Biomarkers and predicting acute kidney injury

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Short title

AKI Biomarkers

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Abstract

Aim:

How can we convert biomarkers into reliable, validated laboratory tests? GFR estimators exist for more than a century. The first utilitarian biomarkers were endogenously produced urea and creatinine. Clinicians then developed simple tests to determine whether or not renal tubular function was maintained. Are there faster and better tests that reflect decreased renal function and increased acute kidney injury (AKI) risk?

Methods:

We inspect earlier, and recently propagated biomarkers. Cystatin C reflects GFR and is not confounded by muscle mass. Direct GFR and plasma volume can now be measured acutely within 3 h. Better yet would be tests that give information before GFR decreases and prior to urea, creatinine, and cystatin C increases. Prospective tests identifying those persons likely to develop AKI would be helpful. Even more utilitarian would be a test that also suggests a therapeutic avenue.

Results:

A number of highly provocative biomarkers have recently been proposed. Moreover, the application of big data from huge electronic medical records promise new directions in identifying and dealing with AKI.

Conclusions:

Pipedreams are in the pipeline; the novel findings require immediate testing, verification, and perhaps application. Future research promises to make such dreams come true.

Key words

Acute kidney injury, biomarkers, artificial intelligence

Introduction

Acute kidney injury (AKI) is defined by a rapid increase in serum (or plasma) creatinine, decrease in urine output, or both. AKI is common and occurs in approximately 15% of patients admitted to hospital, while its incidence in intensive care units has been reported in more than half of patients with a substantial mortality. AKI is not a single disease, but instead consists of a loose syndrome collection of conditions including sepsis, cardiovascular causes, nephrotoxicity, urinary tract obstruction, and in short - anything that can cause glomerular filtration rate (GFR) to be reduced quickly.[1] Can the condition be separated from reversible causes? The importance of delineating any

unknown acute decrease in renal function into “prerenal”, “renal”, and “postrenal” etiologies has not changed since the first renal biomarkers were described and remains the physician’s immediate obligation.

The AKI time framework is 7 days. The current AKI definitions are based on two basic criteria: changes in serum creatinine and how much urine is made (oliguria or not?). From those two variables, we can stage AKI into stage 1 (creatinine >1.5 times baseline and/or urine volume <0.5 ml/kg for 6-12 h); stage 2 (creatinine >2 times baseline or urine volume <0.5 ml/kg for >12 h) or stage 3 (creatinine >3.0 times baseline or urine volume <0.3 ml/kg for >24 h). These definitions are of great value for classification (chart review) but not invariably helpful for physicians working with acutely ill patients. Creatinine is easily measured, while urine production rate is another matter. Choosing Wisely campaigns are aimed to advance a national dialogue on how to avoid unnecessary medical tests, treatments, and procedures. Can physicians receive help for their patients at the bedside or (very) shortly thereafter, particularly along the lines of “choosing wisely”? Biomarkers (better than creatinine) could be of assistance. Arguably, the first utilitarian marker for renal disease, AKI, AKD, or CKD, was proteinuria, which we generally attribute to Bright but was probably described a century earlier.[2] What progress has been made in this area up to the present date (Figure 1)?

Assessment goals

Any clinical test, biomarkers included, requires addressing certain accepted standards. Sensitivity and specificity are terms used to evaluate a clinical test's utility (positive in disease, negative in health). They are independent on the population of interest. Positive and negative predictive values consider the clinical value of a test. These latter parameters are dependent on the prevalence of the disease in the population of interest. The sensitivity and specificity of a quantitative test are also subject to the cut-off value, above or below, which the test is positive. In general, the higher the sensitivity, the lower the specificity (and vice versa). Thereafter, receiver-operator characteristics (ROC) are commonly generated. ROC curves plot false positives against true positives for all cut- off values. The area under the curve (AUC) of a perfect test is 1.0 (right angle) and that of a useless test, no better than tossing a coin (linear line). "Biomarkers" (albeit trendy) are not excused from these rigorous criteria.

Glomerular filtration rate and other markers

Urea and creatinine

Non-protein nitrogen (urea, biuret, and ammonia) was an early marker, but quickly supplanted by urea (in the USA expressed as blood-urea nitrogen, BUN).[3] Thereafter, creatinine soon followed. Van Slyke and colleagues developed the concept of "clearance" and in a remarkable paper compared the clearance of urea and creatinine to phenolsulfonphthalein (PSP) excretion in terms of utility.[4] The authors observed that urea clearance falls below 50% of its normal value before blood creatinine content, blood urea content, or PSP excretion were altered. The group also observed a unique value in determining the urine (U) to plasma (P) ratio (U/P) to delineate certain conditions. Then how did creatinine obtain the vaunted position it holds today?

Rehberg first proposed the use of creatinine to monitor renal function.[5] The measurement at the time was cumbersome in terms of overestimating serum creatinine at values <88 μmol/L, so Rehberg gave creatinine exogenously. We now know that creatinine is excreted slightly. Thus, Rehberg surely saturated the excretory tubular transporter, resulting in a highly accurate GFR estimate; however, Van Slyke had not yet suggested the clearance concept. Since Rehberg, the creatinine era extends to almost 100 years of research and now GFR is estimated by various formulae (eGFR). The subject has been recently reviewed.[6]

Creatinine is a breakdown product of creatine phosphate from muscle and protein metabolism and is released at a constant rate, depending on the body's muscle mass (Figure 2A). Creatine kinase catalyzes the conversion to phosphocreatine. Formation of the anhydride, creatinine, occurs spontaneously during this process (Figure 2B). Creatinine is eliminated by glomerular filtration; however, very little or no reabsorption of creatinine occurs. There is modest tubular secretion, a fraction that relatively increases as GFR decreases. Since Smith, we know that inulin clearance is the gold standard for determining GFR,[7] although renal or plasma clearance of chromium 51-labeled ethylenediaminetetraacetic acid (51Cr-EDTA), diethylenetriaminepentaacetic acid (DTPA), iohexol, and iothalamate are as good.[8]

A glance at the relationship between GFR and serum creatinine (Figure 3A) discloses the fact that creatinine is very good when GFR is sharply reduced, but of lesser utility when renal function is largely preserved.[9] As a result, GFR can be reduced by >50% before clinicians notice the elevation. Clinicians earlier relied on clever strategies

to circumvent confounders in patients with suspected AKI, particularly when urinary output decreased. Since urea is resorbed by the tubules in a flow-dependent manner (oliguria – increased urea absorption), volume depletion (pre-renal azotemia) should feature a sharper increase in plasma urea than creatinine. Measuring U/P urea and U/P creatinine was an early strategy to diagnose AKI, as was determining tubular function in terms of sodium reabsorption. With a single plasma (or serum) sample, coupled with a simultaneous urine sample (no timed collection is necessary), clinicians can distinguish between AKI and volume depletion by calculating the amount of sodium excreted per the amount filtered by dividing the clearance of sodium by the clearance of creatinine, namely the fractional excretion (FENa). Of course, these functional tests are confounded by diuretic treatment and the time interval of administration. A common indignant cause for nephrology consultation in any hospital is: “This patient refuses to respond to Lasix®!” At that point, an intellectual confrontation with AKI is probably already too late. Urinary tract obstruction can now be ruled out in minutes with ultrasound. However, some care and clinical acumen is required in these diagnostic processes and therefore the search for more biomarkers continues.

