New kidney injury biomakers

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INTRODUCTION

Acute kidney injury (AKI) is a common clinical problem. Although measurement of the serum creatinine concentration is widely used for the detection of AKI, it does not permit early diagnosis of acute tubular necrosis (ATN), since tubular injury precedes a significant rise in serum creatinine.

Investigational biomarkers have been evaluated in patients with possible ATN in an attempt to detect tubular injury at an earlier stage.

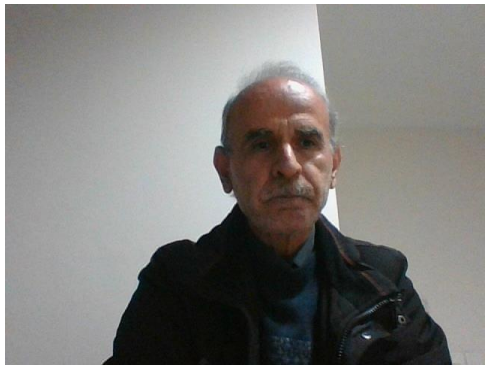
Although the serum creatinine is widely used in diagnosing the presence of AKI, it is a suboptimal biomarker. It is a lagging marker of change in kidney function; therefore, it has poor sensitivity for the early diagnosis of AKI, and, as a marker of glomerular filtration, it is unable to differentiate among the various causes of AKI.

A number of factors may contribute to a lag in rise of serum creatinine after AKI, including dilutional effect of fluid administration and decrease in creatinine generation.

Thus, **different urinary and serum proteins have been intensively investigated as possible biomarkers for the early diagnosis of ATN**.

There are promising candidate biomarkers that report on kidney and tubule function, detect an early and graded increase in tubular epithelial cell injury, and distinguish prerenal disease from ATN.

These novel biomarkers have the potential to reflect physiologic and pathophysiologic processes of the injured kidney.



Some biomarkers are detected in the urine of patients without a diagnostic increase in serum creatinine, which defines a group of patients with "subclinical AKI" who are at risk for adverse outcomes.

Biomarkers are used in clinical investigation to facilitate early randomization to different treatment arms. These vanguard studies using biomarkers may lead to the identification of new therapies and the practical use of biomarkers in routine patient care.

Many candidate biomarkers have been identified.

However, the following steps are necessary before they are used clinically:

- Validation in different settings of AKI (cardiac surgery, sepsis, contrast-induced nephropathy, emergency and pediatric medicine) and in different clinical centers.
- Normal range and cut of points
- Development and testing of rapid assays.
- Development of a panel of tubular biomarkers that can be used in combination with clinical (eg, fluid overload) and/or functional biomarkers (eg, estimated GFR [eGFR] kinetics). It is unlikely that a single will suffice; rather, a panel of biomarkers will be necessary.

Additional barriers for implementing novel biomarkers exist. For example, the **optimal method of reporting biomarker excretion has not been determined**.

Urinary biomarker excretion may be reported as an absolute concentration or normalized to creatinine excretion.

One study that compared these methods showed that the biomarker normalized to creatinine best predicted death, dialysis, or the development of AKI, although the absolute concentration best diagnosed AKI on admission.

Types of Biomarker

1- Dignostic biomarkers:

3- Prognostic biomarkers:

Types of Biomarker

A- Diagnostic Biomarkers:

1- urinary Tubular Enzymes

2- Urinary low molecular weight proteins

3- Neutrophil gelatinase-associated lipocalin (NGAL)

4- Urinary kidney injury molecule-1

5- Urinary interleukin-18

6- Urinary liver-type fatty acid-binding protein

7- Combination of biomarkers

Types of Biomarker

B- Prognostic biomarkers:

1- Soluble urokinase plasminogen activator receptor (suPAR)

2- Dickkopf-3

3- Uromodulin (Tamm-Horsfall protein)

4- Plasma NGAL

5- Urinary insulin-like growth factor-binding protein 7 (IGFBP7)

6- Tissue inhibitor of metalloproteinases-2 (TIMP-2)

Types of Biomarker

Other areas of research for biomarkers of AKI:

1- Proteomics

2- Metabolomics

3- Extracellular vesicles (EVs)

4- MicroRNAs

5- Markers of inflammation – Plasma IL-6 and IL-10

6- Others

DIAGNOSTIC BIOMARKERS

Urinary tubular enzymes — Urinary tubular enzymes consist of

1- proximal renal tubular epithelial antigen (HRTE-1),
2- alpha-glutathione S-transferase (alpha-GST),
3- pi-glutathione S-transferase (pi-GST),
4- gamma-glutamyltranspeptidase (gamma-GT),
5- alanine aminopeptidase (AAP),
6- lactate dehydrogenase (LDH),
7- N-acetyl-beta-glucosaminidase (NAG),
8- alkaline phosphatase (ALP).

