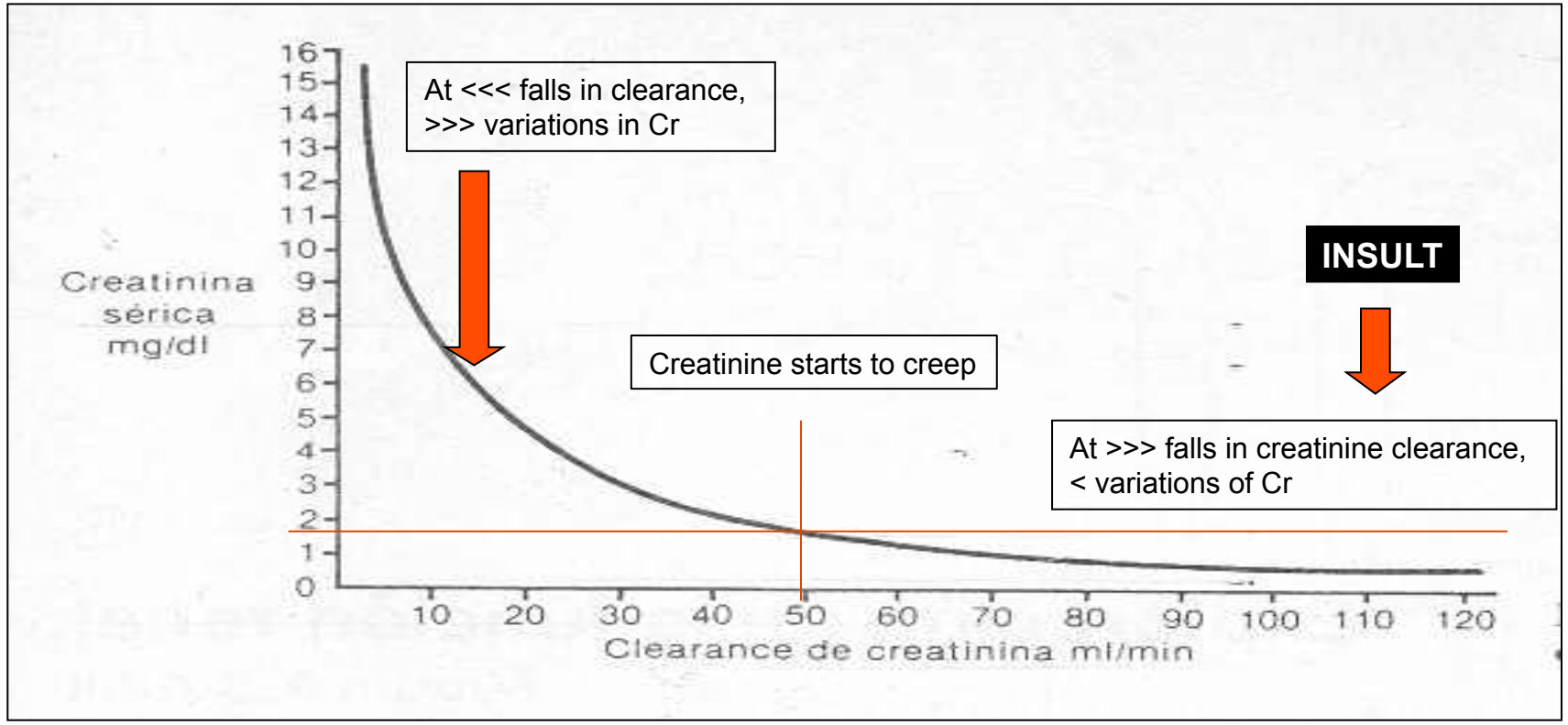
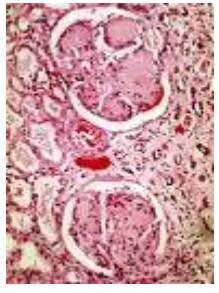
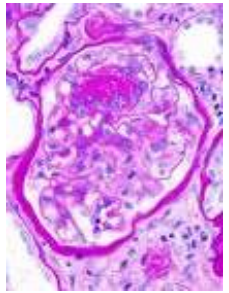
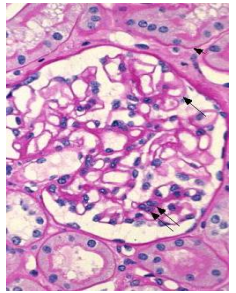


There are only two times in life
NOW and **TOO LATE**







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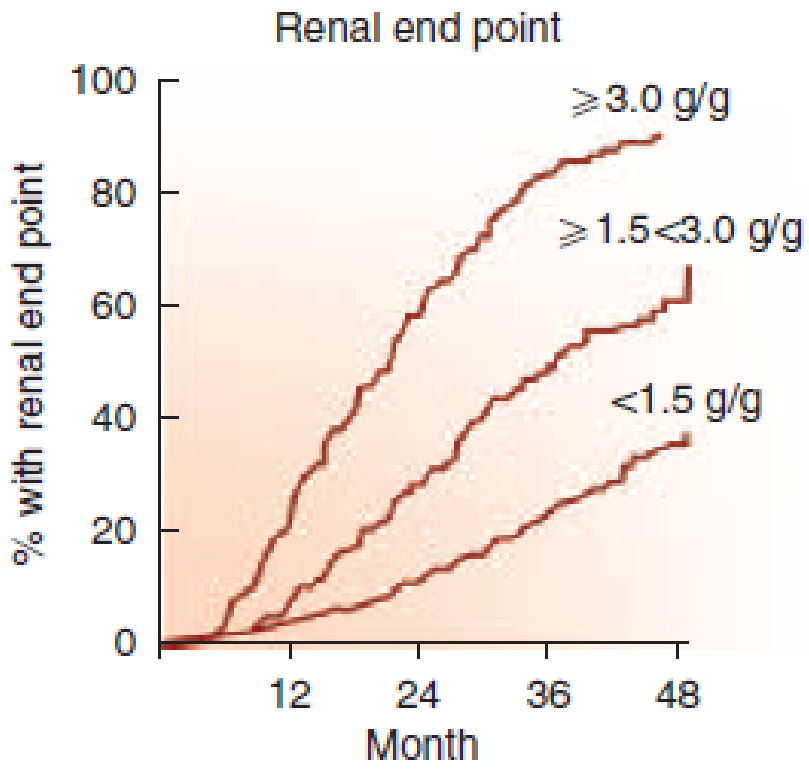

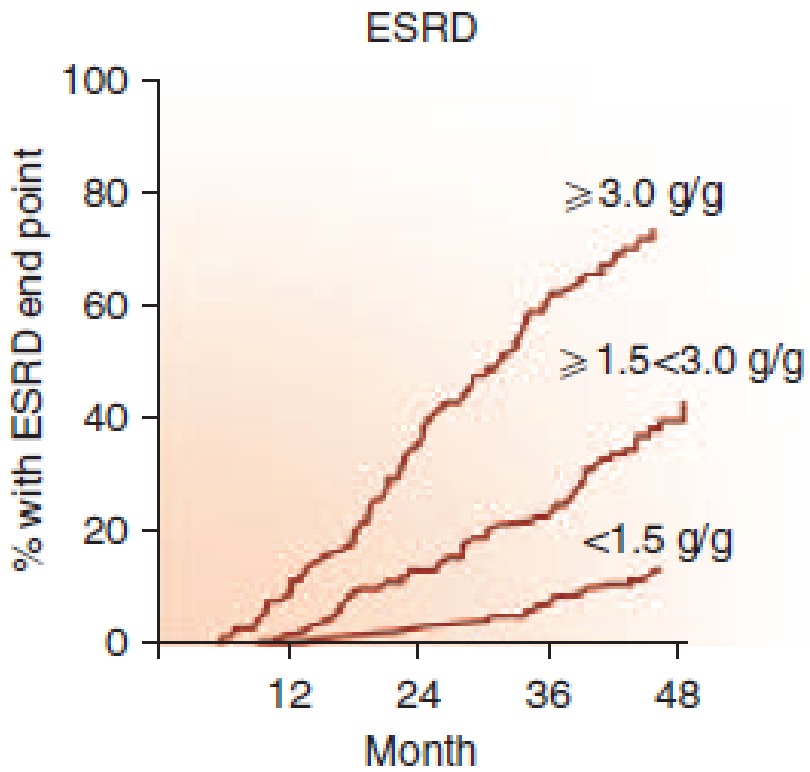

Lattanzio *Kidney International* (2010) **78,** 539–545;