Cystatin C

Cystatin C is a cysteine-protease inhibitor (molecular weight 13.3 kD) that is freely filtered but is degraded and/or resorbed in the renal tubule so that none appears in the urine. Since 1985, cystatin C has been suggested as marker of the renal function.[10] The protease has a "house-keeping" function. Cystatin C is produced at a constant rate in all nucleated cells investigated to date, freely filtered in the renal glomeruli and reabsorbed, and catabolized in the proximal tubules. This catabolic process largely involves megalin. Jensen et al[11] showed that cystatin C binds to megalin and cubulin with high affinity. Megalin deficient mice revealed an increased urinary excretion of cystatin C associated with defective uptake by endocytosis. In rats exposed to ischemia/reperfusion injury urinary cystatin C excretion was increased and associated with a focal decrease in proximal tubule endocytosis with no apparent change in megalin expression. These findings may be of relevance to clinical AKI.

Cystatin C avoids several confounding variables that plague other estimators of GFR. Creatinine is inaccurate at detecting mild renal impairment, and creatinine levels can vary with muscle mass but little (aside from body builders) with protein intake. Urea

levels might change with protein intake and are also urine flow rate dependent. Urea reabsorption is heavily dependent upon protein intake, hydration state, and tubular reabsorption as already observed by Van Slyke et al.[4] Cystatin C side-steps these pitfalls. Direct comparisons of cystatin C- and creatinine-based prediction equations for eGFR, compared to a gold standard, have been made.[12] In those studies, S-Cys C-based prediction equations appeared to be more precise than those of S-Cr for those patients with measured GFR > 60 mL/min/1.73 m^2 and could therefore be of benefit in the earlier detection of renal impairment in certain subpopulations.

But is cystatin C better than creatinine (Figure 3B-C)? Certainly not in cost that favors creatinine over cystatin C by a factor of 10 to 1. A direct comparison between cystatin C and creatinine has been published.[9] Conceivably, in patients with reduced muscle mass because of amputations or in those with degenerative muscle disease, cystatin C might provide more accurate information compared to serum creatinine. Cystatin C may also play a role in assessing renal function in children or in the massively obese. However, as a biomarker, the idea that cystatin C beats creatinine (or urea for that matter) is difficult to defend. Finally, our clinical laboratory reports creatinine values within 20 min. Cystatin C levels (Figure 2C) require much more time.

GFR estimation at the bedside with a visible fluorescent injectate

Rizk and colleagues developed a novel method for rapid bedside GFR measurements that beats creatinine and cystatin C.[13] Simultaneously, the authors estimated effective plasma volume. The markers consist of a rhodamine derivative and small 5-kD fluorescein carboxymethylated dextrans. After a single intravenous injection, both GFR and plasma volume are determined on the basis of the plasma pharmacokinetics of the rhodamine derivative and the fluorescein carboxymethylated dextrans, respectively. The authors relied on iohexol-measured GFR as the gold standard and achieved a correlation with visible fluorescent injectate GFR R^2=0.99. The blood requirement was 1.5 ml and the time-frame 3 h. The added value here is the simultaneous determination of plasma volume, a value of substantial therapeutic importance. Whether or not this method should be employed as a screen in those at high risk or used when simpler biomarkers are suggestive of AKI remains to be determined. In any event, in intensive care units such a technique would clearly be more valuable than eGFR.

Markers that precede creatinine increases

The idea is that the kidneys react to stress or damage by releasing detectable products into the urine or plasma. Since so many mechanisms in the kidneys could be influenced, the candidates to examined are many. The presence of many candidates does not necessarily translate into better diagnostic strategies. But it does result in far more work. A schematic of candidates, tested or hypothetical (Figure 3), is formidable.

Numerous molecules have been identified as potential markers for early AKI detection before creatinine increases can be verified. An early candidate was N-acetyl-beta-D-glucosaminidase (NAG). NAG is a hydrolytic lysosomal enzyme that is present in high concentrations in proximal tubular cell lysosomes. The NAG isoenzyme-B is associated to the lysosomal membrane and excreted in urine during tubular damage. Because of its stability in urine, its relatively large molecular mass which precludes filtration by the glomerulus and its presence in high activity in the tubular lysosomes, elevation of urinary NAG activity has been taken as a marker for renal proximal tubular damage or more precisely, loss of lysosomal integrity. Unfortunately, the threshold for release of tubular enzymes in response to injury may not lead to clinically apparent AKI is quite low.[14] Therefore, urinary NAG has high sensitivity but low specificity for AKI.

Nephrocheck®

Tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor binding protein-? (IGFBP7) reflect cell-cycle arrest, a phenomenon occurring in proximal tubular cells under stress. TIMP-2 and IGFBP7 have been incorporated and commercialized (Astute Medical, San Diego, CA, USA) as a diagnostic test (Nephrocheck). The test gives results within 20 min and the markers peak at 6-24 h. Nephrocheck has been assessed in two single-center trials, leading to Food-and-Drug Administration (FDA) approval. Meersch et al randomized Nephrocheck-positive patients after cardiac surgery to receive specialized care including fluid management and the titration of vasoactive medications. The authors reported a reduction in established AKI.[15] A similar care bundle was given to patients undergoing non-cardiac major surgery after testing positive for the biomarkers.[16] In that study, the rates of moderate and severe AKI were significantly reduced with the intervention. The sample sizes in these studies were small. The need for renal replacement therapy, and mortality were not reduced.

The patients in both studies were at high risk. Nonetheless, the investigators were intrepid by simultaneously testing an intervention.

Kidney injury marker-1 (KIM-1)

KIM-1 is a type 1 transmembrane protein, with an immunoglobulin and mucin domain that is markedly up-regulated in the proximal tubule in the post-ischemic rat kidney. Kim-1 is an apoptotic-cell phagocytosis and scavenger receptor that is most highly upregulated in proximal tubular epithelium in acute and chronic kidney injury.[17] The ectodomain of KIM-1 is shed from cells. Han et al conducted studies to evaluate whether or not KIM-1 is present in human AKI and might serve as a urinary marker of acute renal tubular injury.[18] They reported a soluble form KIM-1 in the urine of AKI patients. Since that report, the utility of KIM-1 in predicting AKI has been avidly pursued. KIM-1 has been most convincingly utilized to identify AKI in the course of drug therapies closely associated with AKI, such as cisplatin-induced nephrotoxicity. Urinary KIM-1 levels are elevated in cisplatin induced AKI and may help in the differential diagnosis of proximal epithelial-cell injuries.[19]

Neutrophil gelatinase associated lipocalin (NGAL)

NGAL is a member of the lipocalin superfamily, 24p3, which induces the formation of renal epithelia.[20] NGAL acts as a growth and differentiation factor in multiple cell types, including developing and mature renal epithelia, and some of this activity is enhanced in the presence of siderophore-iron complexes.[21] The molecule is responsible for iron traffic within renal epithelia.[22] Mishra et al used a transcriptome-wide interrogation strategy to identify renal genes that are induced very early after renal ischemia, whose protein products might serve as novel biomarkers for AKI.[23] NGAL proved the most convincing. Since that time, NGAL has been a serious contender as a utilitarian biomarker for AKI that indeed supersedes creatinine. Nevertheless, weaknesses have been exposed and doubts as to NGAL's utility have been expressed (Figure 4).[24] NGAL levels not only increase unpredictably during evolving AKI, but also during other chronic and acute inflammatory conditions frequently encountered in intensive care units. Such conditions include sepsis, recent coronary bypass surgery, and acute exacerbations of obstructive pulmonary diseases.

