"Early Recognition of Kidney Damage"

INTRODUCTION :

" The Doctor of the future will give no medicine , but will interest her or his patients in the care of Human Frame , in a proper diet and in the cause and prevention of disease "

are the words of Thomas.A.Edison ,to the last words we may add "early recognition of disease "also , yes when prevention of a disease is part of disease management , early recognition of the disease is a crucial next step in curtiling the disease . And this fact is more appropriate to the vital organs of the body especially our kidneys ,our kidneys are real eyes of our body that weeps for ,

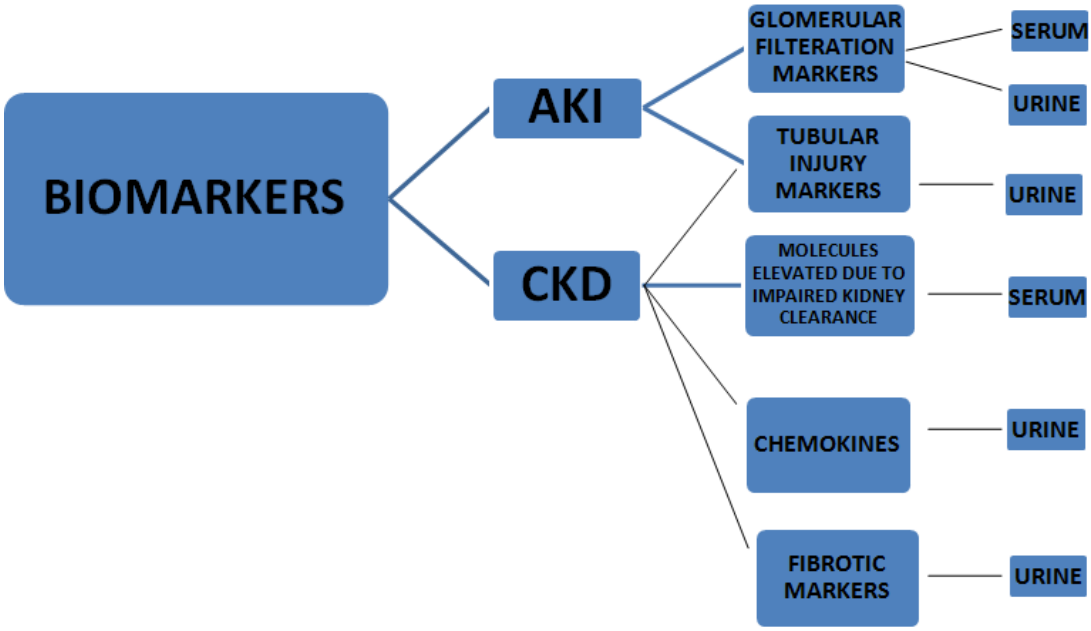
Any drop of blood that is shed
Any crack of tissue that is damaged

Damage to kidney is a global health problem and its incidence is increasing in either forms , that is AKI-Acute Kidney Injury and CKD – Chronic Kidney Injury . WHO states 850000 patients develop kidney disease every year.It posses a heavy toll on global economy .The hit is going to be harder on the developing countries which are socioeconomically deprived and lack a well established health care infrastructure . Importantly progression of kidney disease can be caught hold and controlled if detected early. Hence early recognition and faster therapeutic interventions when the disease have not gone too far into its severity is a charm to an efficient physician and a boon to the affected patient.

Kidney function estimation was commonly made using output monitoring ,serum creatinine (SCr) concentration, blood urea nitrogen (BUN) level and urine analysis. Anyhow these commonly used methods and biomarkers are not optimal to detect injury or dysfunction early enough to allow prompt therapeutic interventions.

Recent advances in technologies in the fields of genomics , proteomics , and metabolomics , have made it easier to interrogate number of potential biomarkers.

.



Acute Kidney Injury(AKI) :

Is a complex disorder with multifactorial etiology associated with adverse outcomes . The mortality and morbidity associated with it are huge in numbers . More timely diagnosis would allow for earlier intervention and could improve patient outcomes. The goal of early identification of AKI has been the primary impetus for AKI biomarker research, and has led to the discovery of numerous novel biomarkers. They also provide valuable insight into the molecular mechanisms of this complex and heterogeneous disease . Barriers in translating the successes of animal studies to efficacy in human clinical trials are

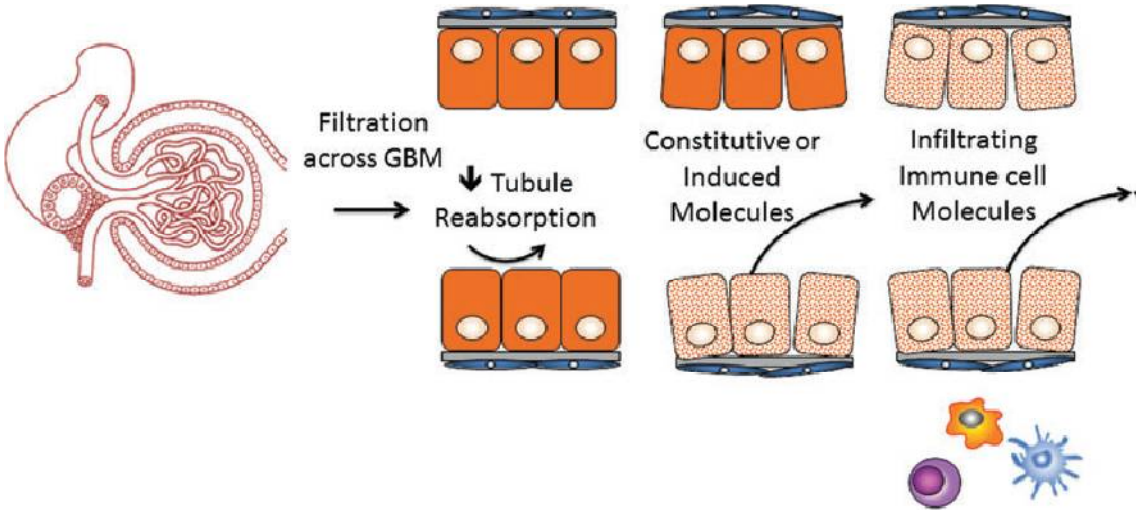
Heterogenicity of our patients with AKI with regards to the etiology of renal injury