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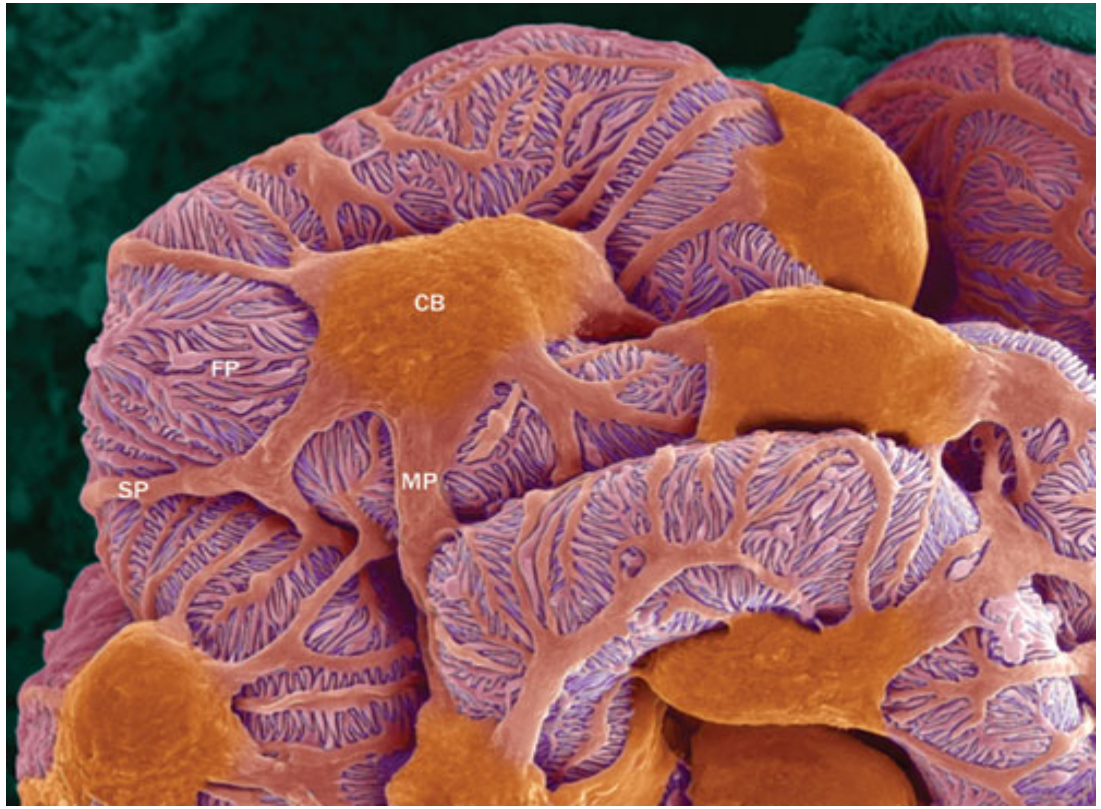
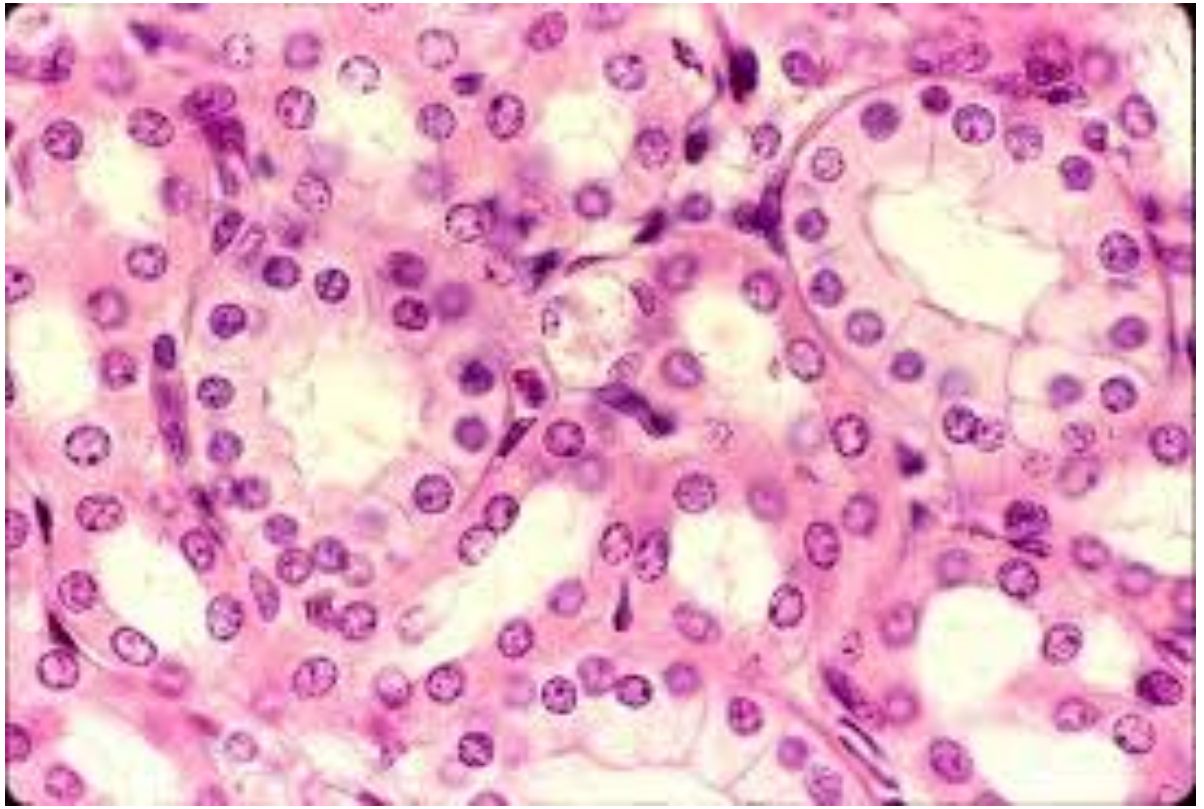
GOAL: ANTEDATE BIOCHEMICAL AVAILABLE TOOLS TO REDUCE KIDNEY DAMAGE AND IDEALLY PREVENT KIDNEY DISEASE, EITHER ACUTE OR CHRONIC





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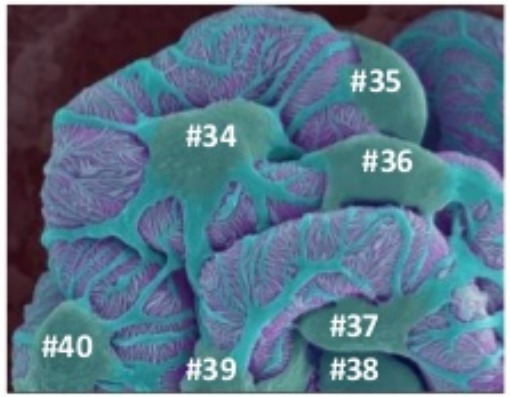
2%
Tryggvason 2011

Each Podocyte Counts!

1,200,000,000 glomeruli



Each glomerulus has 500-600 podocytes.

Podocytes do not efficiently proliferate.

Podocyte loss is cumulative in time

Once a glomerulus loses more than ~20% of its podocytes, it scars down. This injury is irreversible.

120 podocytes/glomerulus

200,000,000 of podocytes

With permission B Najafian FSIG Expert Fabry Conference San Diego 2014

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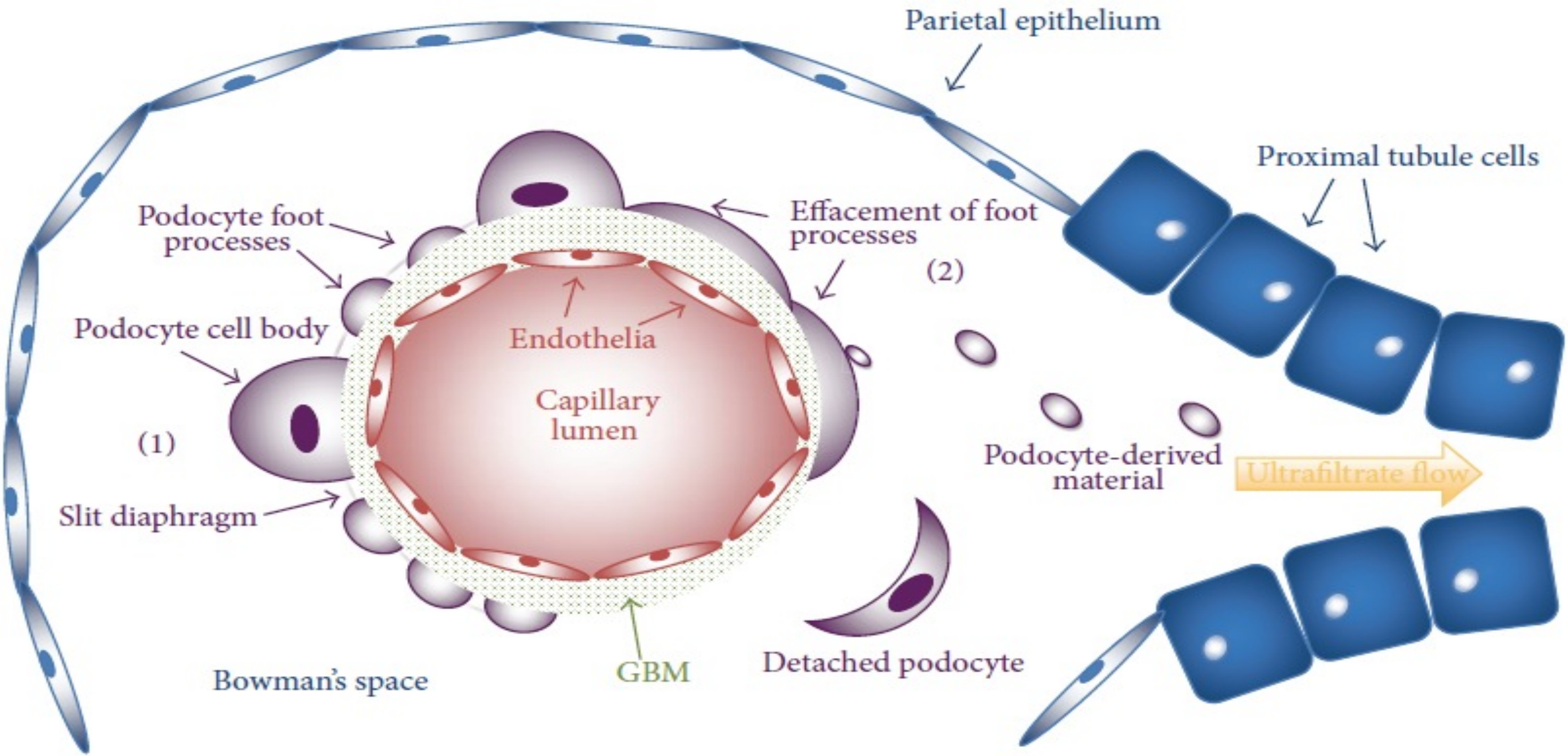

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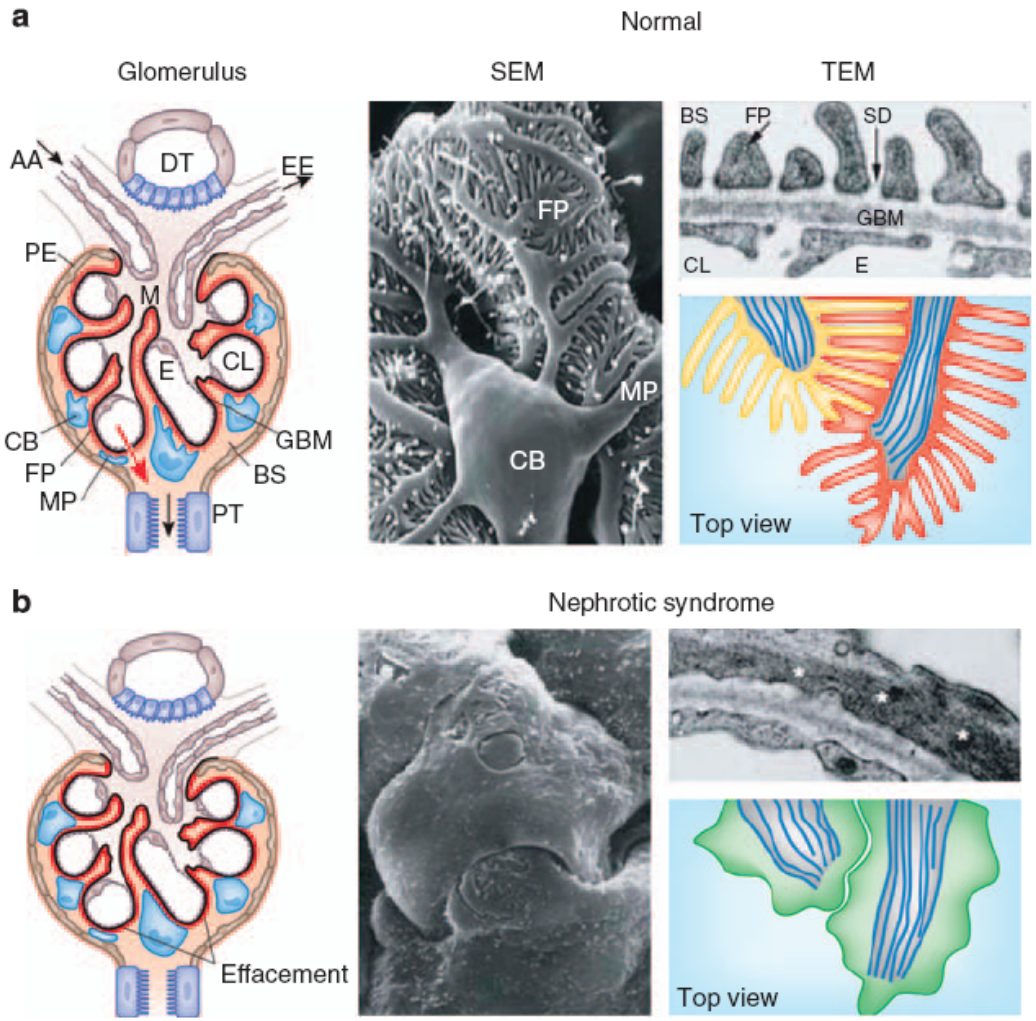