Liver-type fatty acid binding protein (L-FABP)

L-FABP is expressed in human proximal tubules and has an endogenous antioxidative function. L-FABP binds fatty acids and binds them to mitochondria or peroxisomes, where the fatty acids then undergo beta oxidation. L-FABP is expressed not only in the liver, but also in the intestine, pancreas, stomach, lung, and kidney.[25] L-FABP has been studied as a biomarker (ischemia-reperfusion, contrast-induced AKI, transplantation), as well as a potential ameliorating agent. For instance, Matsui et al investigated aristolochic-acid (Balkan nephropathy) nephrotoxicity.[26] Since mice do not express L-FABP in kidney, the authors constructed a transgenic mouse that does so (hl-FABP). Administration of aristolochic acid increased hl-FABP. These mice were protected from Balkan nephropathy compared to wild-type controls without hl-FABP. The novelty here is identifying a biomarker that also harbors a possible therapeutic potential.

Proenkefalin (PENK)

Enkephalins are opioid peptides that are found at high levels in the brain and endocrine tissues. Studies have shown that enkephalins play an important role in behavior, pain, cardiac function, cellular growth, immunity, and ischemic tolerance. PENK is an endogenous opioid polypeptide hormone, which via proteolyic cleavage, produces the enkephalin peptides [met]-enkephalin, and to a lesser extent, [leu]-enkephalin. PENK is expressed in many tissues including the kidney.[27] Beunders et al have proposed PENK as a novel biomarker for renal function.[28] PENK plasma levels showed promise and were highly correlated with iohexol clearance. An influence of PENK on outcome was suggested by the authors but not documented.

Urinary chemokine (C-C motif) ligand 2 (monocyte chemotactic protein-1)

Chemokine (C-C motif) ligand 2 (CCL2) is a chemotactic cytokine that recruits monocytes, releases growth factors and promotes adhesion in vascular endothelium. The CCL2 molecule also recruits memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection. Elevated serum and urinary CCL2 levels and expression of its receptor (CCR2) have been associated with tumorigenesis in human urinary-tract malignancies. The molecule is upregulated in ischemia-reperfusion injury. Moledina et al measured preoperative and postoperative

plasma MCP-1 levels in adults undergoing cardiac operations to evaluate the association of perioperative MCP-1 levels with acute kidney injury (AKI) and death. Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) was a prospective, multicenter, observational cohort study.[29] The authors reported that higher plasma MCP-1 were associated with increased AKI and risk of death after cardiac operations. They suggest that MCP-1 could be used as a biomarker to identify high-risk patients for potential AKI prevention strategies in the setting of cardiac operations.

Dickkopf-3 (DKK-3)

The *dickkopf* (German fathead) *Dkk* gene family encodes secreted glycoproteins represented as four main members in vertebrates.[30] The hallmark of the Dkk1/2/4 family is their ability to modulate Wnt signaling. Wnts, in turn, are an evolutionarily conserved family of growth factors, whose signaling is involved in numerous processes in development, organism maintenance, and disease. In the adult, DKKs are implicated in bone formation and bone disease, cancer, Alzheimer's disease, and now also AKI.

Zewinger et al hypothesized that DKK-3 might reflect stress-induced renal tubular epithelia and that the glycoprotein could provide information regarding the propensity of ongoing tubulo-interstitial disease and short-term eGFR loss.[31] Thus, they actually investigated acute kidney disease (AKD) rather than AKI. First, they observed that DKK-3-to-creatinine concentrations at baseline were higher in CKD patients than controls, supporting the idea that DKK-3 is produced in greater amounts in these patients. Furthermore, DKK-3 predicted the subsequent steepness in decline of eGFR in the patients. Thus, DKK-3 appeared to be a biomarker of persons particularly at risk for a rapidly declining GFR.

In a subsequent article, the group investigated any association between DKK-3, AKI, and subsequent renal functional decline in persons undergoing cardiac surgery.[32] The initial observational study from Saarland University included patients who had cardiac surgery forming a derivational cohort. Thereafter, the investigators studied patients who underwent cardiac surgery at the Cleveland Clinic (RenalRIP trial) forming a validation cohort. The authors found that compared to clinical and other laboratory measurements, urinary concentrations of DKK-3-to-creatinine determinations significantly improved AKI prediction in both cohorts. High urinary DKK-3-to-creatinine concentrations were independently associated with significantly lower renal function at

hospital discharge and reduced GFR after a median follow-up of 820 days. The authors concluded that preoperative urinary DKK-3 is an independent predictor of subsequent AKI risk and a further loss of renal function. Thus, the biomarker could predict AKI before any initiating event, a feature that is unique compared to other markers.

In experimental CKD models, DKK-3 promoted renal tubulointerstitial fibrosis through modulation of the canonical Wnt/-catenin signaling pathway.[33] In clinical studies (above), increased urinary DKK3 levels identified patients at high risk for short-term CKD progression, regardless of the cause of kidney disease, baseline renal function, and albuminuria. A schematic of DKK-3 and kidney is shown (Figure 5).[34]

Soluble urokinase plasminogen activator receptor (suPAR)

Urokinase, also known as urokinase-type plasminogen activator (uPA) is a serine protease originally isolated from urine, but also present in blood and extracellular matrix. The primary substrate is plasminogen, which when cleaved to plasmin triggers a proteolytic cascade that participates in thrombolysis and/or extracellular matrix degradation. The urokinase receptor (CD87 or uPAR) is a multidornain glycoprotein, tethered to cell membranes via a glycosylphosphodidyl- inositol (GPI) anchor. The uPAR interacts with several other proteins, including vitronectin, the uPAR-associated protein (uPARAP), and the integrin family of membrane proteins. When When uPA is bound to the receptor, there is cleavage between the GPI-anchor and the uPAR, releasing soluble receptor (suPAR). The circulating suPAR is a marker of renal disease severity and aggressiveness and has become a biomarker for activation of the inflammatory and immune systems (Figure 6).[35]

The investigators have shown earlier that circulating suPAR is involved in the pathogenesis of focal and segmental glomerulosclerosis and likely acts on podocytes by activating the avβ3 integrin on the cell surface.[36] The group has also shown that the apolipoprotein L1 gene variants present in African Americans are dependent on plasma suPAR levels.[37] APOL1 G1 or G2 genotypes augment avβ3 integrin activation and causes proteinuria in mice in a suPAR-dependent manner. The synergy suPAR and APOL1 G1 or G2 variants on avβ3 integrin activation is a mechanism for CKD.

In a recent tour-de-force, the group showed that suPAR is a marker for, and may be involved in the pathogenesis of, AKI.[38] The investigators measured plasma levels of

suPAR pre-procedurally in patients who underwent coronary angiography (exploratory cohort) and patients who underwent cardiac surgery (confirmatory cohort) and at the time of admission to the intensive care unit in critically ill patients. Pre-procedural high suPAR levels were associated with AKI in the clinical studies, similar to the report involving DKK-3. This state-of-affairs was consistent across a variety of clinical subgroups. The data were underscored with a transgenic suPAR over-expressing mouse model that was subjected to iodinated contrast material. An anti-suPAR antibody ameliorated the effects. The suPAR molecule, binding to avβ3 integrin in a cell system, generated superoxide. The suPAR-treated cells also had a higher rate of non-mitochondrial oxygen consumption, indicating an active involvement of other cellular oxygen-consuming reactions in addition to that catalyzed by the mitochondrial cytochrome C oxidase.