Most of these are released from proximal tubular epithelial cells within 12 hours and four days earlier than a detectable rise in serum creatinine.

No validated cut-off points currently exist to help distinguish prerenal disease from ATN.

DIAGNOSTIC BIOMARKERS

Urinary low-molecular-weight proteins —

1- Alpha1-microglobulin (alpha1-m),
2- beta2-microglobulin (beta2-m),
3- retinol-binding protein (RBP),
4- adenosine deaminase-binding protein (ABP),
5- and urinary cystatin C

They are produced at different sites, filtered at the glomerulus, and reabsorbed at the proximal tubule with no secretion.

Although promising prognostically and to help distinguish prerenal disease from ATN, increased levels may be observed after reversible and mild dysfunction and may not necessarily be associate persistent or irreversible damage.

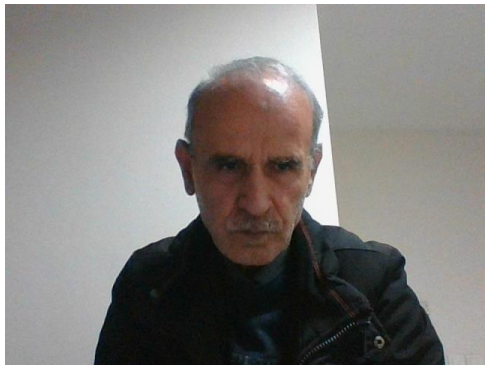
Neutrophil gelatinase-associated lipocalin (NGAL) —

NGAL is markedly upregulated and abundantly expressed in the kidney after kidney ischemia.

In this setting, NGAL may function to **dampen toxicity** by reducing apoptosis and increasing the normal proliferation of kidney tubule cells.

Although its role in clinical care remains uncertain, NGAL is now approved for use as a biomarker of acute kidney injury (AKI) in some countries, including the United States.

In mouse model, the kidneys illuminate following ischemia, indicating NGAL production at the site of injury.
Interestingly, following maneuvers that lead to significant prerenal disease, there was no NGAL illumination, indicating that prerenal disease does not induce NGAL expression.
Thus, NGAL may potentially be useful in differentiating prerenal disease from ATN.



NGAL has been tested in multiple studies of patients at risk for AKI due to sepsis, cardiac surgery, exposure to contrast media, or after kidney transplantation.

In these studies, the average sensitivity and specificity of NGAL measured one to three days prior to AKI diagnosis was 76 and 77 percent, respectively, for cardiac surgery patients and 73 and 80 percent, respectively, for patients admitted to the intensive care unit (ICU).

In a meta-analysis that examined the performance of a variety of novel AKI biomarkers in 110 studies with over 38,000 patients, biomarkers utilizing urinary and/or serum NGA the most accurate at predicting AKI

Examples of specific studies are as follows:

●Urinary NGAL levels were determined in 196 children following cardiopulmonary bypass. Ninety-nine patients sustained AKI, defined as a >50 percent increase in serum creatinine. **Urinary NGAL levels obtained at two hours following procedure correlated with severity and duration of AKI, length of stay, requirement for dialysis, and death.**

●Urinary NGAL levels were determined in 635 consecutive patients assessed in the emergency department.

Thirty patients had AKI, defined as the new onset of a 50 percent increase in the serum creatinine level (compared with historic baseline) or a 25 percent decrease in estimated glomerular filtration rate (eGFR). **The mean urinary NGAL level was significantly elevated in pat AKI compared with those with normal kidney function, ch kidney disease (CKD), or prerenal azotemia.**

●Urinary NGAL among other biomarkers was measured in patients coming to the emergency department and at later time points in the ICU. Urinary NGAL diagnosed AKI up to 48 hours. Testing later did not affect the AKI diagnostic performance.