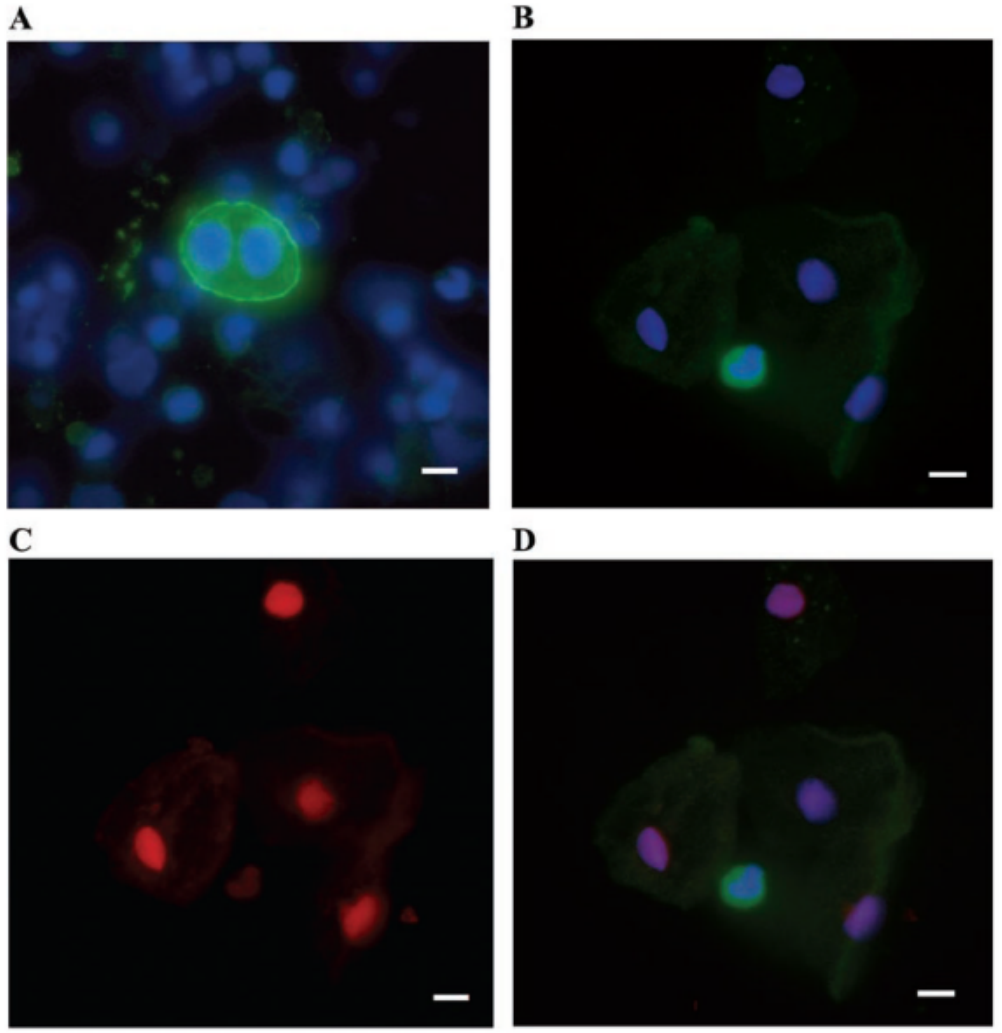

Trimarchi H et al. Study submitted to ERA-EDTA 2015

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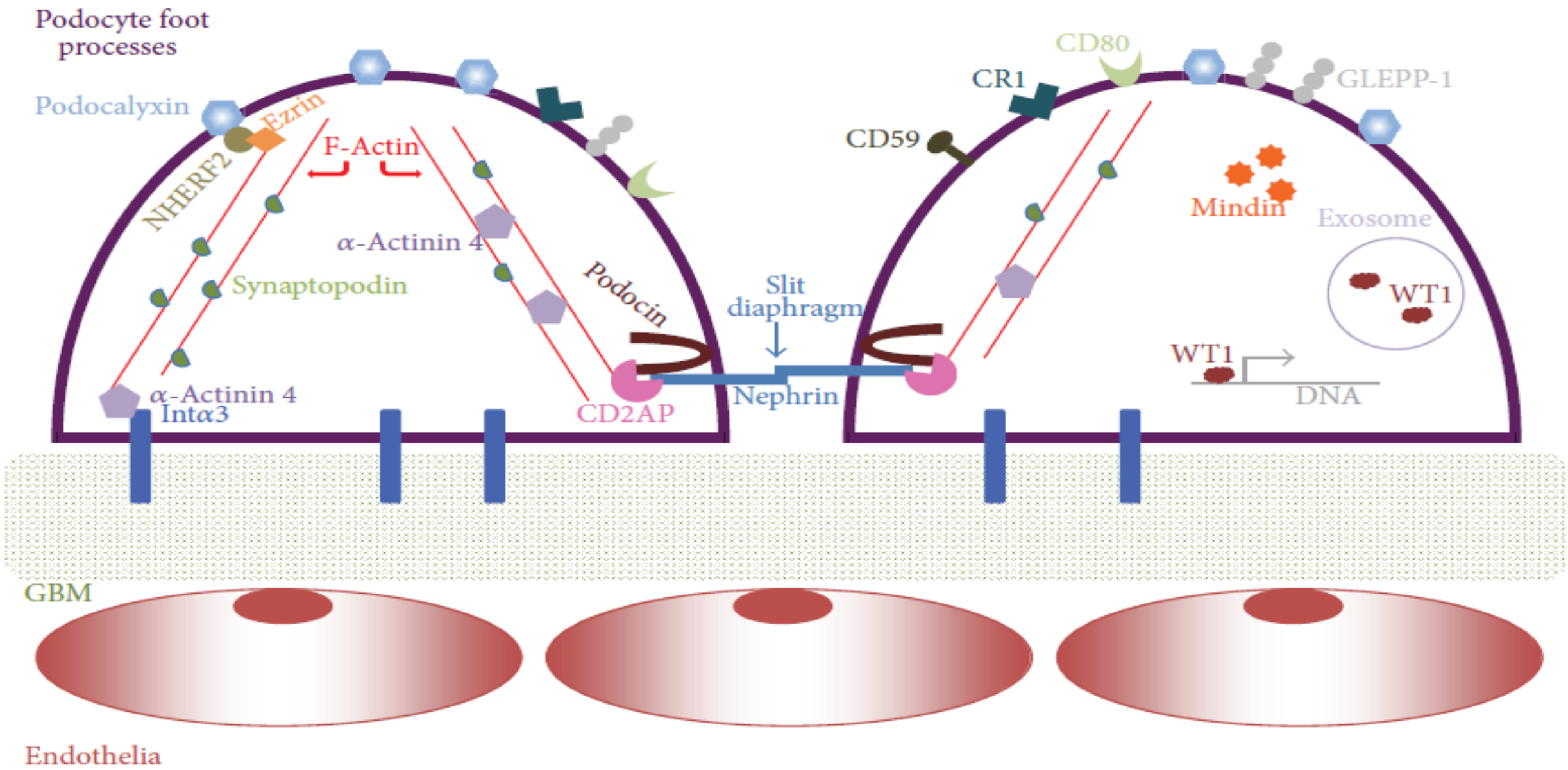
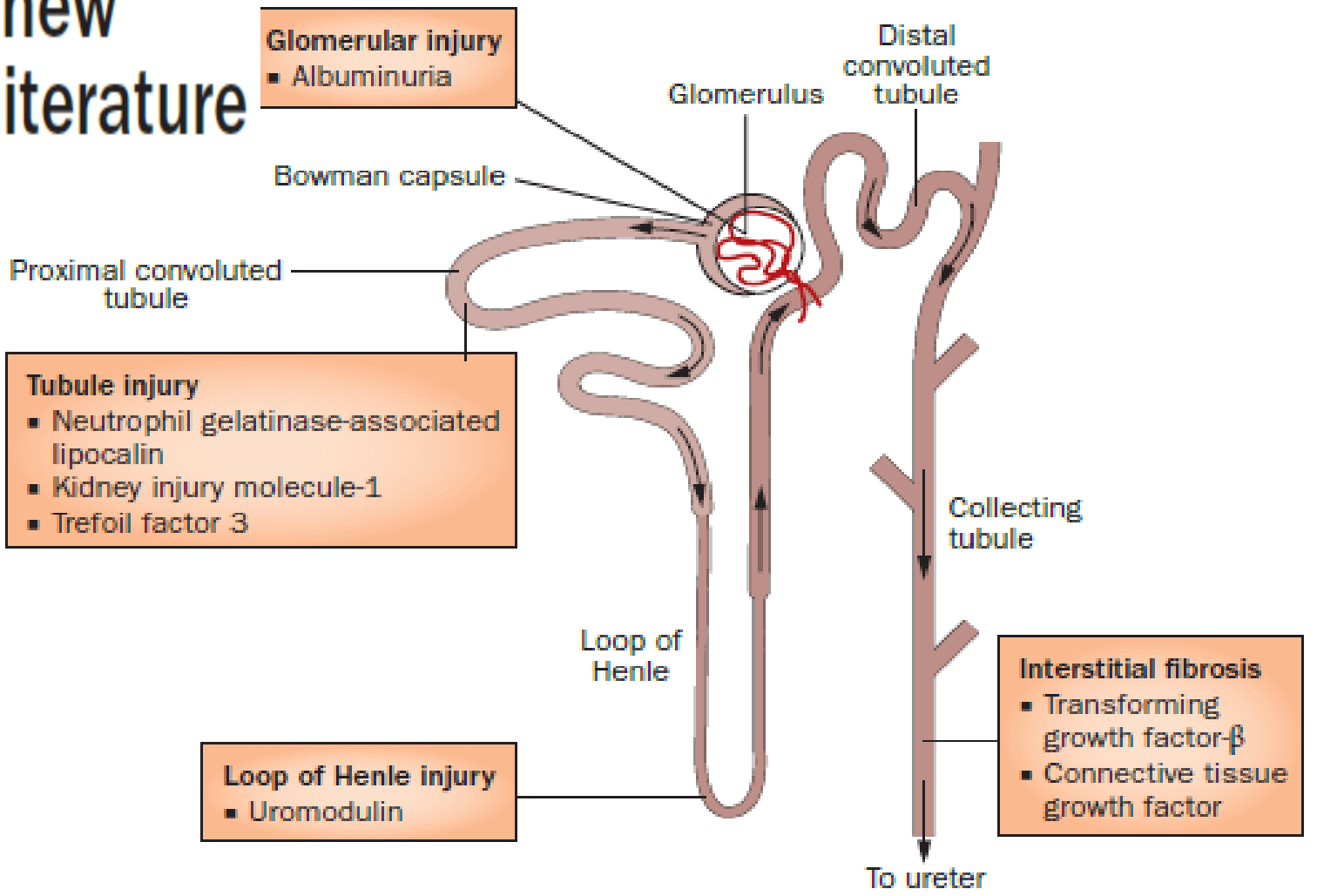