A prospective study to identify biomarkers (RUBY)

A multi-center international prospective observational study to identify biomarkers of the persistence of stage 3 AKI as defined by the KDIGO criteria would appear highly desirable. The RUBY (not an eponym) was such an investigation. Patients in the intensive care unit (ICU) with moderate or severe AKI (KDIGO stage 2 or 3) were enrolled.[39] A total of 336 patients could be analyzed. The authors selected potential candidate biomarkers from proteins associated with apoptosis, necrosis, endothelial injury, cell–cell and cell–matrix adhesion, cytoprotection, oxidative processes, cell-cycle regulation, inflammation, tubular injury, immune function, and fibrosis for biologic plausibility. Urinary C–C motif chemokine ligand 14 (CCL14) was the most predictive of persistent stage 3 AKI with an area under the receiver operating characteristic curve (AUC) of 0.95. As a matter of fact, CCL14 beat CH13L1, plasma cystatin C, plasma proenkephalin, urinary NGAL, and urinary L-FABP.

CCL14 is a member of the chemokine family of small molecules that were initially recognized for roles in leukocyte chemotaxis and are implicated in tissue injury and repair processes. CCL14 (HCC-1) belongs to the CC chemokine family. CCL14 activates monocytes but does not induce chemotaxis. Could CCL14 be a mediator of AKI and non-recovery? A possibility might be that inflammatory (such as tumor necrosis factor-α

and others) signals from injured tubules, release CCL14. Such a state-of-affairs could promote further Th1 inflammatory signals.

Whither to now?

If one's own knowledge is not enough, perhaps artificial intelligence could help.[40] A retrospective cohort study was conducted in medical, surgical, and mixed ICUs at Mayo Clinic in Rochester, Minnesota. The investigators stated their primary objective as predicting AKI using extant clinical data following ICU admission. They incorporated known AKI risk factors and routinely measured vital characteristics and laboratory results. The model achieved an AUC of 0.88 on validation. The results suggest a sensitivity of 92%, a specificity of 68% and detected 30% of AKI cases at least 6 h before the standard time criteria (AKI stages 1-3). For discrimination of AKI stages 2 to 3, the model had 91% sensitivity, 71% specificity, and 53% detection of AKI cases at least 6 hours before AKI onset. The idea here is to apply a self-improvable modifiable algorithm to improve AKI diagnoses. Such algorithms could incorporate all biomarkers known to date.

This idea has been carried much further. Tomasev et al developed a deep learning approach for the continuous risk prediction of future AKI deterioration in patients.[41] They built their AKI model on recent work that incorporates adverse events from electronic health records. Their model was developed on a large, longitudinal, dataset of electronic health records (US Veterans Administration Electronic Health Records) that covers diverse clinical environments, comprising 703,782 adult patients across 172 inpatient locations and 1,062 outpatient sites. The model predicted 56% of all inpatient episodes AKI, and 90% of all AKI episodes that required subsequent administration of dialysis, with a lead time of up to 48 h and a ratio of 2 false alerts for every true alert. All of this remarkable result was without any of the biomarkers mentioned here.

Conclusions

The “elephant-in-the-room” question is: “Does this biomarker research improve outcomes?” The answer is, “it might!” Research from future prospective clinical studies should help us further. Investigators must settle upon a “gold-standard” test and then compare the new candidates in terms of performance with the Bland-Altman plot

statistic. Improved phenotyping of the patients is mandatory, which requires a utilitarian electronic medical records system. Particularly exciting are putative biomarkers that have direct therapeutic implications.

Prospective studies such as the RUBY study are mandatory.[39] Haase-Fielitz and colleagues recently tested the notion that drawing attention to AKI might improve care.[41] Hospitalized patients with increasing creatinine values meeting KDIGO guidelines for AKI were randomized into those identified with an early warning computerized message recommending a nephrology consult and supplied with an AKI identifier or to usual care. The nephrologists confirmed the diagnosis, made medication recommendations (usually to discontinue), optimized circulating fluid volume, and arranged for follow-up. Compared to usual care, AKI complications were reduced, diagnoses were more commonly established, and potentially deleterious medications discontinued. This study defines how creatinine (the gold standard) stacks up. Now, the investigators are in a position to test other markers against this standard in a prospective fashion. In that way, robust statistics can be applied and the markers tested against creatinine.

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Interest conflicts

None

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Figure legends

Fig. 1. Shown is a nephron along which putative "biomarkers" are approximately located. Hope is it that these products arrive in the urine before clinical evidence of decreased GFR (increase in serum creatinine) is apparent. All are dependent upon urine production. Thus, oliguria is a strong confounder.

Fig. 2. The pivotal enzyme, creatine phosphokinase, metabolizes substrates to phosphocreatine and to the by-product, creatinine (A). As a result, an anhydride (loss of water) is formed that we term "creatinine" (B). The daily-produced product is freely filtered, nonprotein bound, and appears in the urine. The resorption is essentially zero and the secretion is (relatively) trivial.

Fig. 3. The relationship between serum creatinine and glomerular filtration rate (renal work) is hyperbolic (A). Therefore, lower concentrations are of lesser value in accurately reflecting GFR than higher concentrations. Cystatin C has some advantages over creatinine in selected populations (B). However, cystatin C shows similar limitations to creatinine in those subjects with largely-preserved renal function. Cystatin C (ribbon diagram) is a "second-generation" GFR marker, also endogenously produced (C). This marker is produced by our total (nuclear-containing) cell mass (adapted from Porrini et al.[9]).

Fig. 4. Many studies suggest that NGAL levels in urine or plasma can be regarded as a biomarker for AKI. Under normal conditions, NGAL levels are relatively low in urine and plasma. However, NGAL levels in AKI patients increase rapidly from basal levels to quickly reach diagnostic levels. However, NGAL has numerous sources, several interacting partners, and relies on megalin for urinary clearance. The complexity is illustrated by the fact that urinary NGAL levels may also predict the progression of CKD (Adapted from Martennson and Bellamo.[24]).

Fig. 5. Urinary DKK3 is a biomarker of ongoing renal tubular cell injury, i.e. a renal tubular cell 'stress' indicator within the 'kidney injury continuum'. ECM, extracellular matrix (Adapted from Schunk et al.[34]).

Fig. 6. (A) Schematic of the structure of uPAR, the mechanism of cleavage and the formation of suPAR. D1, D2, and D3 represent the three homologous domains of suPAR. (B) The suPAR molecule binds to avβ3 integrin. In a human proximal tubular cell line, suPAR had profound effects on mitochondrial respiration and resulted in superoxide production (adapted from Hayek et al.[37]).