Patient comorbidities

Late timing of presentation to us and initiation of interventions

Delayed interventions have been attributed to deficiency of creatinine as an AKI biomarker . The goal of most AKI biomarker research has been the discovery of a “kidney troponin”,which could be a sensitive and specific early marker of renal injury.

MECHANISM OF URINARY BIOMARKERS IN KIDNEY INJURY :



Biomarkers are renal and non renal derived molecules that report on the functional status of kidney filteration and tubular injury. Markers may represent non renal molecules filtered , secreted or reabsorbed , molecules that constitutive or upregulated or molecules from infiltrating immune cells

Functional biomarkers	Tubular enzymes	Upregulated proteins
Creatinine	Alanine aminopeptidase (AAP)	KIM-1
Cystatin C	Alkaline phosphatase (AP)	Clusterin
β2-microglobulin	α-glutathione-*S*-transferase (α-GST)	Neutrophil gelatinase-associated lipocalin (NGAL)
α1-microglobulin	γ-glutamyl transpeptidase (γΓT)	IL-18
Retinol-binding-protein (RBP)	*N*-acetyl-β-glucosaminidase (NAG)	Cysteine-rich protein (CYR-61)
Microalbumin		Osteopontin
		FABP
		Sodium/hydrogen exchanger isoform (NHE3)
		Exosomal fetuin A

KIDNEY FUNCTION MEASUREMENT :

mGFR :

Establishing the true GFR is difficult because the filtration process simultaneously takes place in millions of glomeruli and filtrate composition and volume change when passing through the kidney. GFR is measured (mGFR) indirectly as the clearance of filtration markers that are eliminated by the kidney only by glomerular filtration. As such, an ideal substance is one that is freely filtered at the glomeruli and neither secreted nor reabsorbed by the renal tubules. Inulin is an exogenous filtration marker derived from a fructose polymer and is a physiologically inert substance and is considered an ideal substance for mGFR. Although inulin clearance is considered the gold-standard method for mGFR, it is cumbersome and expensive to measure .Kidney excretion of 51Cr-EDTA or iothalamate, and plasma removal of 51Cr-EDTA or iohexol, using inulin clearance as reference, were sufficiently accurate methods to measure GFR

eGFR :

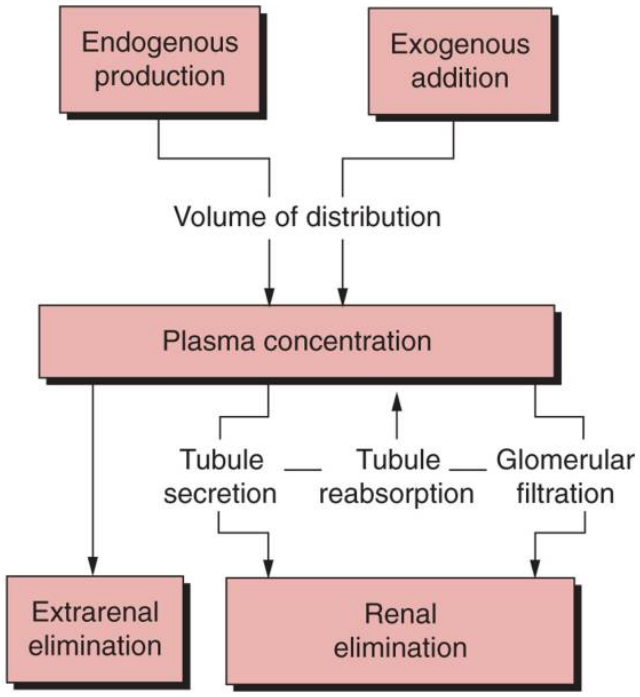
Routinely, GFR is usually estimated from prediction equations which are based on endogenous serum markers like creatinine or CysC in addition to demographic variables such as age, sex and race . Measured GFR is reserved for situations where eGFR may be inaccurate such as patients in non-steady state, or individuals that possess different characteristics compared to those where the estimating equation was created such as old age, loss of muscle mass ,obesity, chronic illness .