Sekulic M. Pathol Res Int 2013; ID782395

RENAL FUNCTION BIOMARKERS

Biomarkers for incident CKD: a new framework for interpreting the literature

Michael G. Shlipak and Erica C. Day



Shlipak, M. G. & Day, E. C. *Nat. Rev. Nephrol.* **9**, 478–483 (2013)

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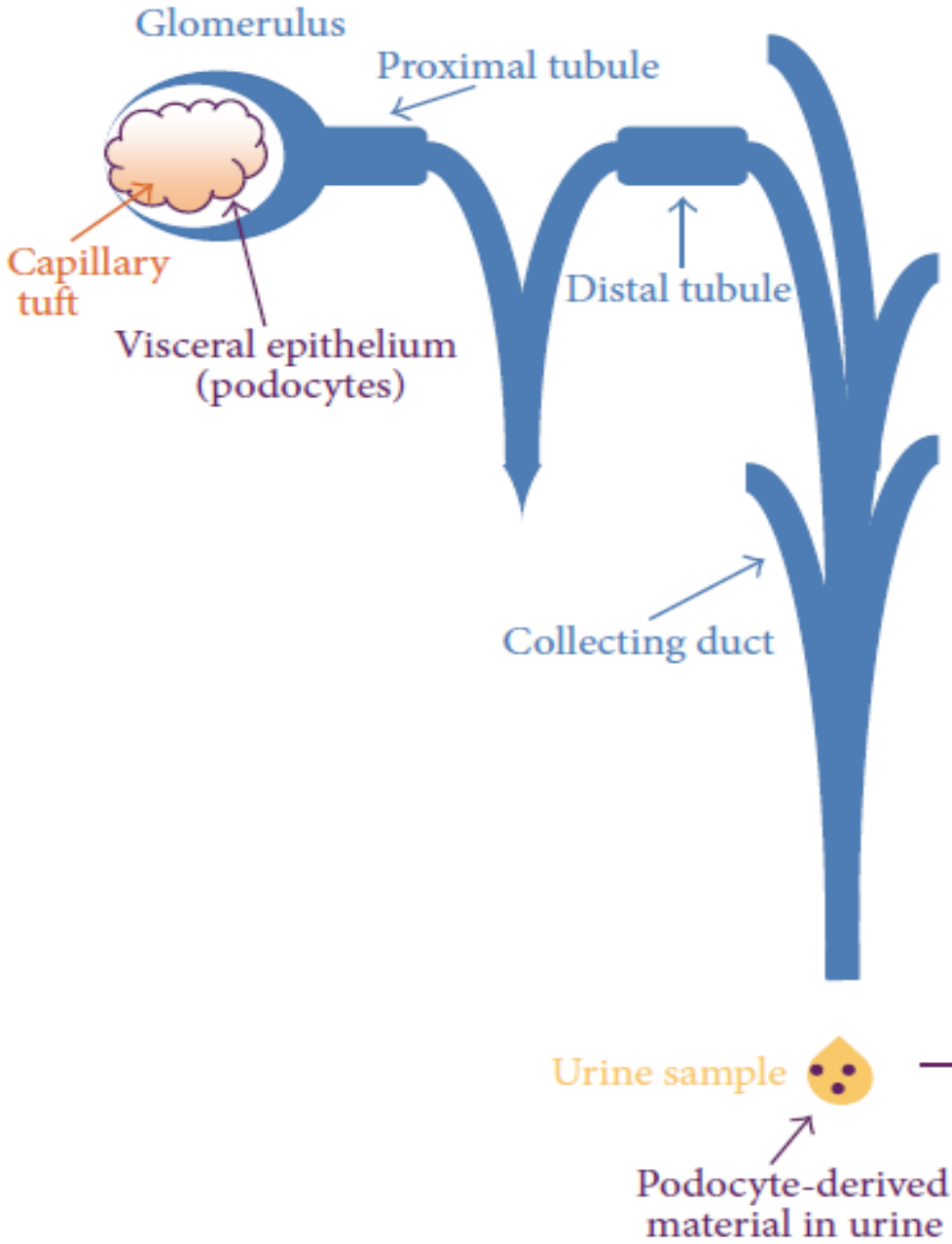

Identified by. . .

- Immunofluorescent microscopy
- Western blot
- Enzyme-linked immunosorbent assay (ELISA)
- Flow cytometry
- Mass spectrometry
- Polymerase chain reaction (PCR)

Look for. . .

Proteins:
  - podocalyxin, nephrin, podocin, CR1, CD80, synaptopodin, GLEPP-1, mindin, alpha 3 integrin, CD59, and Wilms tumor protein 1 (WT1)
- Messenger ribonucleic acid (mRNA):
  - nephrin, podocin, synaptopodin, podocalyxin, *CD2AP, ACTN4, PTPRO, WT1, and B7-1*
- Exosomal transcription factor (WT1)
- Podocalyxin positive granular structures (PPGS)

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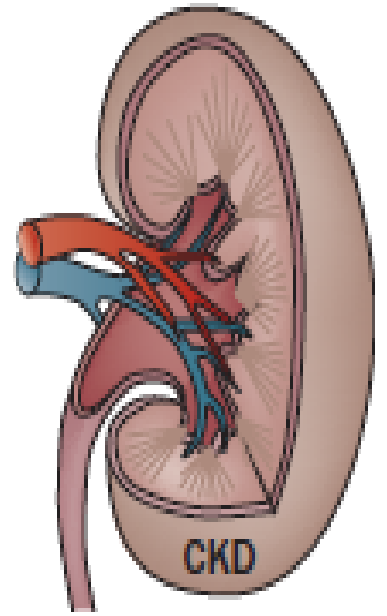
Systemic biomarkers
- Inflammation/procoagulant state
- RAAS activation

Susceptibility biomarkers
- Demographic characteristics: age, sex, ethnicity
- Genetic variation
- Environmental exposures: low birth weight, toxins

Kidney injury biomarkers
- Neutrophil gelatinase-associated lipocalin
- Kidney injury molecule-1
- Uromodulin
- Transforming growth factor-β
- Connective tissue growth factor

Markers of early loss of GFR
- Creatinine
- Cystatin C



Shlipak, M. G. & Day, E. C. *Nat. Rev. Nephrol.* **9**, 478–483 (2013)

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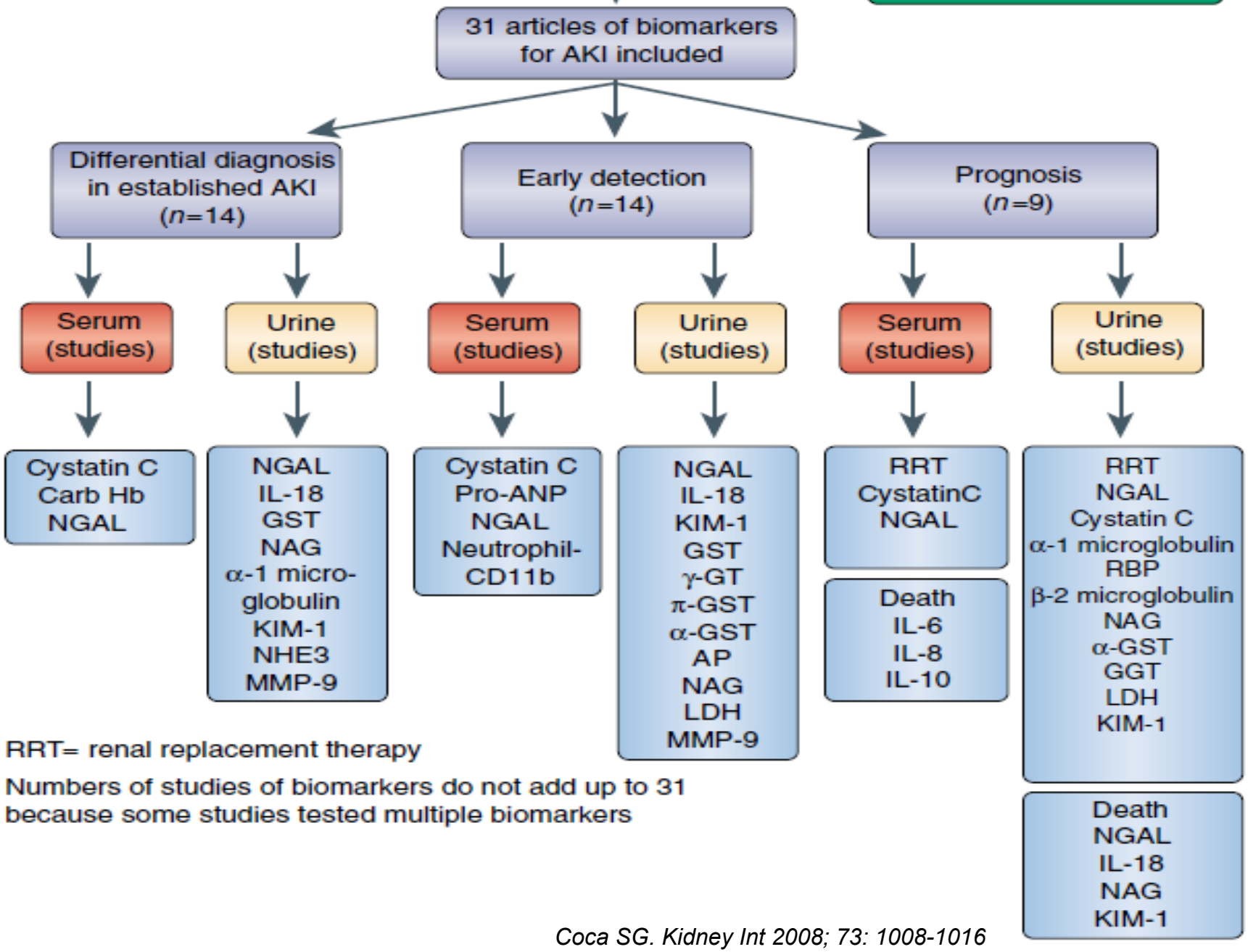