●Urinary NGAL, monomeric NGAL (mNGAL), interleukin (IL)-18, and other conventional urinary biomarkers for AKI (albumin, beta-2 microglobulin, fractional excretion of sodium) were measured at diagnosis and on days 3, 7, and 14 in 320 patients with AKI and decompensated cirrhosis. **Among all biomarkers, urinary NGAL measured at day 3 had the greatest accuracy for differentiating ATN from all other types of AKI.**

Urinary kidney injury molecule-1 —

Kidney injury molecule-1 (KIM-1), is low in normal kidneys but in high levels in the proximal tubule cells of kidneys with ischemic or toxic injury.

The ectodomain of KIM-1 is a soluble fragment that can be measured in urine by immunoassay. KIM-1 has been tested in a number of cohorts serving as a sensitive and specific biomarker for AKI.

●**KIM-1**-In a prospective study that included 123 adults undergoing cardiac surgery, urinary KIM-1, NGAL, cystatin C, hepatocyte growth factor, pi-GST, alpha-GST, and fractional excretion of sodium and urea were measured preoperatively, postoperatively, and at the time of the clinical diagnosis of AKI.

At various postoperative time points, cystatin C, NGAL, KIM-1, alpha-GST, and pi-GST all demonstrated ability to diagnose stage 3 AKI.

Preoperative KIM-1 and alpha-GST predicted the development of stage 1 and stage 3 AKI, possibly reflecting subclinical proximal tubular injury present at this time.

- **KIM-1-**

In one study of 38 patients, KIM-1 differentiated ATN from other forms of AKI and CKD.

The normalized urinary KIM-1 levels were significantly higher in patients with ischemic ATN (2.92) compared with levels in patients with other forms of AKI (0.63) or CKD (0.72). After adjustment for age, sex, time between the initial insult, and sampling of the urine, a one-unit increase in normalized KIM-1 was associated with a greater than 12-fold (odds ratio [OR] 12.4, 95% CI 1.2-119) risk for the presence of ATN.

●**KIM-1** was tested in a **case-control study of 20 children** who underwent cardiopulmonary bypass surgery both with and without complicating AKI.

Urinary KIM-1 was increased 6 to 12 hours following cardiopulmonary bypass and remained elevated up to 48 hours in patients who sustained a >50 percent increase in serum creatinine within the first 48 hours but not in children who had normal kidney function.

The increase in KIM-1 was paralleled by that of NGAL.

Urinary interleukin-18

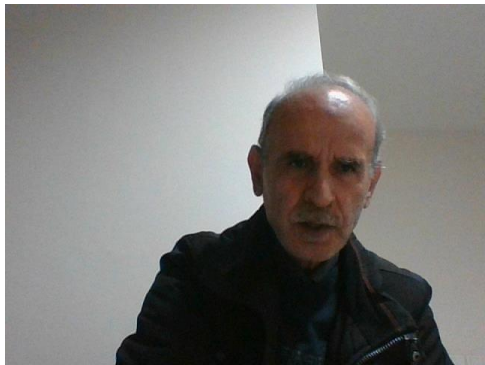
Urinary interleukin (IL)-18 has been shown to be elevated in patients with ATN compared with patients with prerenal azotemia, urinary tract infection (UTI), or CKD. Its predictive utility for AKI following cardiac surgery could not be demonstrated in one prospective, observational study of 100 adult patients. In addition, in a large study population of 1439 critically ill patients, IL-18 had a poor-to-moderate ability to predict AKI, kidney replacement therapy, or 90-day mortality. Further studies are needed to clarify the role of urinary IL-18 as a biomarker.

Urinary liver-type fatty acid-binding protein —

Urinary excretion of urinary liver-type fatty acid-binding protein (L-FABP) reflects stress of proximal tubular epithelial cells and correlates with severity of ischemic tubular injury.

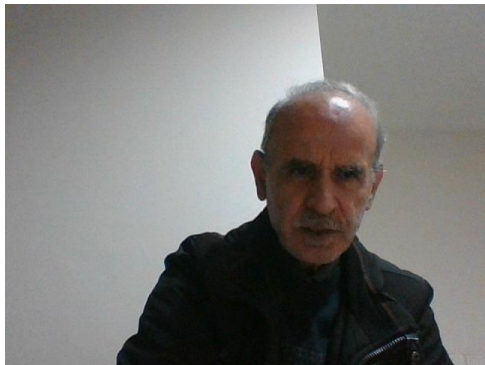
A meta-analysis of 15 prospective cohort studies demonstrated that, in hospital–based cohorts of patients at risk of AKI, L-FABP can discriminate for the diagnosis of AKI and predict the need for dialysis and in-hospital mortality. Further validation is needed in large cohort studies

Combination of biomarkers — Several biomarkers are now investigated in panels, some including clinically available markers such as a urinary microscopy score. In a prospective study of 90 adults with cardiac surgery, KIM-1, NAG, and NGAL were evaluated for early detection of AKI in the postoperative phase. The combined analysis of these three markers enhanced the sensitivity of early detection of postoperative AKI.