Glomerulus

Cystatin C
β2 microglobulin
α1 microglobulin
Albumin
Creatinine
Urea

Proximal Tubule

KIM-1
Clusterin
NAG
NGAL
TIMP-2
IGFBP7
GST-α
Netrin-1
RBP
IL-18
HGF
PENK
CCL2
CCL12
L-FABP
Albumin

Distal Tubule

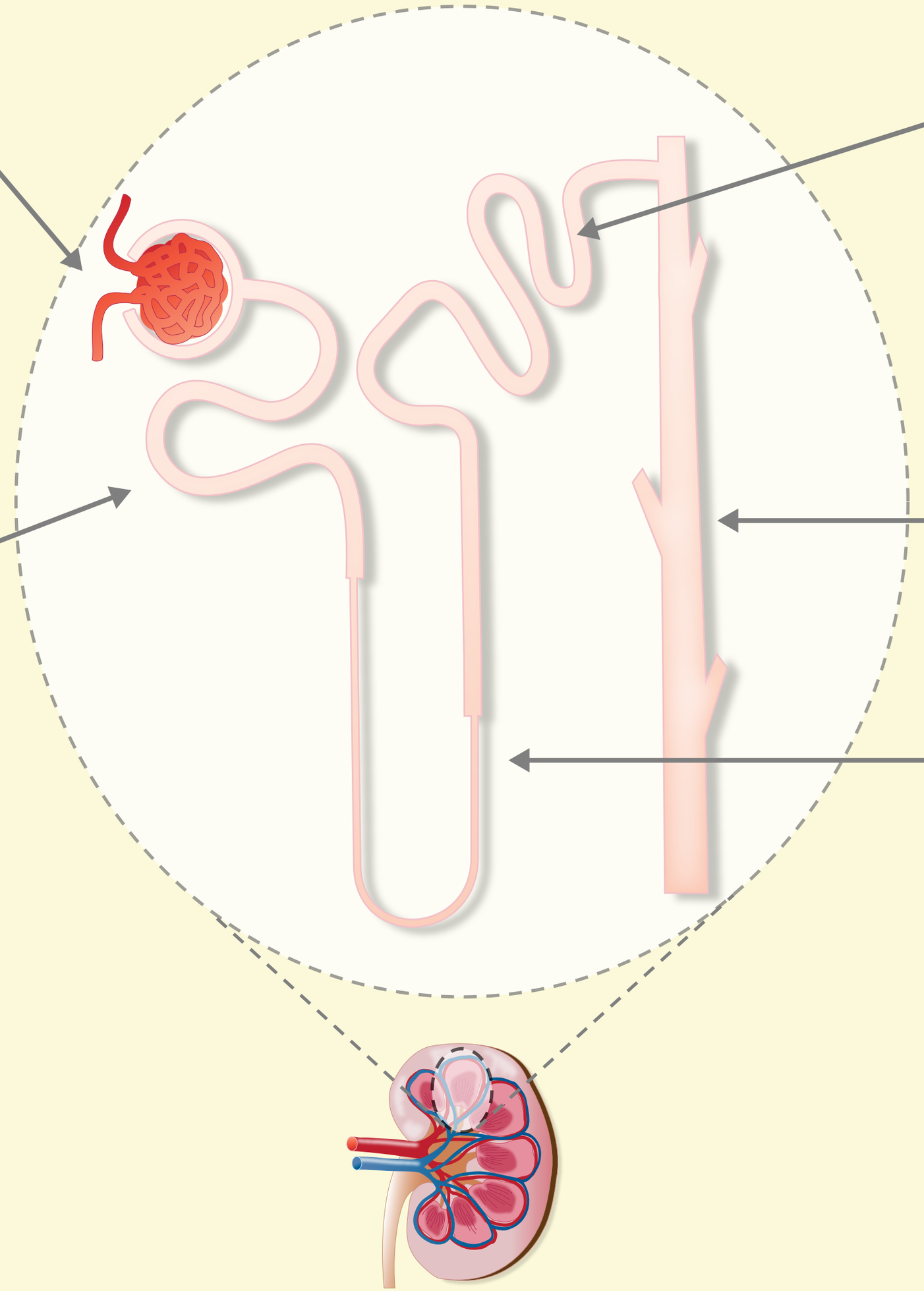
Osteopontin
Clusterin
GST-μ/π
NGAL
H-FABP
Calbindin D28