RRT= renal replacement therapy

Numbers of studies of biomarkers do not add up to 31 because some studies tested multiple biomarkers

Coca SG. Kidney Int 2008; 73: 1008-1016

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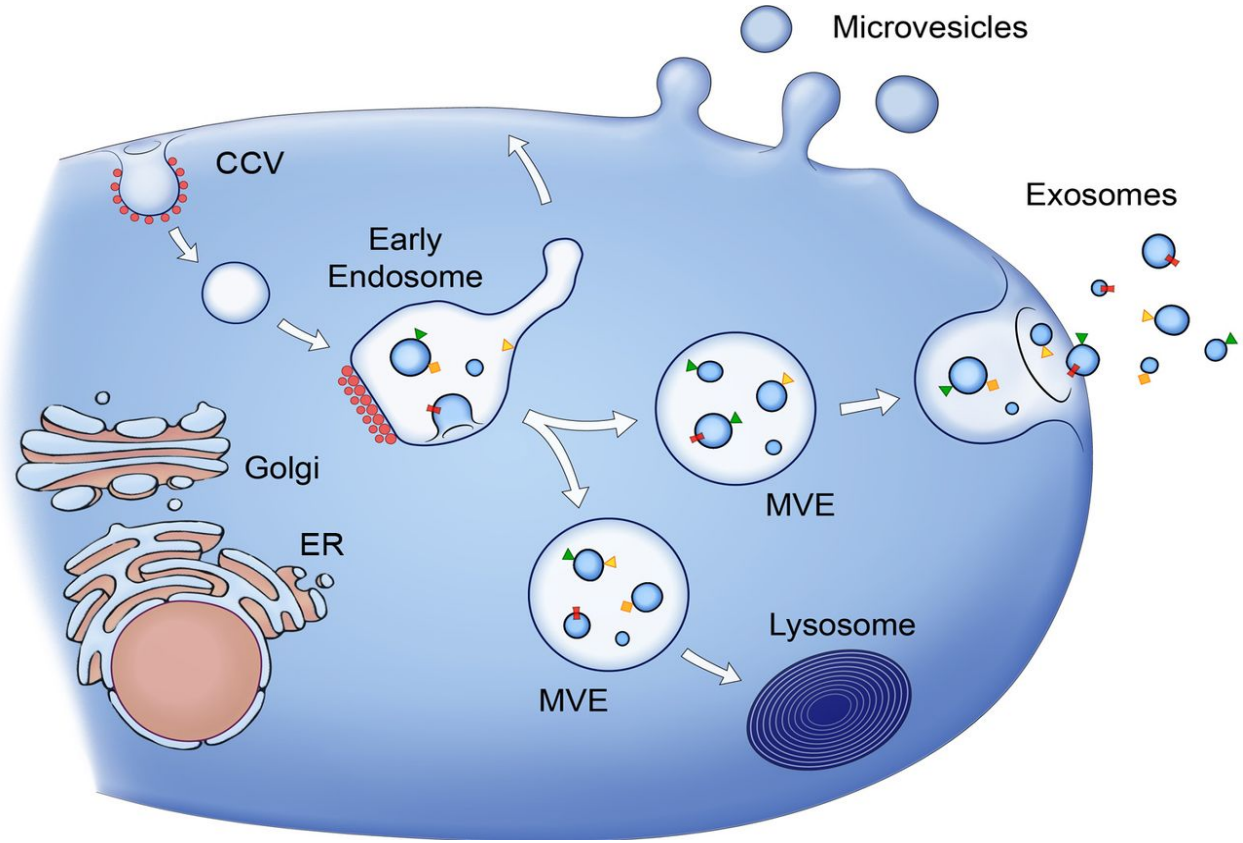
EXTRACELLULAR VESICLES (EVs)

Different types of vesicles: Exosomes (EXs), Ectosomes (MVEs), Apoptotic bodies:

EXs are 30–150nm vesicles derived from the inward budding of endosomal membranes, resulting in the progressive accumulation of intraluminal vesicles (known as EXs) within large multivesicular bodies.
Released by fusion with the plasma membrane

Ectosomes, also referred as Microvesicles (MVEs) are 100–1000 nm and are produced by the direct budding of the plasma membrane.

Dying cells also shed membranous vesicles, called apoptotic blebs, with heterogeneous shape and size.



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Hence, the different subcellular origin of EVs accounts for their specific composition and function.

EVs contain a specific subset of common proteins
related to **biogenesis and trafficking**
and also **a specific signature** from their **cell or tissue of origin**, including protein and nucleic acids.

Therefore, the study of the proteome and the nucleic acid content of EVs may provide information about the cell or tissue of origin and, importantly, their physiological state.

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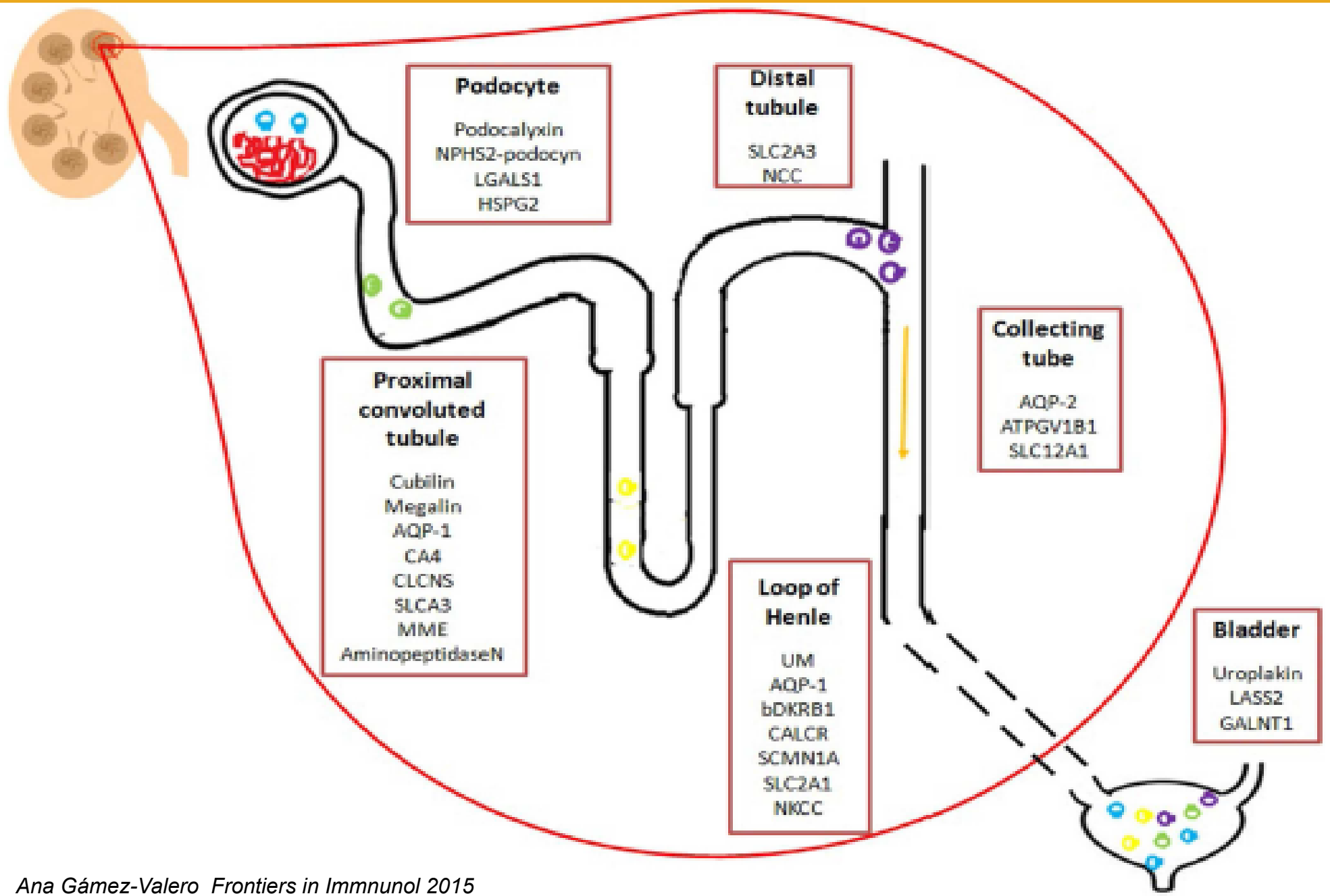

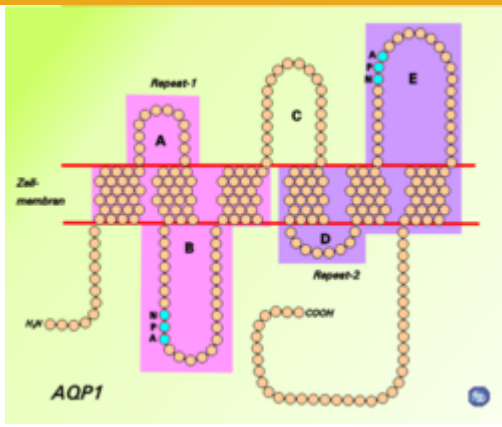

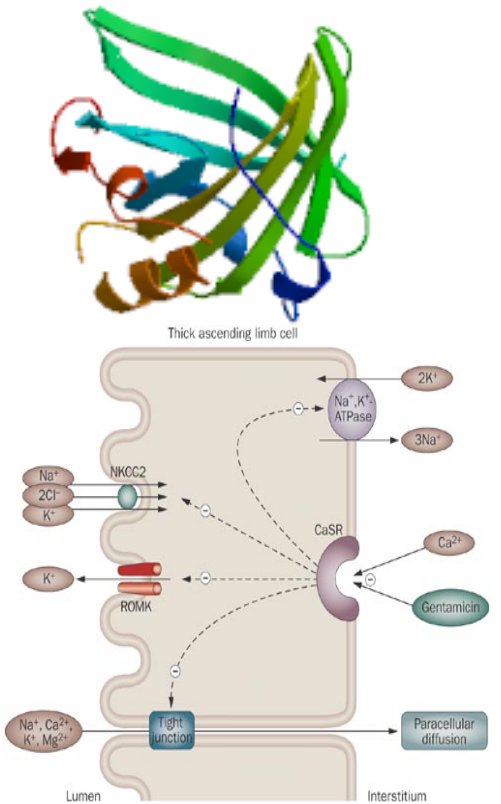

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Table 1. Detection of uEV markers of glomerular and tubular damage and fibrosis in human renal diseases and animal models