●In another prospective, observational study, NGAL, cystatin C, and conventional markers like creatinine and urea were measured at different time points before and after cardiac surgery in 100 adult patients.

NGAL and cystatin C (in patients with initial eGFR >60 mL/min/1.73 m^2) were independent predictors of AKI. Similar findings were reported in another study of pediatric patients.



●In a multicenter, prospective, cohort study, five biomarkers were measured in 1635 unselected emergency department patients at time of hospital admission.

Urinary NGAL and urinary KIM-1 predicted a composite outcome of dialysis initiation or death during hospitalization.

These biomarkers could detect a subpopulation with low serum creatinine on admissions that was at risk of adverse events.

Other areas of research for biomarkers of AKI

- **Proteomics –protein profiling: the study of the interactions, function, composition, and structures of proteins and their cellular activities. Proteomics provides a better understanding of the structure and function of the organism than genomics**.

The role of protein profiling with laser mass spectrometry in urinary samples of patients with AKI is being investigated.

This method allows identification of new and early markers of AKI. In a derivation cohort of cardiac bypass surgery patients,

a panel of 204 urinary peptides related to hemolysis, inflammation, immune cells, cell growth, and survival wa identified as a potential predictor of AKI. In two validation this novel peptide signature outperformed several single bio and clinical scores.

- Metabolomics –

Metabolomics is the study of **small-molecule metabolites that are produced by the body and provide insight into physiological and pathophysiological conditions.**

Metabolomic analysis can be readily performed in biofluids such as blood and urine, and, because there are fewer metabolites than there are genes, mRNA, and proteins, analyses are simpler.

This method may allow for identification of new markers in AKI

●Extracellular vesicles (EVs) –

EVs are a heterogenous population of small submicron membrane fragments released from multivesicular bodies (exosomes, <100 nm) or shed from various cell types into different body fluids (microvesicles, 100 to 1000 nm).

They carry markers of their parent cells that are utilized to identify their origin.

In particular, urinary EVs contain proteins from various nephron segments providing nephron-specific information.

In addition, EVs represent an important mode of intercellular communication by serving as vehicles for transfer between cells of membrane and cytosolic proteins, lipids, and genetic inform

Therefore, these EVs have important implications with r biomarkers and mechanisms of disease.

- MicroRNAs –

Serum microRNAs are being explored in patients with AKI. MicroRNAs were profiled in 77 patients with AKI and 18 critically ill patients with acute myocardial infarction. **Circulating microRNAs were altered in patients with AKI**. MicroRNA-210 predicted mortality in this patient cohort. Other pilot studies are indicating other sets of microRNAs to be altered days before increase in serum creatinine.

- Markers of inflammation – Plasma IL-6 and IL-10 have been measured in adults undergoing cardiac surgery. IL-6 and IL-10 were elevated after surgery and associated with higher risk for AKI. In a substudy of the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) cohort, plasma IL-6 and IL-10 were measured in 106 children undergoing cardiopulmonary bypass.

Preoperative plasma IL-6 concentrations were associated with the development of stage 2 and

●Others – Additional novel markers that have been evaluated include serum cystatin C levels, isoform 3 of the sodium-hydrogen exchanger (NHE3), perforin and granzyme B, CXCR3-binding chemokines, endothelin, ProANP (1Y 98), tryptophan glycoconjugate and cysteine-rich protein 61 (CYR61), fatty acid-binding protein, TNF receptor-I, plasminogen activator inhibitor-1, netrin-1 [79], activating transcription factor 3 (ATF3), and MCP-1. Prospective studies need to be done to evaluate the utility of these biomarkers.