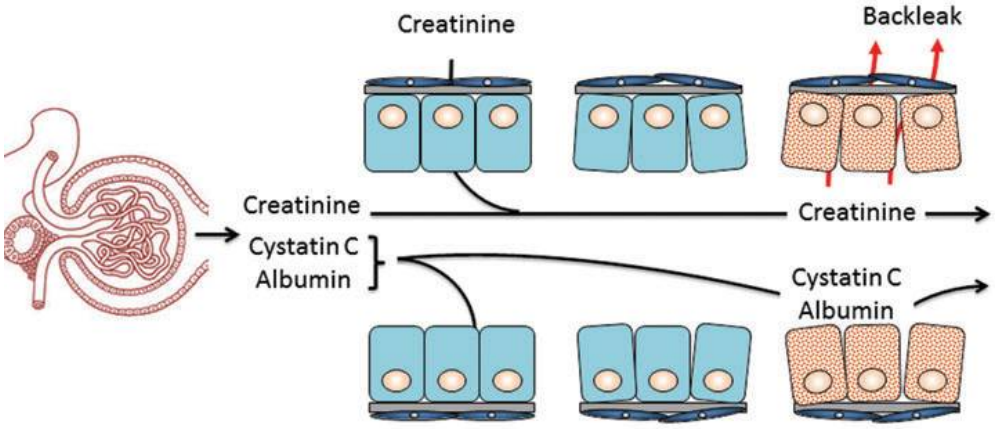
GLOMERULAR INJURY MARKERS

SERUM GLOMERULAR FILTRATION MARKERS :

Creatinine:

SCr derives from creatine degradation , it is freely filtered but is not reabsorbed or metabolized however a significant percentage of creatinine in the urine derives from proximal tubular secretion. One of the requirements for utilizing estimating equations based on SCr is stable kidney function. In addition, non-GFR determinants, such as variation in production associated to dietary intake, or changes in muscle mass, variation in tubular secretion and extrarenal creatinine excretion (associated with advanced kidney disease) need to be accounted when utilizing creatinine . Another important factor that limits the accuracy of equations is the variability in SCr measurement. The recognition that small variations in SCr translates in significant changes in kidney function has prompted to standardize creatinine determinations throughout clinical laboratories which occurred in 2006





CysC:

CysC has come to light as another marker of kidney function during the past decade. CysC is a non-glycosylated protein produced by all nucleated cells. CysC is freely filtered, reabsorbed and completely metabolized in tubular cells and therefore is not subjected to tubular secretion. Compared to creatinine, CysC has a more stable rate of production; however CysC serum levels are also influenced by non GFR determinants, such as uncontrolled thyroid disease, corticosteroid use, age, sex, ethnicity, smoking and adipose tissue .In addition, CysC predicts outcomes and the association is stronger than SCr. CysC level to have an important association with mortality across the GFR range, including individuals with GFR between 60 and 90 mL/min per 1.73 m2, grouped as “preclinical kidney disease”. It is a better predictor of adverse cardiovascular and non cardiovascular outcomes compared to to SCr.

ESTIMATING EQUATIONS :

The most commonly used equations include Cockroft Gault (CG), 4-modification of diet in renal disease (MDRD), 2009 CKDEPI and more recently the equation that combines creatinine and CysC. The CKD-Epidemiology Collaboration equation performs better than the MDRD equation , especially at GFR above 60 mL/min per 1.73m2. Equations combining CysC and SCr perform better than the equations using either CysC or SCr alone and are recommended in situations where CKD needs to be confirmed. Combining creatinine, CysC and urine albumin to creatinine ratio improves risk stratification for kidney disease progression and mortality.

Blood urea nitrogen:

BUN increases as GFR declines however is less valuable than the SCr since the BUN can vary independently of the GFR. The production rate of urea is not stable and increases with rich protein diets or tissue breakdown such as bleeding, muscle trauma or steroid administration. On the other hand a very low protein diet or liver failure can decrease BUN without affecting GFR.

β **Trace Protein :**

Also called prostaglandin D synthase ,a biomarker for GFR. It belongs to lipocalin family ,and is produced in CSF. Eliminated by glomerular filtration . Its concentration not affected by immunosuppressive medications ,hence very useful in evaluating kidney function in kidney transplant recipients , in whom cystatin C may falsely be elevated due to steroid treatment

URINARY GLOMERULAR INJURY MARKERS :

Glomerular disease are associated with abnormalities in podocyte structure , hence called as podocytopathies . These podocyte defects can occur in immunological and non immunological forms of glomerular disease like ischemic injury , toxin induced injury , minimal change disease , FSGS , membranous glomerulopathy diabetic nephropathy , lupus nephritis.

Podocyte count :

After undergoing structural changes podocyte detach from the glomerular basement membrane (GBM) and are excreted in the urine . The number of podocytes shed in the urine is higher in patients with active glomerular disease . Podocyte number in urine correlate with disease activity (assessed with renal biopsy). But it is technically demanding , time consuming. Another problem is urinary sediments contain whole viable podocytes as well as cell debris . Indirect assessment of podocyte count by PCR and ELISA are also tried

Podocalyxin :

It is the most commonly used protein for detecting podocyte in urine . Urinary podocalyxin has been reported as a marker of activity in a number of diseases , like IgA nephropathy , Henoch- Schonlein purpura , diabetic nephropathy , lupus nephritis , FSGS , PSGN and preeclampsia.