Collecting Duct

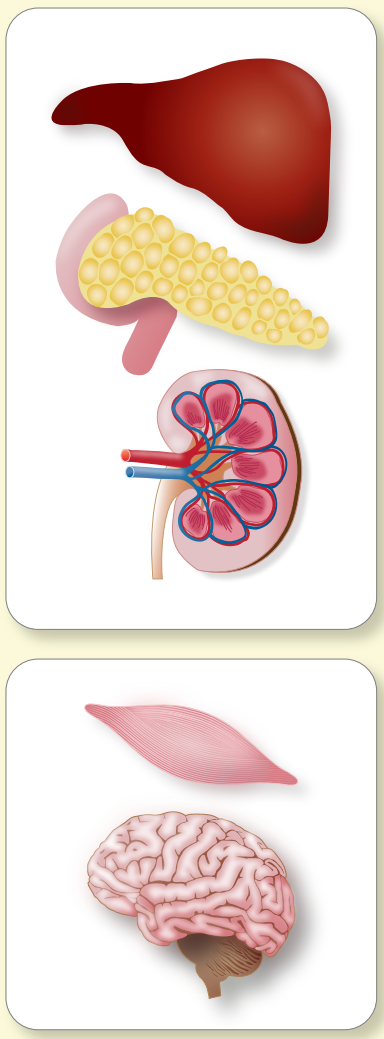
Calbindin D28

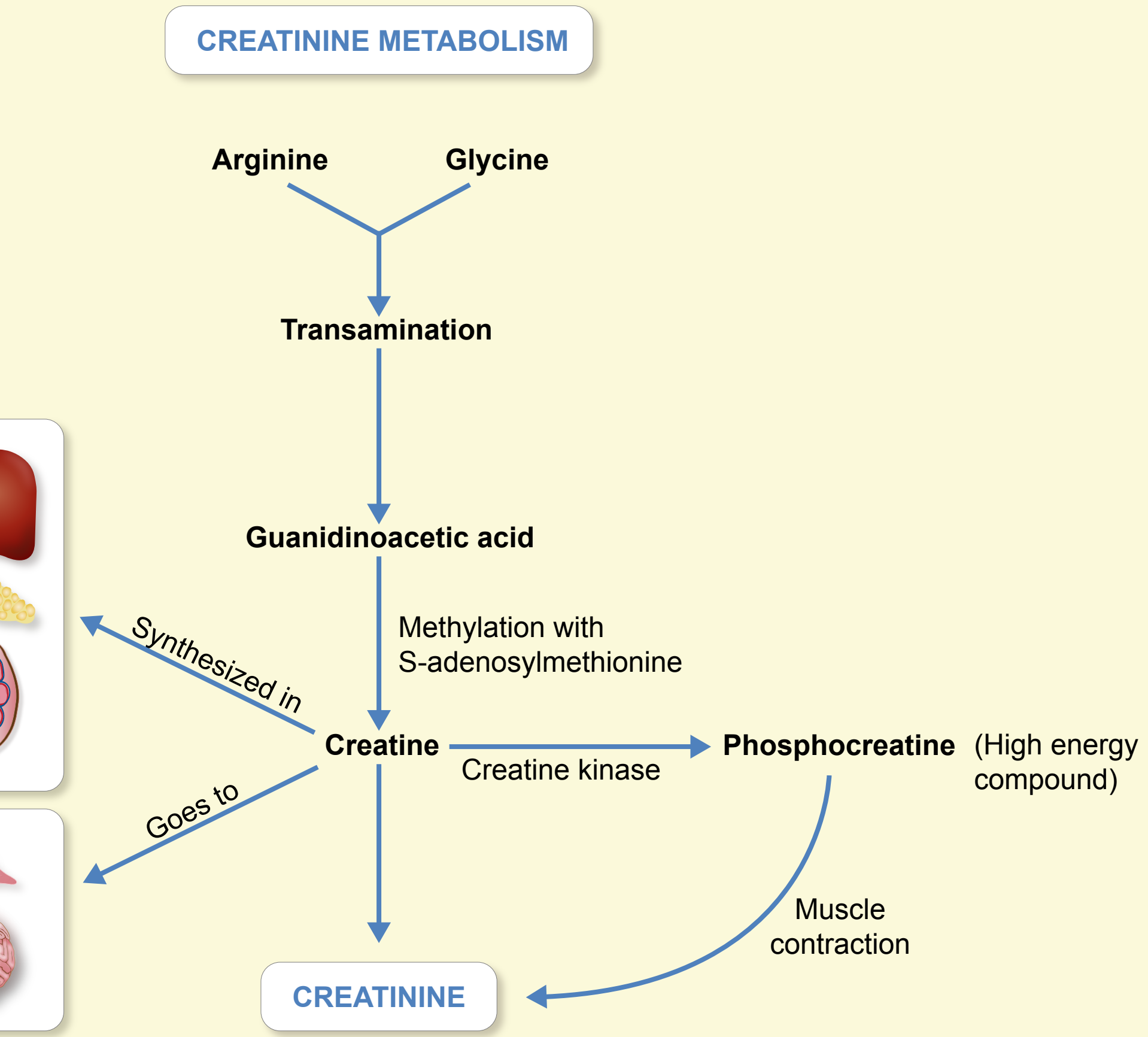
Henle's Loop

Osteopontin
NHE-3

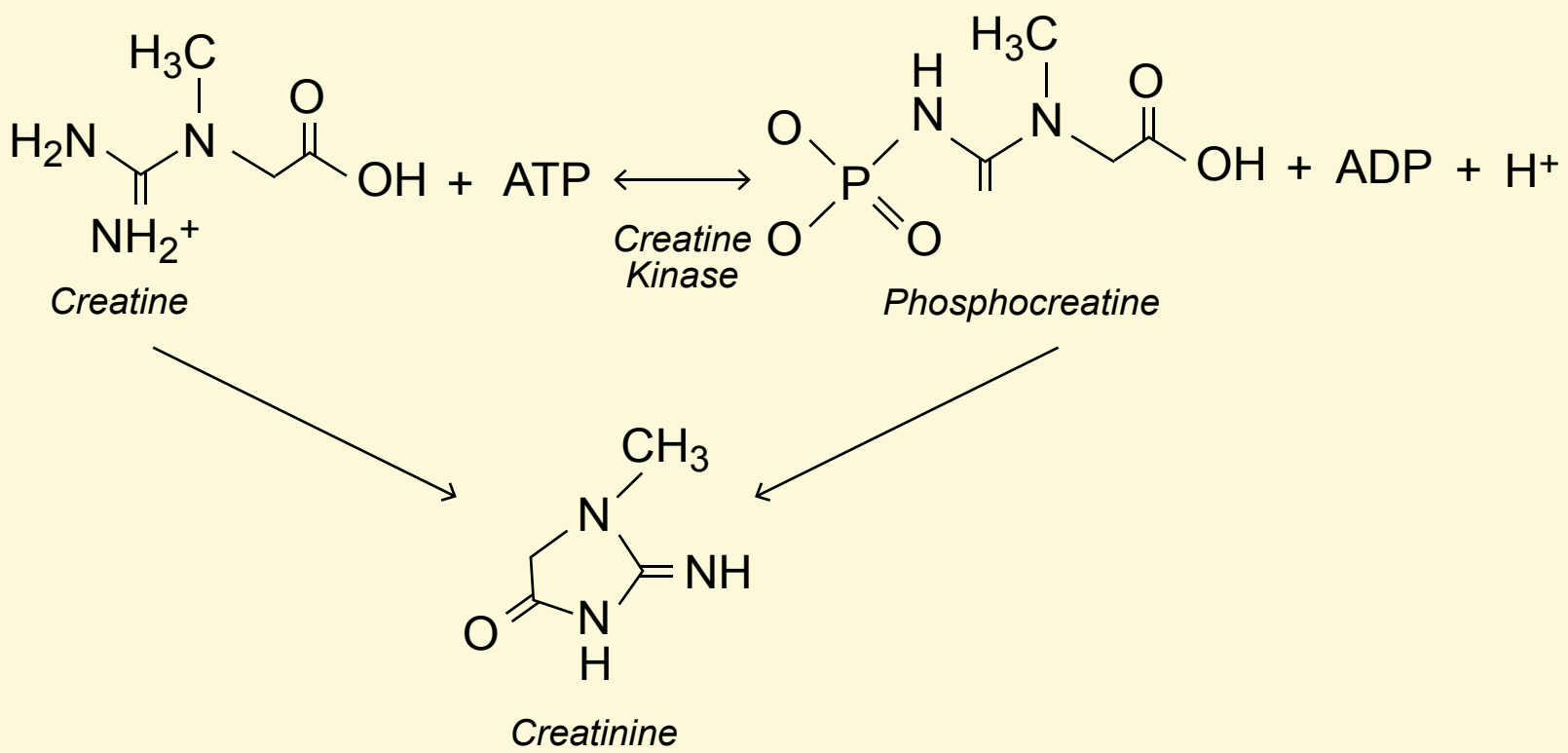


A

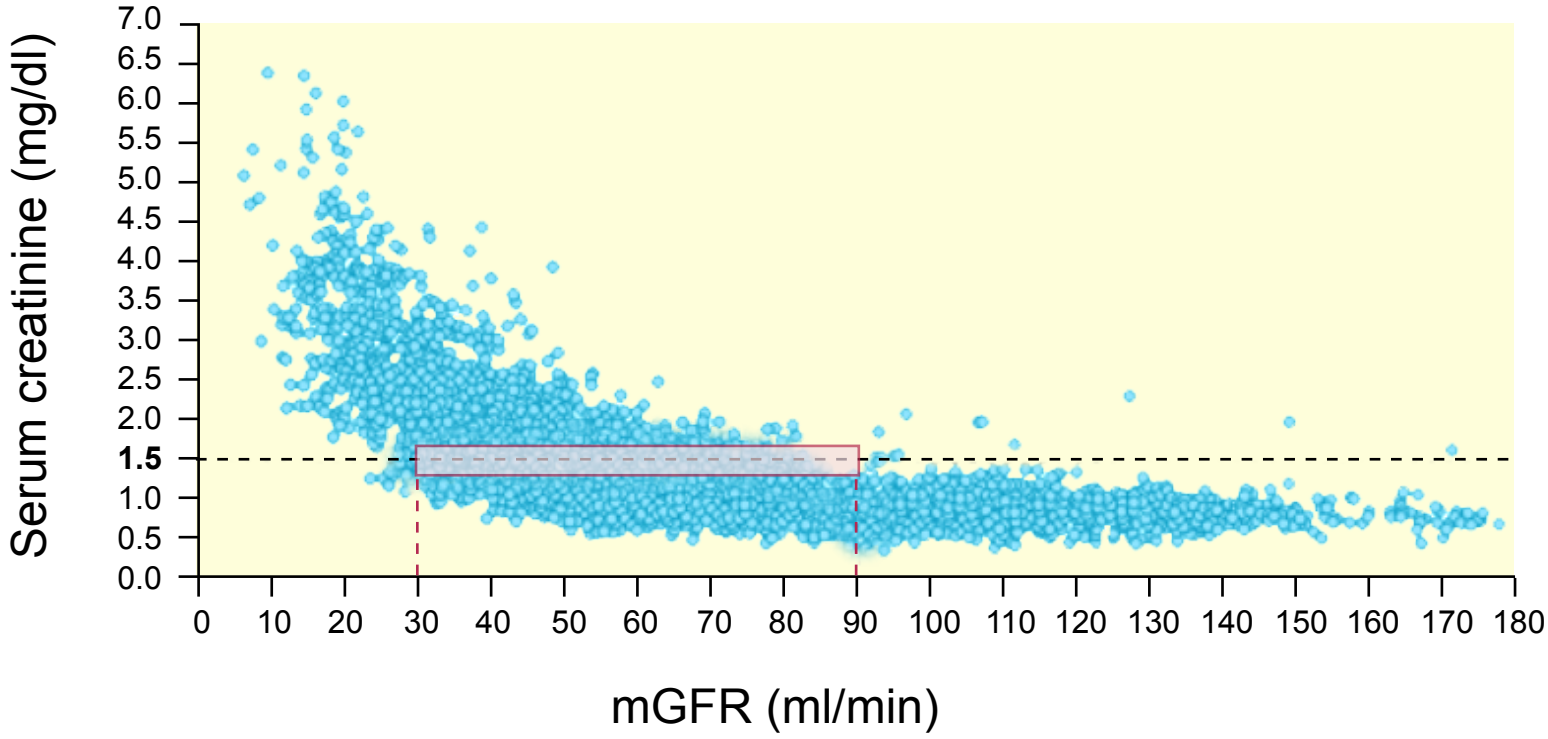



CREATININE METABOLISM
Arginine
Glycine
Transamination
Guanidinoacetic acid
Methylation with
S-adenosylmethionine
Synthesized in