Disease	Humans/animal models	uEVs marker	References
Tubular damage			
Acute kidney injury	I/R injury in rats	ATF3	[18]
		Fetuin-A	[6]
		AQP1	[19]
	Cisplatin-induced AKI in rats	ATF3	[18]
		Fetuin-A	[6]
	Humans	ATF3	[18]
		Fetuin-A	[6]
		AFT3 (mRNA)	[20]
Kidney transplant	Humans	AQP1	[19]
		NGAL	[21]
		NKCC2/NCC	[22]
Glomerular injury and chronic renal damage			
Focal segmental glomerulosclerosis	PAN-induced glomerulosclerosis in rats	WT-1	[18]
	Podocin/Vpr transgenic mice	WT-1	[18]
	Humans	WT-1	[18]
Diabetes nephropathy	Humans	WT-1	[23]
		AMBP, MLL3 VDAC	[24]
		miR-145	[25]
IgA nephropathy	Humans	α-1-antitrypsin ceruloplasmin	[14]
Chronic kidney disease	Humans	CD2AP (mRNA)	[26]
		miR-29	[26]
		OPG	[27]
Obstructive nephropathy	Humans	TGFβ	[28]

Clin Kidney J (2015) 8: 23–30
doi: 10.1093/ckj/sfu136
Advance Access publication 30 December 2014

Ckj Review

Extracellular vesicles in the urine: markers and mediators of tissue damage and regeneration

Andrea Ranghino[1,2,*], Veronica Dimuccio[1,*], Elli Papadimitriou[1,*] and Benedetta Bussolati[1]

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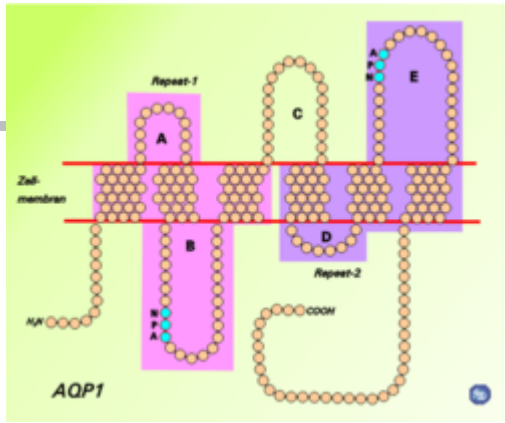
Am J Physiol Renal Physiol 297: F1006–F1016, 2009.
First published July 29, 2009; doi:10.1152/ajprenal.00200.2009.



Decreased abundance of urinary exosomal aquaporin-1 in renal ischemia-reperfusion injury

Hiroko Sonoda,[1] Naoko Yokota-Ikeda,[2] Sayaka Oshikawa,[1] Yosuke Kanno,[1] Kazuya Yoshinaga,[3] Kazuyuki Uchida,[4] Yuuji Ueda,[5] Kouichi Kimiya,[6] Shigehiro Uezono,[2] Akira Ueda,[2] Katsuaki Ito,[1] and Masahiro Ikeda[1]

Aquaporin-1 (AQP1), a water channel protein, is abundantly expressed in renal epithelial cells of the proximal tubules and the descending thin limb.

In renal ischemia-reperfusion, an important cause of AKI, **AQP1** is decreased.

AQP1 has been shown to be secreted into urine as an exosomal protein.

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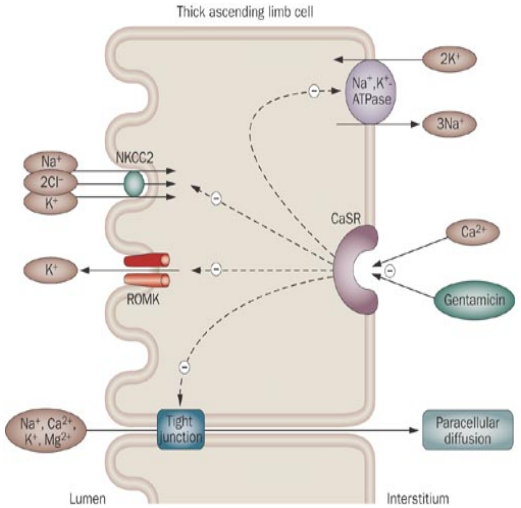
Renal Sodium Transporters Are Increased in Urinary Exosomes of Cyclosporine-Treated Kidney Transplant Patients

Am J Nephrol 2014;39:528–535

Cristina Esteva-Font[a, d] Elena Guillén-Gómez[a, d] Joan Manuel Diaz[b, d]
Luís Guirado[b, d] Carmen Facundo[b, d] Elisabet Ars[a, d] Jose A. Ballarin[c, d]
Patricia Fernández-Llama[c, d]



The content of **NKCC2** and Na-Cl co-transporter **(NCC)** in the uEVs in kidney transplanted patients was found to be significantly increased in cyclosporine-treated patients compared with the controls.

Cyclosporine-mediated hypertension could be acomplished via **NKCC2 and NCC** transporters

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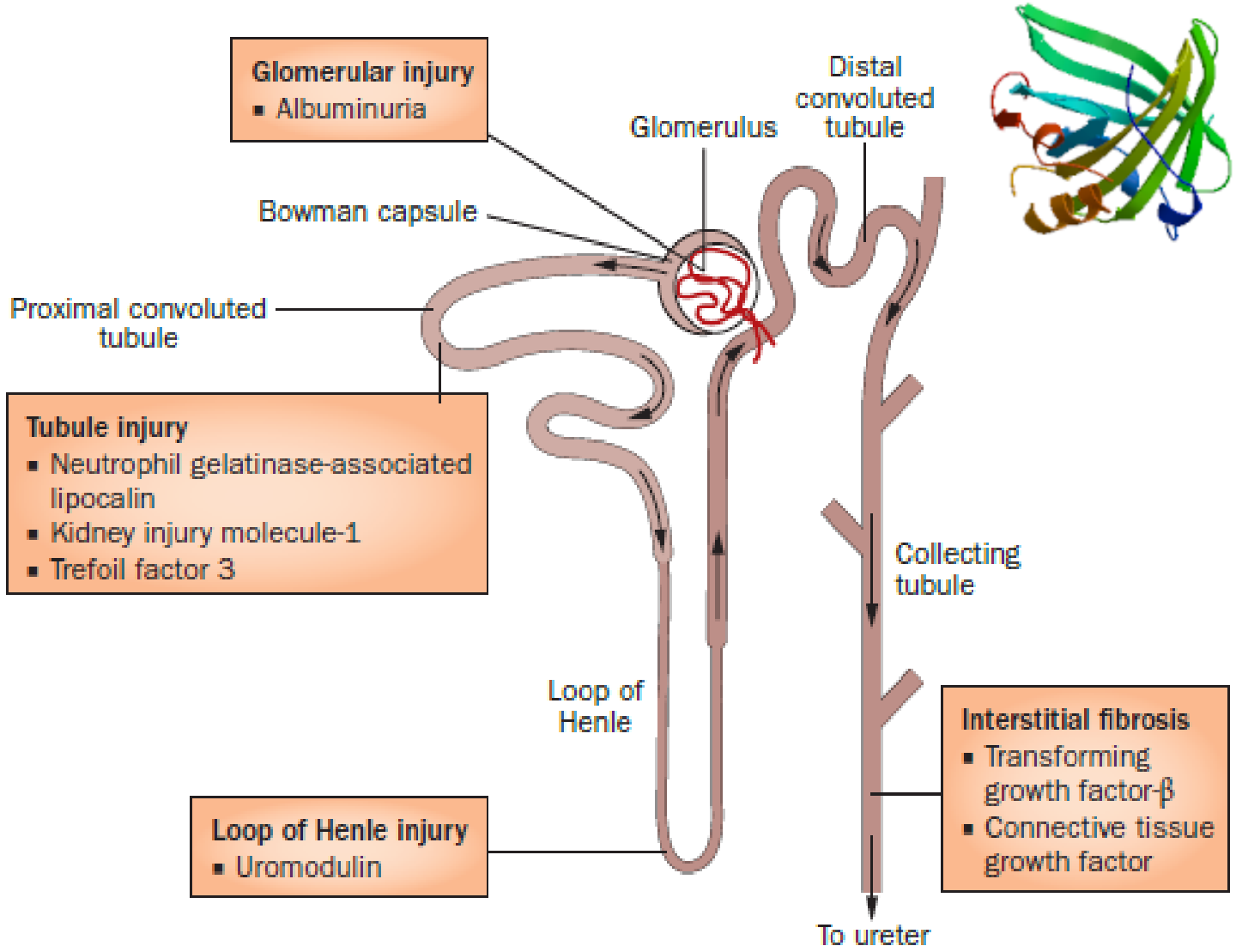
Analysing the **uEVs** collected from renal transplanted patients, neutrophil gelatinase- associated lipocalin (**NGAL**) protein, an emerging biomarker of AKI and of delay graft function, is abundant in the **uEVs** of all transplanted patients.

High levels of **NGAL** protein were found in the isolated **uEVs** as compared with the cellular fraction and were elevated in the **uEVs** of patients with DGF, suggesting that the exosomal **NGAL** might be a valid tool to evaluate the allograft damage.



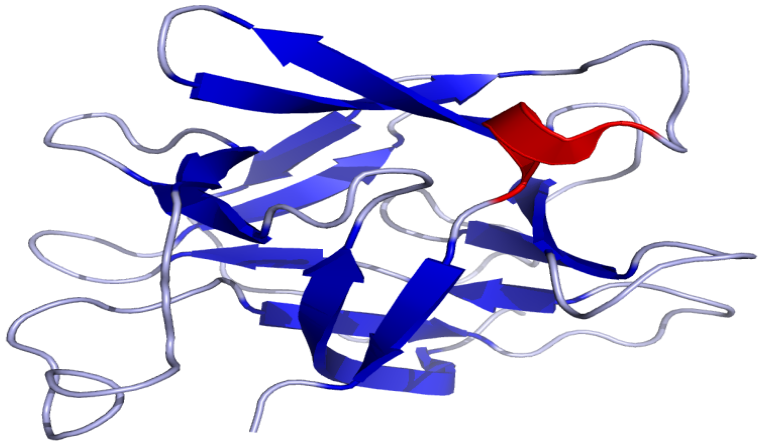
Alvarez S, Suazo C, et al. Transplant Proc 2013; 45: 3719–3723

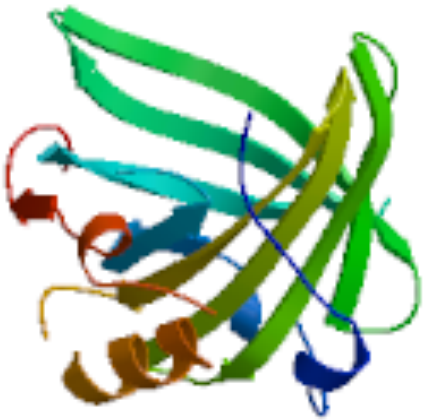
RENAL FUNCTION BIOMARKERS

IL-18 and Urinary NGAL Predict Dialysis and Graft Recovery after Kidney Transplantation

Isaac E. Hall,*† Sri G. Yarlagadda,‡ Steven G. Coca,*† Zhu Wang,*† Mona Doshi,§ Prasad Devarajan,‖ Won K. Han,¶ Richard J. Marcus,** and Chirag R. Parikh*†

J Am Soc Nephrol 21: 189–197, 2010





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"Current methods for predicting graft recovery after kidney transplantation are not reliable."