URINARY TUBULAR INJURY MARKERS :

Components of urine have been used to quantitate tubular cell injury in a more specific and sensitive fashion .These markers have demonstrated to be of extreme value in detecting kidney injury in the setting of AKI . Also some of these biomarkers such as , Kidney Injury Molecule (KIM 1), Neutrophil Gelatinase –Associated Lipocalin (NGAL) , Liver type Fatty Acid Binding Protein (FABP 1), have been shown to be useful in both AKI and CKD

Urine microscopy :

This is one bedside test that has stood for the test of time . Urine from patients with tubular injury typically contains proximal tubular epithelial cells, proximal tubular epithelial cell casts, and mixed cellular casts patients with predominantly prerenal azotemia have hyaline or fine granular casts in their urine .Studies have shown that the increase in urinary casts excretion correlates well with AKI . Anyway , the sensitivity of this test as an early indicator of tubular injury in the kidney remains controversial.

α -1 Microglobulin :

It is a low molecular weight glycoprotein of lipocalin superfamily . Urine and serum levels have been found to be elevated in patients with renal tubular disease . α -1 Microglobulin is freely filtered at the glomerulus and completely reabsorbed and catabolised by normal proximal tubule. Age influences the urine levels of α -1 Microglobulin .

B2-microglobulin:

B2-microglobulin (B2-M) is a small molecule that constitutes a class 1 HLA, is present in all nucleated cells in the body. It has the characteristic that it is freely filtered in the glomeruli and is reabsorbed and metabolized in the proximal tubule. Levels of B2-M are elevated in kidney disease, malignancies, autoimmune diseases, infections and aging. There is data to demonstrate that plasma B2-M is a good endogenous marker of GFR and that in the context of GFR decline **the increase of serum B2-M occurs prior than SCr**. B2-M has been associated with death in a cohort of 1034 elderly subjects and is superior than CysC . It has the potential to distinguish prerenal azotemia from acute tubular necrosis and can detect subclinical AKI or predict AKI.

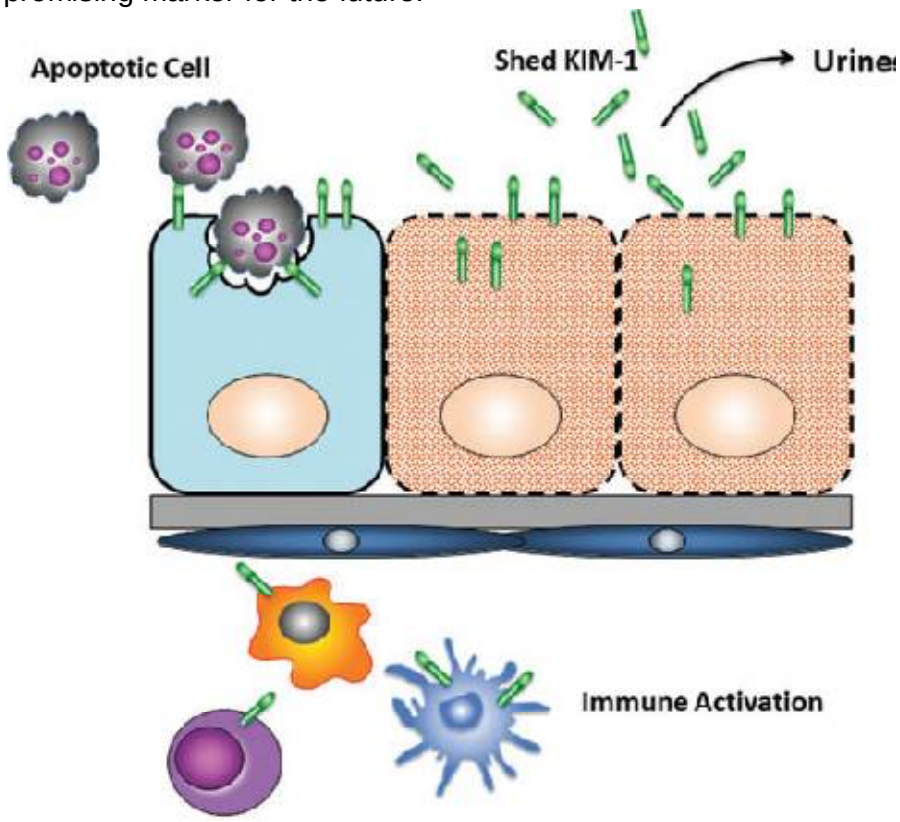
New biomarkers for kidney damage :

Although albuminuria is a powerful biomarker, it may occur after the damage has occurred or may not be present in other types of kidney damage such as tubulointerstitial disease and hypertensive kidney disease. This has led to the search for new biomarkers .

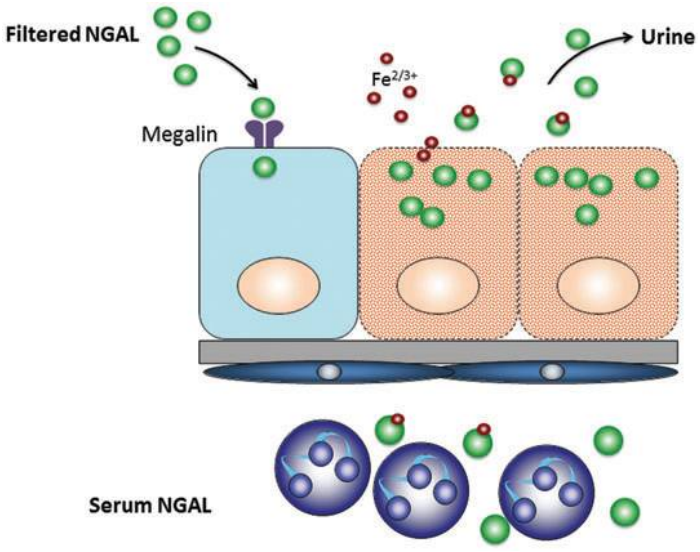
Kidney injury molecule :