Creatine
Goes to
Creatine kinase
Phosphocreatine
(High energy
compound)
Muscle
contraction
CREATINE

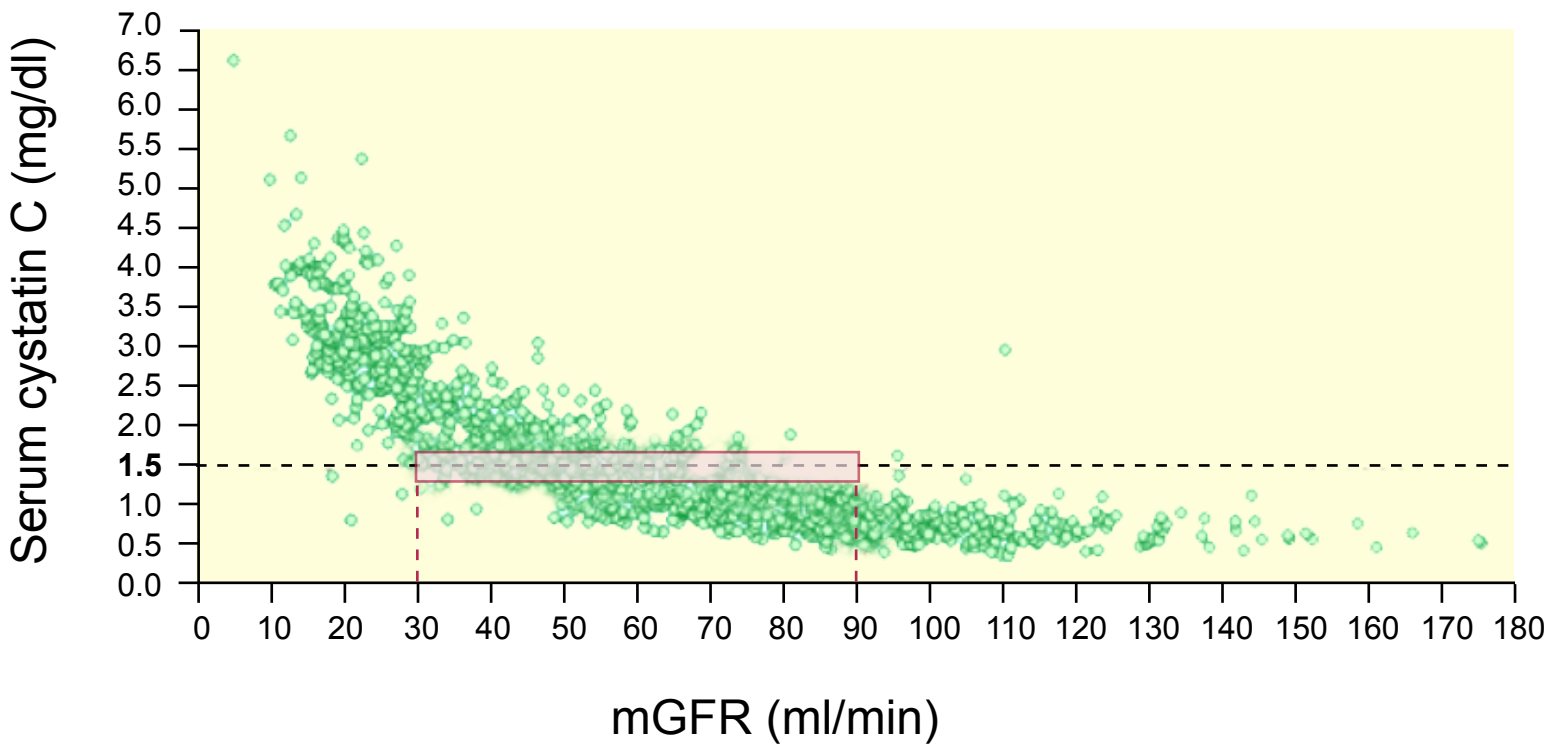
B


H3C
H2N
N
O
OH + ATP
NH2+
Creatine
Creatine
Kinase
H
N
H3C
N
O
O
P
O
O
OH + ADP + H+
Phosphocreatine
CH3
N
NH
O
N
H
Creatinine

A


Serum creatinine (mg/dl)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
mGFR (ml/min)