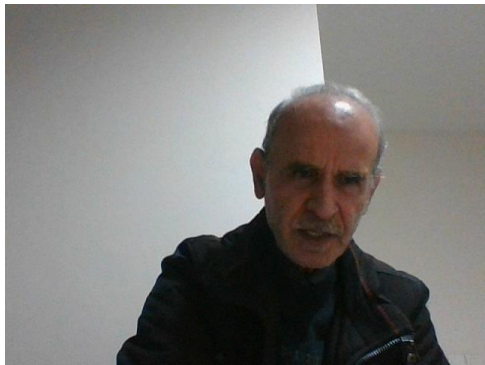
PROGNOSTIC BIOMARKERS —

Most of the biomarkers described above allow early detection of AKI but do not predict severe acute kidney injury (AKI).

Soluble urokinase plasminogen activator receptor (suPAR) — suPAR is the circulating form of the membrane-bound uPAR, a glycosyl-phosphatidylinositol-anchored protein normally **expressed on endothelial cells, podocytes, and, with induced expression, on monocytes and lymphocytes**.

In the setting of inflammation, uPAR is shed by immune cells following proteolytic cleavage and travels as the soluble form, suPAR.

suPAR is thought to represent a biomarker of immune activation and inflammation.



As a biomarker, suPAR was predictive of progressive decline in kidney function in a number of studies that included healthy participants and patients with CKD of various etiologies.
suPAR was also predictive of AKI in multiple cohorts.
In one study, for example, high levels of suPAR were associated with risk of mild to moderate AKI within the first seven days after cardiac surgery, coronary angiography, or ICU admission.

Dickkopf-3

The preoperative urinary concentration of dickkopf-3 (DKK3), a urinary cytokine and tubular stress biomarker, has been used to identify surgical patients at high risk for AKI.

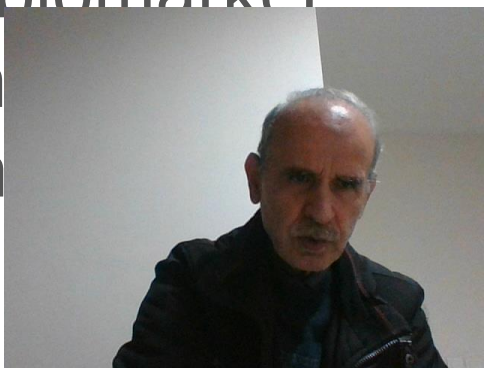
In over 700 patients who were scheduled to undergo cardiac surgery, elevated urinary DKK3 to creatinine ratios were associated with an increased risk of postoperative AKI, independent of baseline kidney function.

Furthermore, compared with clinical and other labora
urinary DKK3 to creatinine ratios improved AKI predic

Uromodulin (Tamm-Horsfall protein) — Uromodulin is a 95-kD glycoprotein produced by the thick ascending limb and the distal convoluted tubule and is thought to bind pathogenic bacteria and prevent stone formation.

Mice deficient of uromodulin are more susceptible to ischemia-reperfusion kidney injury.

In a post-hoc analysis of a prospective cohort study of 218 adult patients undergoing cardiac surgery, lower urinary uromodulin/creatinine ratio was associated with higher odds for AKI. In children undergoing cardiopulmonary bypass surgery, preoperative urinary uromodulin also has been assessed as a predictive biomarker. Children in the lowest quartile of urinary uromodulin levels h markedly increased risk of postoperative AKI compared with the highest quartile.



Urinary insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2) — Sepsis- and ischemia-induced cell injury and repair are associated with cell cycle regulation.

IGFBP7 and TIMP-2, two urinary biomarkers identified in a discovery study, are expressed in epithelial cells and act in an autocrine and paracrine manner to arrest cell cycle in AKI.

In the Sapphire validation study of over 700 critically ill patients, the primary endpoint was moderate to severe AKI (Kidney Disease: Improving Global Outcomes [KDIGO] stage 2 to 3) within 12 hours of sample collection. These markers performed well in patients with sepsis (with area under the receiver operating characteristics curve [A 0.82) and postsurgery (AUC 0.85) in comparison with traditiona biomarkers and improved risk stratification for AKI well ahead o manifestations (azotemia and oliguria).

Furosemide stress test

— The furosemide stress test (FST) was developed as a clinical tool to assess the risk for AKI progression. FST entails administration of intravenous furosemide (1 mg/kg in furosemide-naïve and 1.5 mg/kg in non-naïve) to euvolemic patients with stage 1 or 2 AKI. A urine output of >200 mL over two hours after administration of furosemide indicates FST responsiveness. In observational studies, FST-unresponsiveness was predictive of progression to stage 3 AKI, need for kidney replacement therapy, and higher inpat mortality.