Kidney injury molecule (KIM-1) is a transmembrane protein is a type 1 transmembrane protein whose expression has been upregulated after kidney injury. KIM-1 is an early biomarker for proximal tubular damage since it is expressed in the urine during the first 12 h of the

tubular injury. Experimental and clinical studies have demonstrated high KIM-1 expression in areas of fibrosis and inflammation , polycystic kidney disease, , interstitial fibrosis of human allografts. In addition baseline plasma KIM-1 levels correlated with rate of eGFR decline after adjustment for baseline urinary albumin-to-creatinine ratio, eGFR, and Hb1Ac . KIM-1 may represent a promising marker for the future.

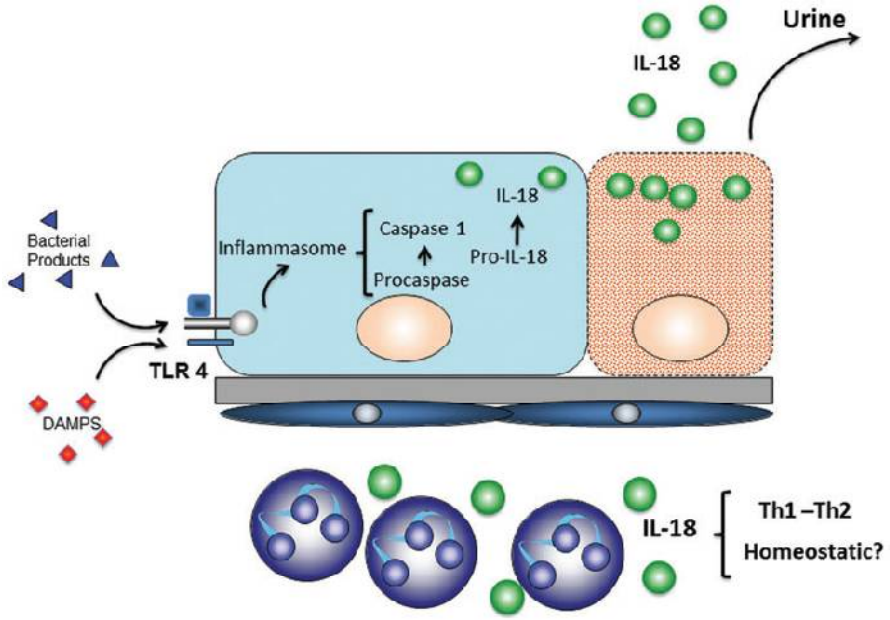


Neutrophil gelatinase-associated lipocalin:



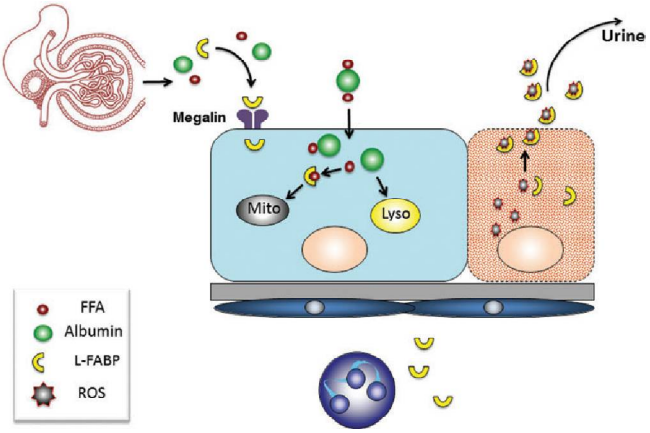
NGAL is produced by neutrophils and is expressed to a limited degree in the liver, spleen and kidney. It functions includ inhibiting bacterial growth, scavenging iron and inducing epithelial cell growth. A small amount of NGAL is filtered and taken up by the proximal tubule through megalin. Upon injury, NGAL (a stress response protein) is upregulated and released into the urine and plasma. Its protective effect when infused may be related to its ability to scavenge iron as depicted or through its ability to induce cell growth .NGAL has been an established marker for acute kidney injury however its role in CKD is less studied . In patients with IgA nephropathy urinary NGAL level was high and was also associated with disease severity. In patients with glomerular proteinuria above 1 g/24h and in patients with polycystic kidney disease, NGAL levels were higher compared to controls and significantly correlated to SCr

INTERLEUKIN-18 :