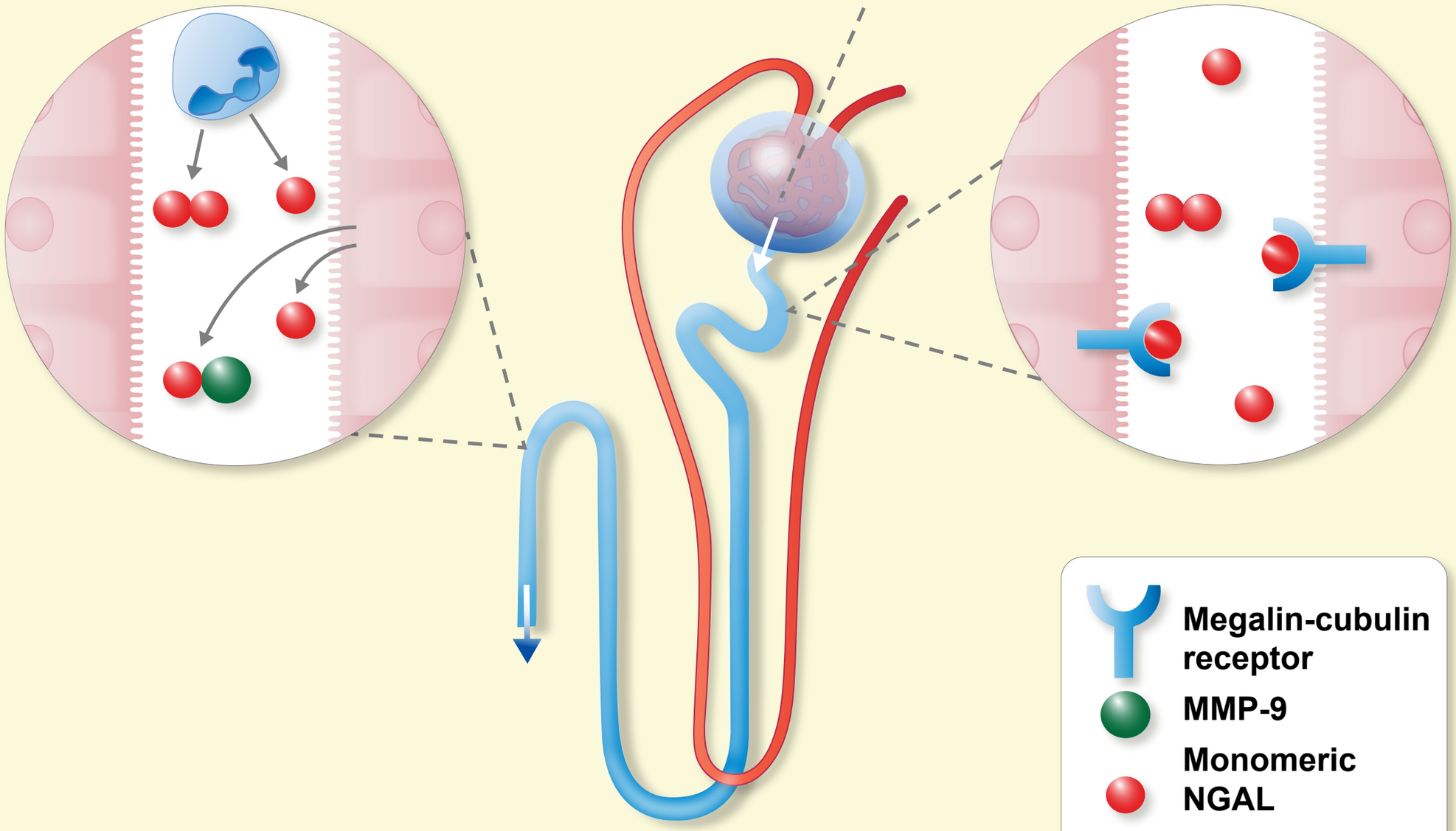
B


Serum cystatin C (mg/dl)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
mGFR (ml/min)

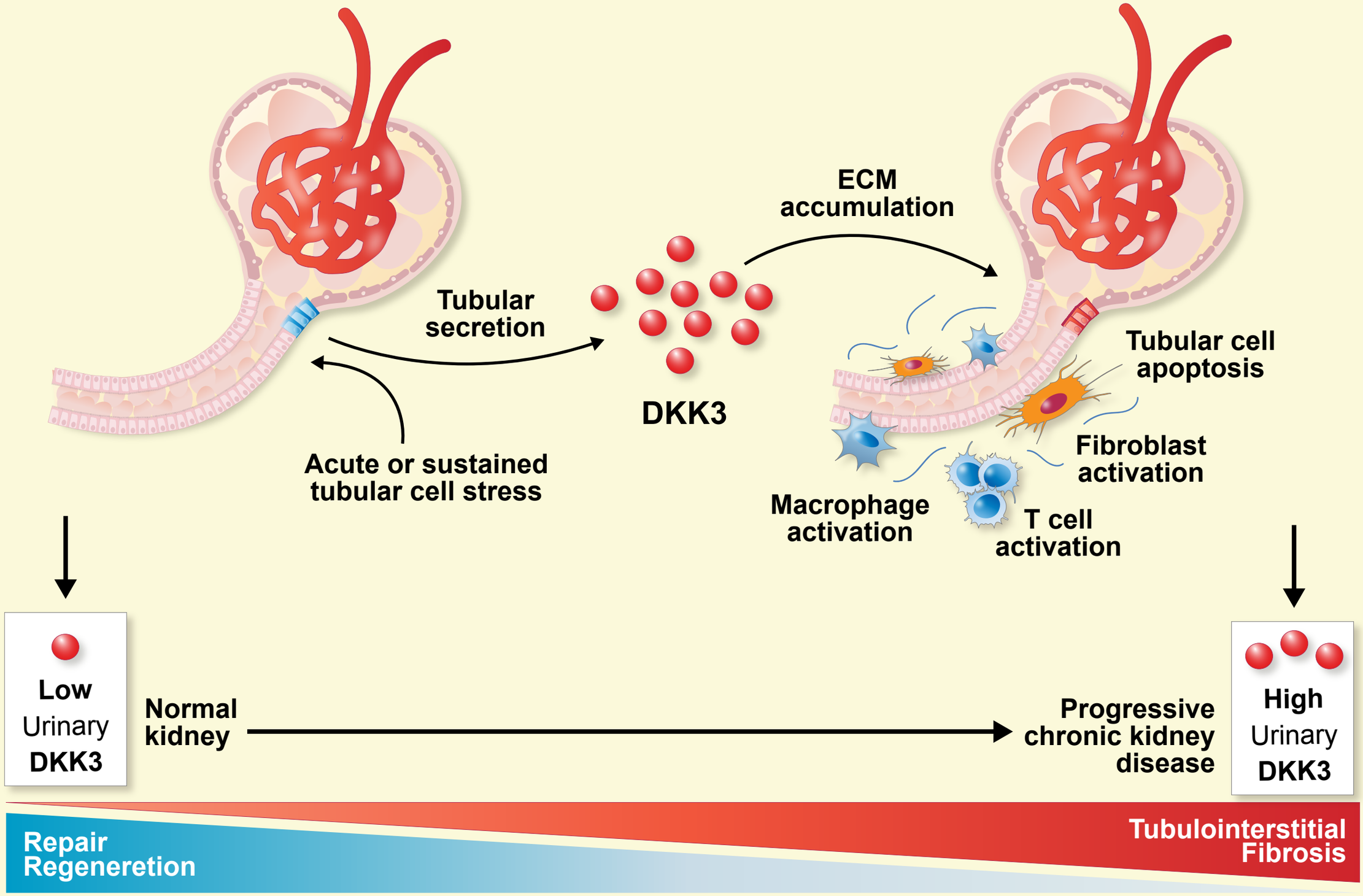
C

SYSTEMATIC INFLAMMATORY RESPONSE:

- ↑ NGAL synthesis by extrarenal tissues
- ↑ NGAL release from circulating neutrophils



- Megalin-cubulin receptor
- MMP-9
- Monomeric NGAL
- Homodimeric NGAL
- Heterodimeric NGAL


Tubular secretion
DKK3
ECM accumulation
Tubular cell apoptosis
Fibroblast activation
Macrophage activation
T cell activation
Acute or sustained tubular cell stress
Low Urinary DKK3
Normal kidney
Progressive chronic kidney disease
High Urinary DKK3
Repair Regeneretion
Tubulointerstitial Fibrosis

Cell membrane

GPI anchor

uPA

D2

D3

D1

uPAR

Linker region

Cleavage sites

GPI-anchor cleavage

D2

D3

D1

suPAR

Cleavage sites

s-uPAR

D1

D2

D3

c-uPAR

Integrin

D2

D3

α

β