91 Deceased-donor kidney transplant patients to evaluate: NGAL, IL-18, and KIM-1 as biomarkers for predicting dialysis within 1 wk of transplant and subsequent graft recovery.

Urine samples were collected for 3 days after transplant and analyzed the biomarkers.

Classification of graft recovery:

delayed graft function (DGF) 34, slow graft function (SGF) 33, or immediate graft function (IGF) 24.

Median NGAL and IL-18 levels, but not KIM-1 levels, were statistically different among these three groups at all time points".

J Am Soc Nephrol 21: 189–197, 2010

NOVARTIS PHARMACEUTICALS

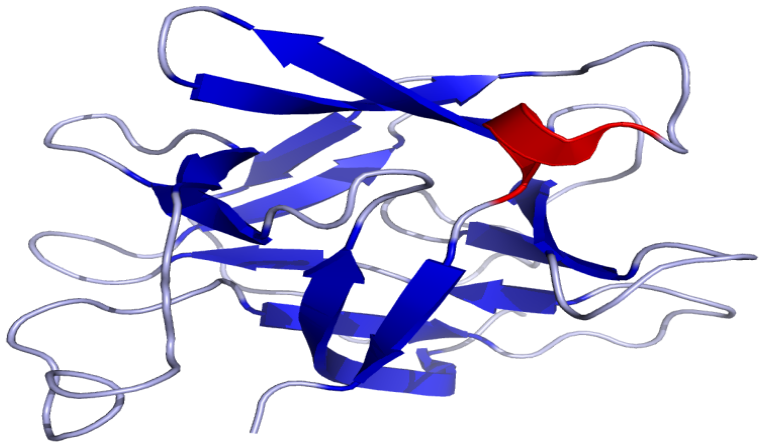
RENAL FUNCTION BIOMARKERS

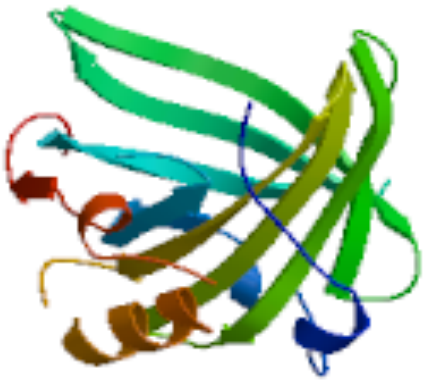
"The abilities of NGAL or IL-18 to predict dialysis within 1 wk were moderately accurate when measured on the first postoperative day, whereas the fall in serum creatinine (Scr) was not predictive."

Elevated levels of NGAL or IL-18 predicted the need for dialysis after adjusting for recipient and donor age, cold ischemia time, urine output, and Scr.

NGAL and IL-18 quantiles also predicted graft recovery up to 3 mo later

Urinary NGAL and IL-18 are early, noninvasive, accurate predictors of both the need for dialysis within the first week of kidney transplantation and 3-mo recovery of graft function".





J Am Soc Nephrol 21: 189–197, 2010

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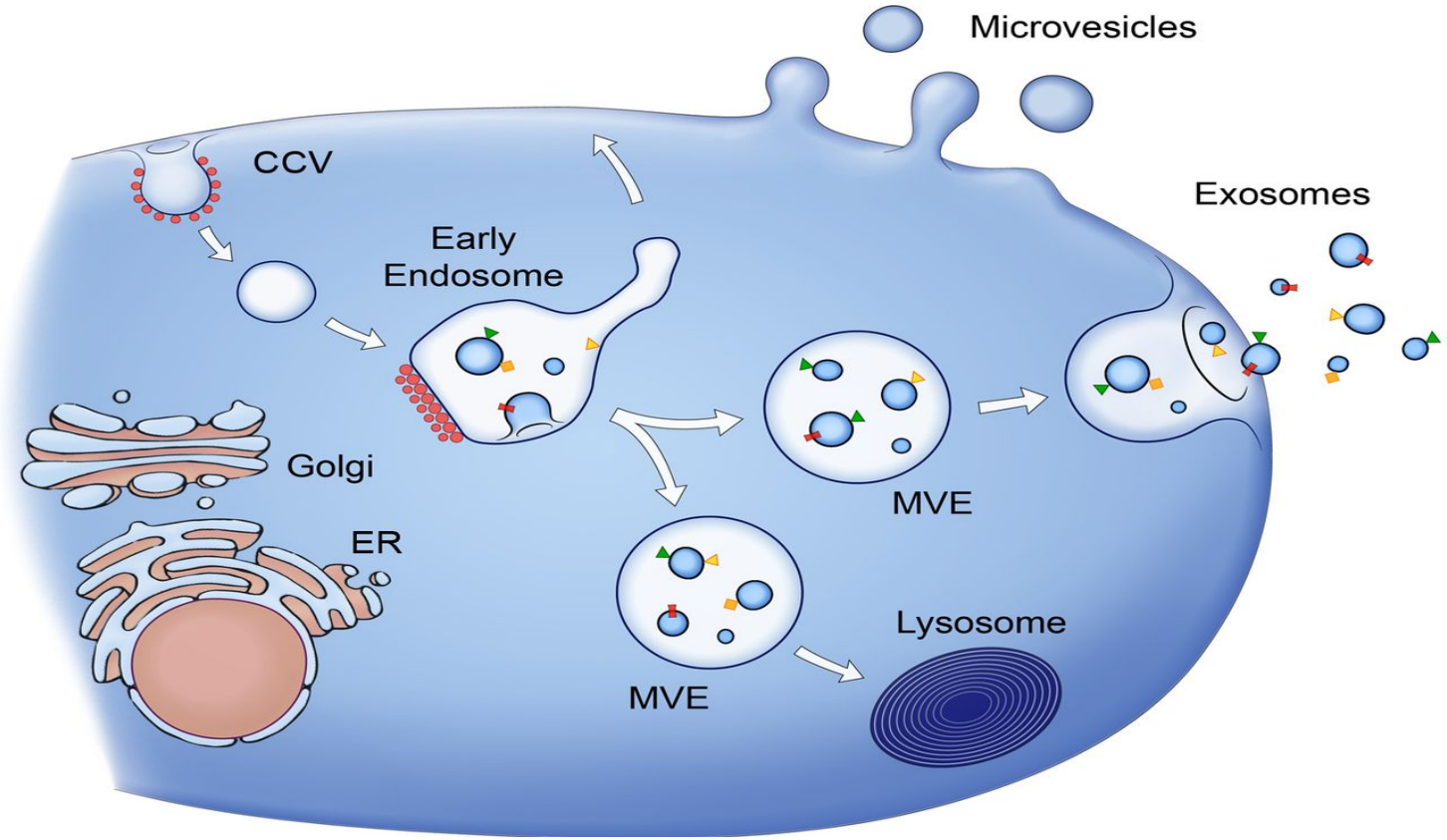
RENAL FUNCTION BIOMARKERS

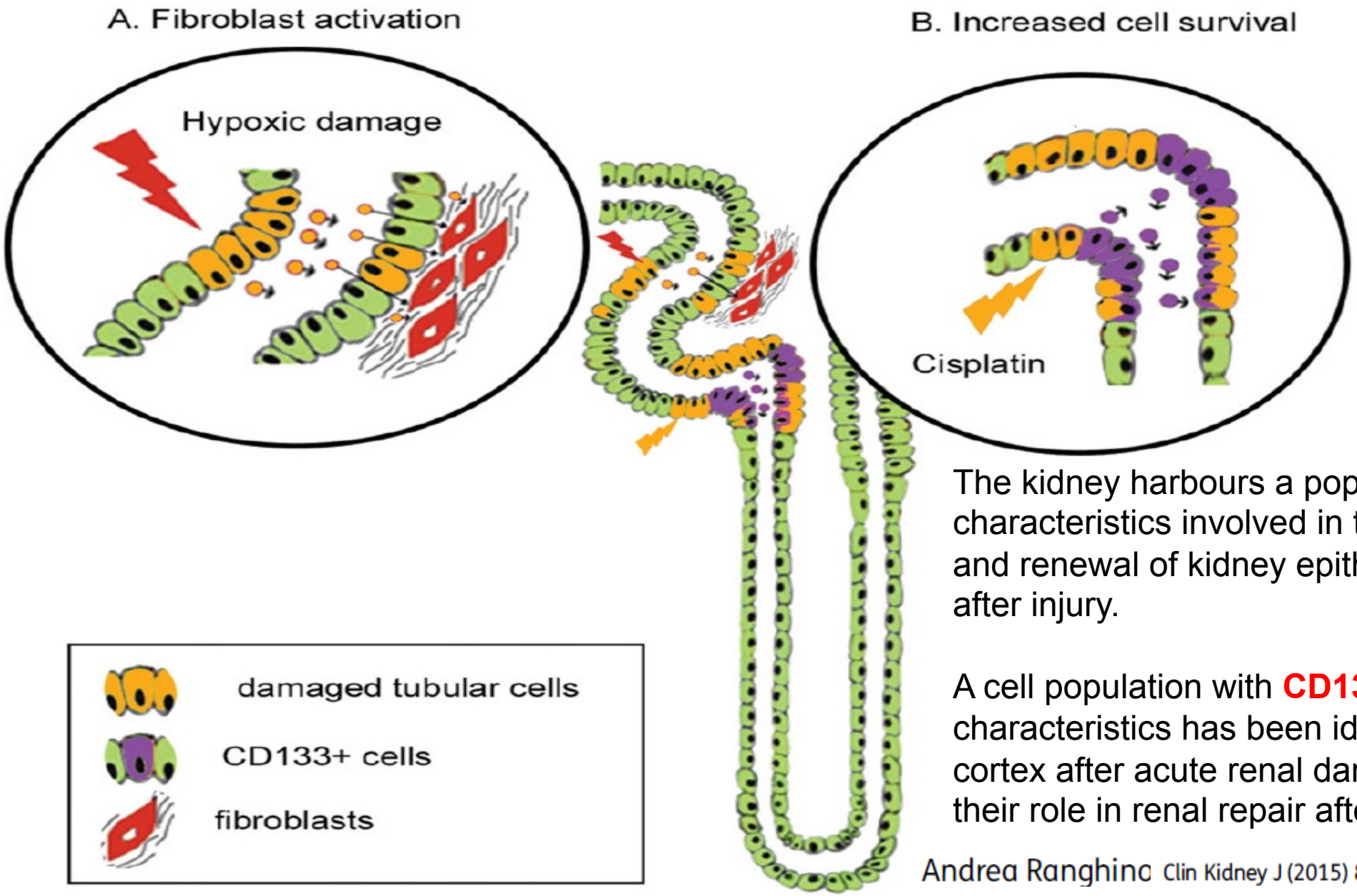
Evs in urine: Markers of damage
Provide information on
the physiological
state of the kidney and
on the intrinsic mechanisms
of its homeostasis and repair.