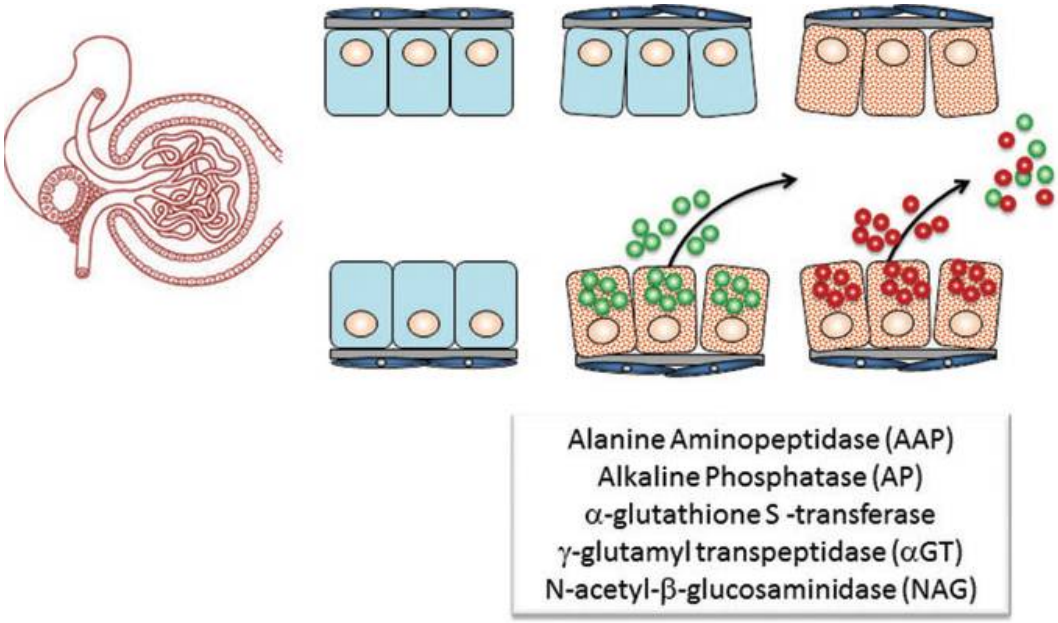
IL-18 is produced by immune cells and by active epithelial cells. Following activation of toll like receptor 4 (TLR4), activation of inflammasome leads to cleavage of pro-caspase 1 to caspase-1. This in turn cleaves pro-IL-18 into the active IL-18 molecule. IL-18 has proinflammatory properties or may have homeostatic properties . It is induced and cleaved in the proximal tubule, and subsequently easily detected in the urine after ischemic AKI in animal models. Urinary IL-18 and NGAL were shown to represent sequential AKI biomarkers in children undergoing cardiac surgery. In patients who developed AKI 2 to 3 days after surgery, urinary NGAL was induced within 2 hours and peaked at 6 hours, whereas urine IL-18 levels increased around 6 hours and peaked at more than 25-fold at 12 hours postsurgery

LIVER FATTY ACID-BINDING PROTEIN :

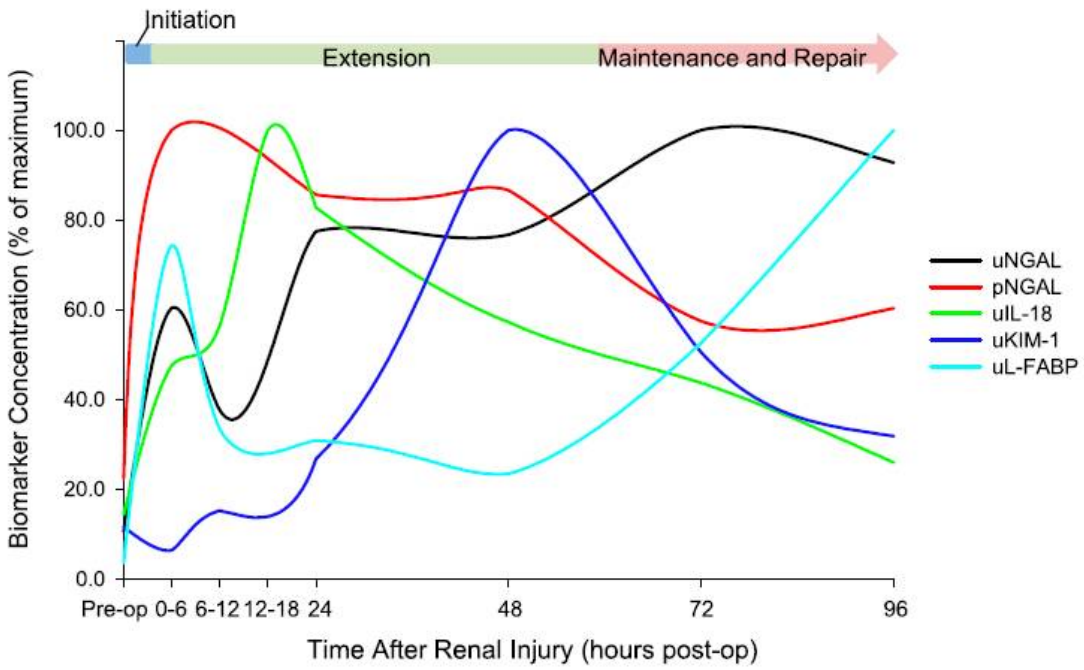


L-FABP are bound to serum albumin and are reabsorbed into the proximal tubule bound to serum albumin. Filtered L-FABP
is taken up by the proximal tubule and acts as a carrier protein and transports free fatty acids to mitochondria and peroxisomes for metabolism. Upon stress and ischemia–reperfusion there is an upregulation of L-FABP, which binds lipid hydro-peroxides and other reactive oxygen—which together are released into the urine.