RESEARCH ARTICLE

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Illumination of Hydroxyl Radical in Kidney Injury and High-Throughput Screening of Natural Protectants Using a Fluorescent/Photoacoustic Probe

Han Gao, Lei Sun, Jiwei Li, Qilin Zhou, Haijun Xu, Xiao-Nan Ma, Renshi Li,* Bo-Yang Yu, and Jiangwei Tian**

The hydroxyl radical (•OH) is shown to play a crucial role in the occurrence and progression of acute kidney injury (AKI). Therefore, the development of a robust •OH probe holds great promise for the early diagnosis of AKI, high-throughput screening (HTS) of natural protectants, and elucidating the molecular mechanism of intervention in AKI. Herein, the design and synthesis of an activatable fluorescent/photoacoustic (PA) probe (CDIA) for sensitive and selective imaging of •OH in AKI is reported. CDIA has near-infrared fluorescence/PA channels and fast activation kinetics, enabling the detection of the onset of •OH in an AKI model. The positive detection time of 12 h using this probe is superior to the 48-hour detection time for typical clinical assays, such as blood urea nitrogen and serum creatinine detection. Furthermore, a method is established using CDIA for HTS of natural •OH inhibitors from herbal medicines. Puerarin is screened out by activating the Sirt1/Nrf2/Keap1 signaling pathway to protect renal cells in AKI. Overall, this work provides a versatile and dual-mode tool for illuminating the •OH-related pathological process in AKI and screening additional compounds to prevent and treat AKI.

the timely diagnosis and treatment of AKI critical for preventing and managing kidney diseases. In vitro diagnostic methods have been employed to monitor renal function and prevent AKI. However, the current diagnostic approach for kidney disease in clinical settings relies on static analysis of blood urea nitrogen (BUN) and serum creatinine (sCr),[2] which only increase after a 50% decrease in glomerular filtration rate (GFR).[3] Non-invasive imaging strategies, such as single-photon emission computed tomography (SPECT) a
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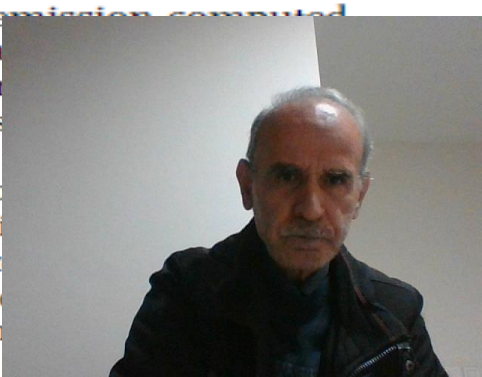

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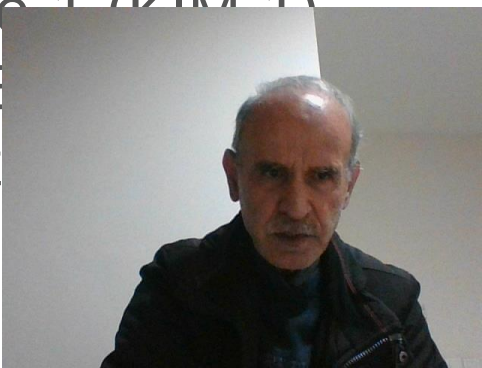
SUMMARY

●**Investigational biomarkers of acute kidney injury** – Acute kidney injury (AKI) is a common clinical problem. Although measurement of the serum creatinine concentration is widely used for the detection of AKI, it does not permit early diagnosis of acute tubular necrosis (ATN), since tubular injury precedes a significant rise in serum creatinine. Investigational biomarkers have been evaluated in patients with possible ATN in an attempt to detect tubular injury at an earlier stage.

●**Steps necessary for clinical use** – Various urinary and serum proteins have been intensively investigated as possible biomarkers for the early diagnosis of ATN. Before any such proteins are used clinically, validation in different settings of AKI and the development and testing of rapid assays are necessary. In addition, it needs to be shown if there is an association between levels of biomarkers and outcome.

●**Diagnostic biomarkers** – Promising biomarkers for the diagnosis of AKI include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), urinary interleukin (IL)-18, and liver-type fatty acid-binding protein (L-FAB

●**Prognostic biomarkers** – The development of biomarkers that predict occurrence and/or severity of AKI would allow for personalized interventi or minimize AKI. Assays proposed to identify patients at high risk of AKI,



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