The kidney harbours a population of cells
with progenitor characteristics involved in
the continuous regeneration and renewal
of kidney epithelia as well as in
its repair after injury.

Andrea Ranghino Clin Kidney J (2015) 8: 23–30

RENAL FUNCTION BIOMARKERS



The kidney harbours a population of cells with progenitor characteristics involved in the continuous regeneration and renewal of kidney epithelia as well as in its repair after injury.

A cell population with **CD133** expression and progenitor characteristics has been identified and increases in the cortex after acute renal damage, suggesting their role in renal repair after injury.

Andrea Ranghino Clin Kidney J (2015) 8: 23–30

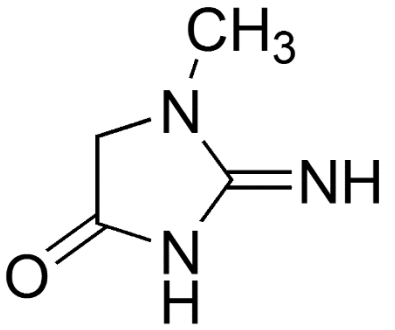
NOVARTIS PHARMACEUTICALS

RENAL FUNCTION BIOMARKERS

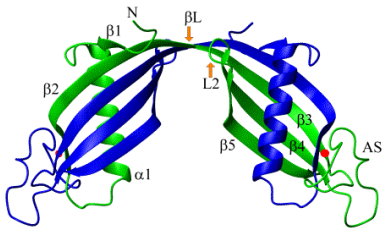
ORIGINAL ARTICLE

JNEPHROL 2012; 25(06): 1003-1015

DOI: 10.5301/jn.5000083



Creatinine- vs. cystatin C-based equations compared with ^{99m}TcDTPA scintigraphy to assess glomerular filtration rate in chronic kidney disease



Conclusions: At GFR <60 ml/min, CKD-EPI and Hoek equations appeared to best correlate with ^{99m}TcDTPA. In controls and at early stages of CKD, creatinine-based equations correlated better with ^{99m}Tc-DTPA, with CKD-EPI being the one with the best degree of agreement.

Trimarchi H et al. J Nephrol 2012; 25(06): 1003 -1015

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Primary focal and segmental glomerulosclerosis and soluble factor urokinase-type plasminogen activator receptor

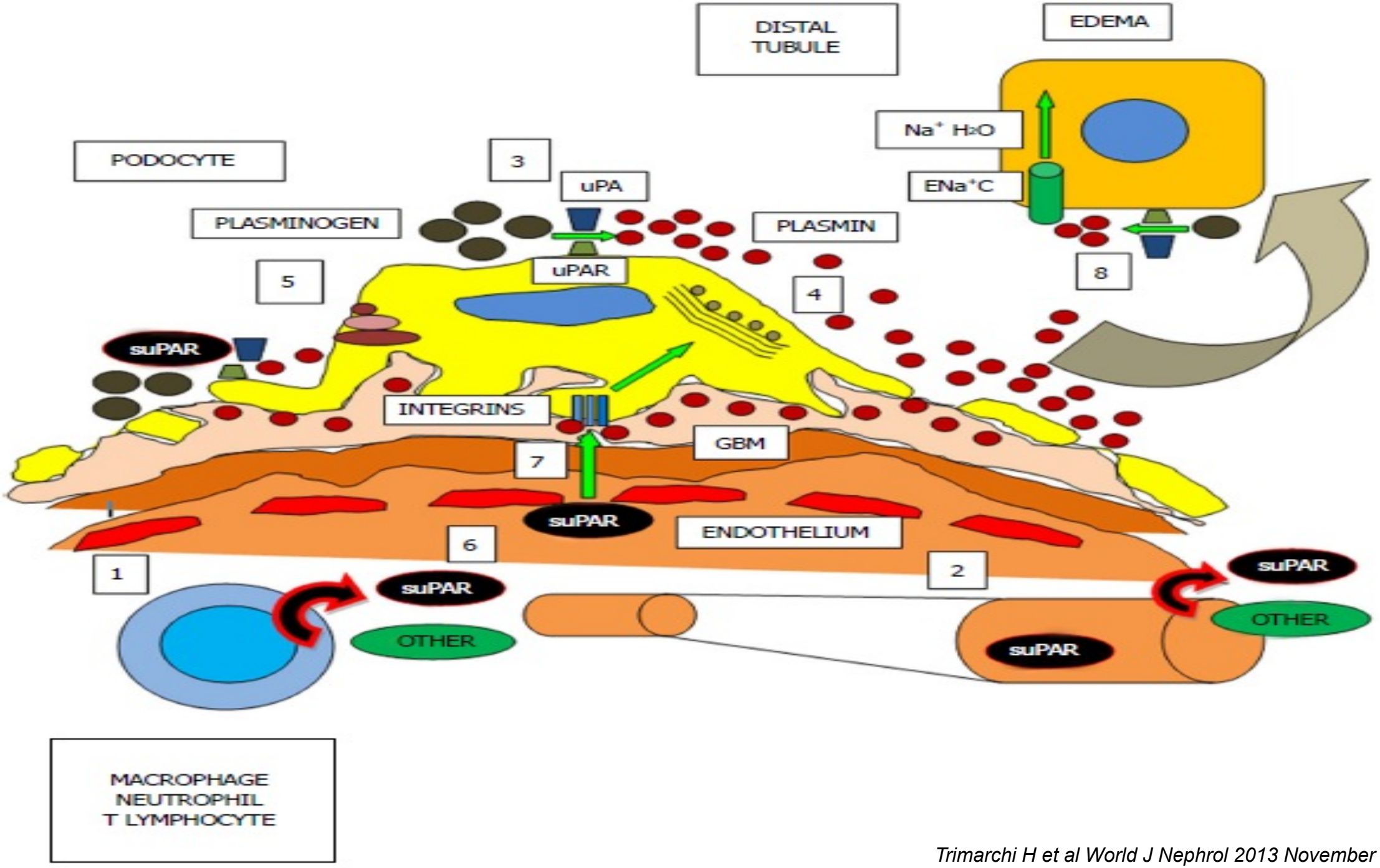
Trimarchi H et al World J Nephrol 2013 November 6; 2(4): 103-110

Plasmin and Plasminogen Activator Inhibitor Type 1 Promote Cellular Motility by Regulating the Interaction between the Urokinase Receptor and Vitronectin

Waltz DA. JCI 1997; 100: 58-67

Plasmin(ogen) Promotes Renal Interstitial Fibrosis by Promoting Epithelial-to-Mesenchymal Transition: Role of Plasmin-Activated Signals

Guoqiang Zhang *J Am Soc Nephrol* 18: 846–859, 2007.

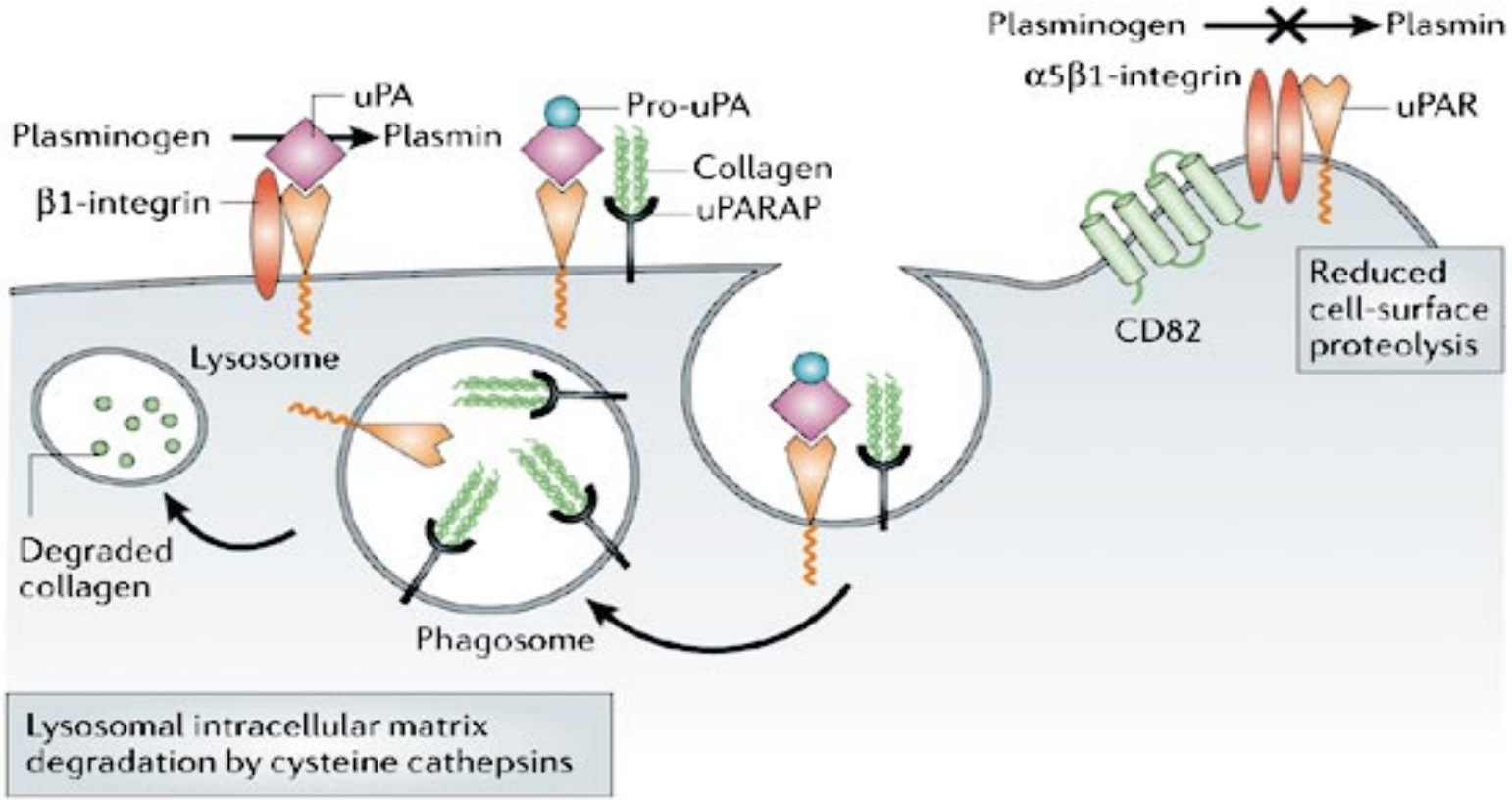


Trimarchi H et al World J Nephrol 2013 November 6; 2(4): 103-110

RENAL FUNCTION BIOMARKERS

Amiloride off-target effect inhibits podocyte urokinase receptor expression and reduces proteinuria

Bin Zhang Nephrol Dial Transplant (2012) 27: 1746–1755



NOVARTIS PHARMACEUTICALS

RENAL FUNCTION BIOMARKERS

WHY DO WE NEED RENAL FUNCTION BIOMARKERS?

NOVARTIS PHARMACEUTICALS