N-ACETYL- β - D-GLUCOSAMINIDASE :



Enzymatic injury biomarkers AAP, AP, γ- gG and NAG are present in the epithelial cells and are released into the urine following cellular injury. Within the kidney, NAG originates from the lysosomes of the proximal tubule cells and can be measured in the urine using a colorimetric assay. Increased
urinary concentration of NAG is a sensitive marker for proximal tubule injury with loss of lysosomal integrity



Changes inAKI biomarker concentration over time after renal injury.

CKD:

GFR in CKD :

GFR is the most important marker of kidney function. Unfortunately GFR cannot be easily measured in most clinical or research settings , and therefore estimating equations are based on filtration markers such as serum creatinine (SCr) and cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. Further , new potential biomarkers have arisen with the promise of detecting kidney damage prior to the commonly used markers of kidney disease.

Biomarkers in CkD :

All forms of CKD are associated with tubulointerstitial injury . As previously described markers of tubular injury like KIM-1 , NGAL , L-FABP , have shown to predict outcomes of CKD . In addition , elevated systemic levels of molecules that have impaired kidney clearance or increased production in CKD (eg asymmetric dimethylarginine , fibroblast growth factor 23) as well as chemokines (eg monocyte chemoattractant protein – 1) and fibrotic markers (connective tissue growth factor , TGF β -1, and collagen 4).

Fibroblast growth factor 23:

FGF-23 is protein secreted by bone osteocytes. It is reported that increasing levels of FGF-23 associated with decline in kidney function or initiation renal replacement therapy after a follow-up of 2 and 4.4 years respectively. In addition, in patients undergoing renal replacement therapy, elevated FGF-23 levels have been associated with CardioVascular outcomes such as left ventricular hypertrophy and increased risk of mortality . This association is independent of phosphate levels and CKD stage.

Asymmetric dimethylarginine:

ADMA is an aminoacid. As kidney function deteriorates ADMA levels increaseand this has been associated to kidney parenchymal damage through the decrease in dimethylargininedimethylamino-hydrolase.

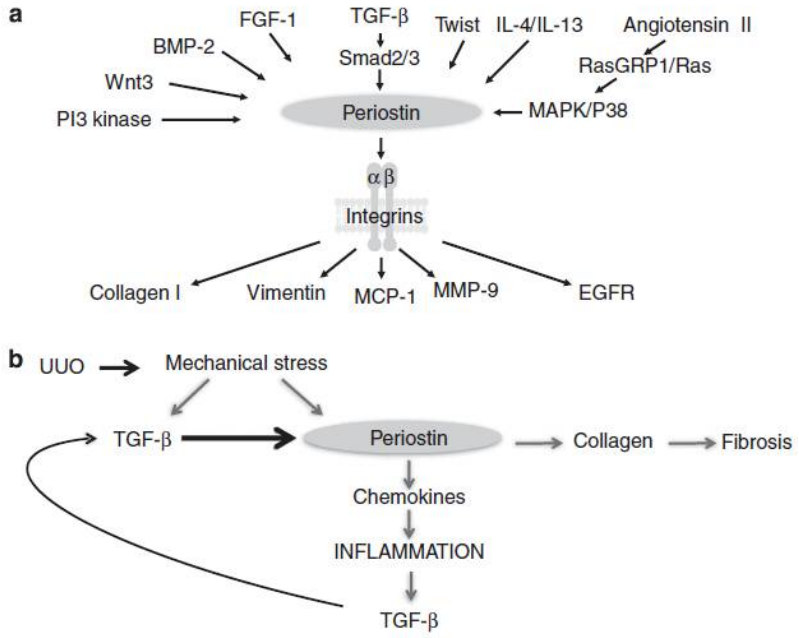
MCP-1:

MCP-1 belongs to the group of inflammatory Chemokines . Expression of MCP-1 is up regulated in kidney diseases that have a sustained inflammatory response, such as in diabetic nephropathy and lupus nephritis.

Urine retinol-binding protein 4:

Urine retinol-binding protein 4 (uRBP4) is a 21 KDa protein derived of plasma RBP4 (pRBP4), belongs to lipocalin family and is produced mainly in the liver but also in adipose tissue where it performs as an adipokine that has been linked to insulin resistance and obesity . Unlike other biomarkers such as NGAL and KIM-1, uRBP4 is currently the most sensitive functional biomarker of proximal tubule.

PERIOSTIN As A Novel Markers Of CKD:

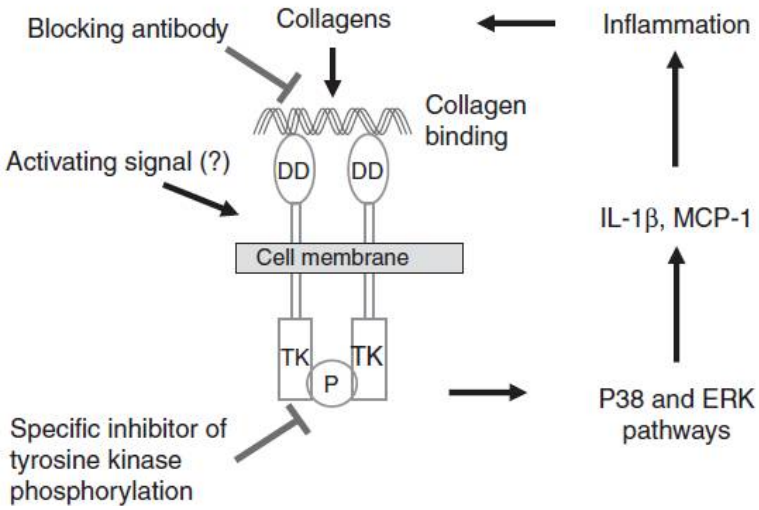


Physiopathological actions of periostin activation.

(a) The 'periostin network': in vitro data showed that periostin can be highly induced by a variety of signaling pathways; it can interact with integrins to stimulate mechanisms promoting inflammation, extracellular matrix formation, and cell phenotype changes.

(b) Proposed mechanism of periostin action in renal epithelial cells following unilateral ureteral obstruction (UUO) injury: periostin is induced early in renal epithelial cells and interacts with the TGF-b signaling pathway to promote inflammation, extracellular matrix remodeling, and subsequently the progression of interstitial fibrosis . It could be a novel marker and target of therapy in CKD

DISCOIDIN Domain Receptor 1 :



Mechanisms showing the detrimental amplifying action of DDR1 to deteriorate renal function. A yet unidentified cell signal induces locally de novo expression and activation of DDR1. Subsequently, DDR1 is dimerized and phosphorylated, and this activation stimulates pro-inflammatory pathways, which in turn trigger collagen synthesis. Collagens are ligands of DDR1 and further stimulate DDR1 and so on. Recent studies demonstrated that DDR1 is an important mediator in renal inflammation and fibrosis.

KIDNEY'S WELL BEING BECOMES SOCIAL WELL BEING :

When we meet a kid we ask the child " which class are you studying ? " in our country but in japan they ask " Have you got your " Nyō kensa " done". The introduction in Japan of routine urinalysis for pre-school and school age children has greatly facilitated the discovery of renal disease like MPGN, FGS, and IgA nephropathy in asymptomatic children . In a developing country like ours if we could diagnose kidney damage earlier and avoid CKD and its costlier managements , it would be a boon to our poor patients

FUTURE PERSPECTIVES :

A clinical situation analogous to AKI, acute myocardial infarction, the medical evaluation of which has progressed over the past few decades from detection of Q-waves by electrocardiogram through a series of serum biomarkers with increasing sensitivity and predictive value.

In stark comparison, the diagnosis, treatment, and prognosis of AKI have not changed appreciably in the past 5 decades. The use of serum creatinine measurements to institute promising interventions for AKI is analogous to waiting 2 to 3 days before intervening in patients with acute myocardial infarction or acute neurologic stroke.

Fortunately, we are closing in on the kidney troponins and the AKI biomarker panel. Incredibly, the adaptive response of the stressed kidney itself, with the rapid and robust induction of select genes whose protein products have provided us with highly promising biomarkers. These include NGAL, IL-18, and KIM-1. These biomarkers have completed the initial validation stage, and have entered the prospective screening stage in the biomarker development process. These biomarkers will revolutionize renal and critical care . The ability to predict AKI or who would progress to CKD or ESRD enables early initiation of therapies . These could change the dismal outcomes associated with this clinical problem .

REFERENCES :

1 . Venkata sabbisetti and Joseph V.Bonventre Biomarkers in Acute and Chronic Kidney Disease Brenner and Rector's The Kidney, 8th ed

2 . **Yang Q, Eskurza I, Kiernan UA, Phillips DA, Blüher M, Graham TE, Kahn BB**. Quantitative measurement of full-length and C-terminal proteolyzed RBP4 in serum of normal and insulinresistant humans using a novel mass spectrometry immunoassay. Endocrinology 2012

3 . Salvador Lopez-Giacoman, Magdalena Madero Biomarkers in chronic kidney disease, from kidney function to kidney damage World J Nephrol 2015 February 6; 4(1)

4 . Jennifer R. Charlton1,2, Didier Portilla3 and Mark D. Okusa A basic science view of acute kidney injury biomarkers Nephrol Dial Transplant (2014) 29: 1301–1311 doi: 10.1093/ndt/gft510 Advance Access publication 2 January 2014

5 . Muh Geot Wong1 and Carol A. Pollock1 Biomarkers in kidney fibrosis: are they useful? 2014 International Society of Nephrology

6 . Panos Kavvadas1, Jean-Claude Dussaule1 and Christos Chatziantoniou1 Searching novel diagnostic markers and targets for therapy of CKD2014 International Society of Nephrology

7 . Joseph L. Alge* and John M. Arthur*† Biomarkers of AKI: A Review of Mechanistic Relevance
and Potential Therapeutic Implications
Clinical Journal of the American Society of Nephrology

8 . Prasad Devarajan, MDThe Future of Pediatric Acute
Kidney Injury Management—Biomarkers Semin Nephrol 28:493-498 © 2008 Elsevier Inc.

9 . Claudio Ronco AKI Biomarkers www (Who, Where, When):
You Cannot Treat What You Do Not Know November 11, 2014 Blood Purif 2014;38:I–II
DOI: 10.1159/000368919

10 . **Naylor HM**, Newcomer ME. The structure of human retinolbinding protein (RBP) with its carrier protein transthyretin reveals an interaction with the carboxy terminus of RBP. Biochemistry 1999; **38**: 2647-2653 [PMID: 10052934 DOI: 10.1021/bi982291i]

11 . Sakai T, Kitagawa T .Screening system for asymptomatic renal disease in children in Japan. 1990 Dec;32(6):677-81. Acta Paediatr Jpn .