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## **Urinary Biomarkers: Recommendations of the Joint European/United States Workshop for Future Research**

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### **INTRODUCTION**

At the conclusion of the Joint European and United States Workshop on Urinary Biomarkers, a series of questions were raised regarding the direction of future research in the field of urinary biomarkers. Based on the discussions of the prior sessions, a number of conclusions were reached and a series of recommendations were made.

### **CONCLUSIONS AND RECOMMENDATIONS**

#### **Conclusion #1**

There are differences between markers of exposure, markers of susceptibility, and markers of effect (1). Among the markers of effect, a distinction must be made between physiological, anatomical and biological markers. Also, there may well be differences between markers of the initial response to injury and markers that reflect cell or tissue repair. At the present time, it is not possible to identify a universal urinary biomarker or group of biomarkers that mirror the final common pathway of the renal expression of injury. However, recent advances in urinary biomarkers have created opportunities for scientists, clinicians and public health professionals to collaborate in developing standardized methodologies to assess renal function following toxic exposures.

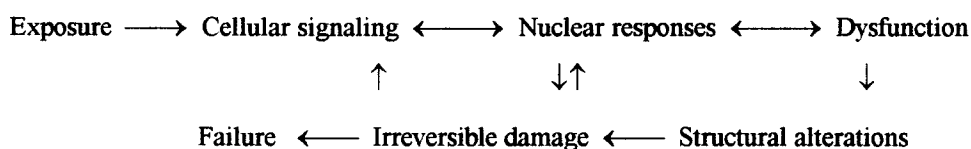
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### Recommendation #1

A collaborative effort among investigators should be encouraged to develop protocols by which new markers can be tested in parallel with a standard panel of urinary biomarkers. This step is necessary to determine their ability to detect renal injury and their utility in predicting the extent of recovery.

### Conclusion #2

With regard to the behavior of the new markers in people with various kinds of exposure, there is a need for longitudinal studies defining the various steps in the cascade of responses (effects) that follow exposure. The cascade of response includes different stages that interact.



### Recommendation #2

In addition to the characterization of markers of exposure, markers of susceptibility, and markers of effect, it is imperative to further categorize the markers of effect so that it is known where they fit in the cascade of the response to exposure.

### Conclusion #3

There is considerable variation in the interpretation of urinary biomarkers and the meaning of renal dysfunction. For example, following cadmium exposure, tubular proteinuria has been shown to predict the development of renal failure as defined by an elevation of the serum creatinine concentration. In the case of exposure to elemental mercury, renal damage appears to be entirely reversible in the absence of an idiosyncratic immunological response leading to the development of the nephrotic syndrome. This highlights the controversy about the meaning of renal "dysfunction" since the appearance of urinary biomarkers predicts renal failure from cadmium but may indicate only reversible renal effects following exposure to mercury. Even the long-term implications of tubular proteinuria following exposure to lead remains unclear.

### Recommendation #3

In considering the cascade of the response to exposure, it needs to be determined which of the urinary markers is indicative of cellular signaling with a nuclear response and reversible dysfunction and which of the urinary markers is predictive of structural damage and irreversible changes. Indeed, various questions need to be answered about the significance of urinary biomarkers in established disease states.

- What is the meaning of elevated values in a specific disease state or after exposure to a toxin?

- Which biomarkers should be used for the study of a particular disease or toxin?
- If a biomarker is present in urine, which part of the nephron is affected?
- At what stage of renal disease is the patient, if a cascade of urinary biomarkers exists - how early or late in the disease process should patients be studied?
- How reproducible (and practical) are methods of measurement?
- What is the correlation of a biomarker with the eventual progression to end-stage renal disease (ESRD)?
- What is epidemiological evidence for association of marker with the disease and assessment of relative risk from different biomarker concentrations?

#### **Conclusion #4**

There are standard tests which, when combined, are useful to assess glomerular and tubular function. According to the National Research Council (2), consideration should be given to the use of a battery of relatively simple and noninvasive tests. Initial results should be obtained by testing for proteinuria with dipsticks and measuring urinary concentrating ability as judged by the urine osmolality following a period of fluid restriction. The level of the glomerular filtration rate can be estimated by measurement of the serum creatinine concentration. For more sensitive determination of tubular integrity, monitoring for an increase in urinary enzyme or low-molecular weight protein excretion is preferred.

The Renal Test Battery used by the National Center for Environmental Health (3) has evolved over time. The measurements of the serum enzymes gamma-glutamyl transferase and lactate dehydrogenase, along with the determination of selected low-molecular weight protein beta-2-microglobulin, have been dropped because of problems of stability and interpretation. The urinary tests currently in use to define effects on various parts of the nephron include measurement of the high-molecular weight protein albumin, the low-molecular weight retinol-binding protein, the lysosomal enzyme N-acetyl- $\beta$ -D-glucosaminidase (NAG) and the membrane-bound enzyme alanine amino-peptidase (AAP). While the maximum urine osmolality obtained under hydropenic conditions is a good measure of the distal tubule concentrating operation, tests of concentrating ability in large-scale field surveys are problematic because of the inability to be sure that subjects maintain rigorous restriction for twelve hours prior to urine collection.

#### **Recommendation #4**

Among the new markers, consideration should be given to the addition of cystatin C as a marker of the glomerular filtration (4). Simple assays should be developed for structural proteins that may appear in the urine. These include laminin fragments (5) and fibronectin (6). The measurement of urinary Aquaporin-2 may be helpful under certain circumstances (7). The analysis of urinary protein excretion can be improved using 2-dimensional electrophoretic techniques (8). This technique holds the promise that protein appearing in the urine can be separated into glomerular versus tubular origin without the need for concentrating the specimen. Other markers that are worthy of exploration include: epidermal growth factor (EGF) (9), interleukin-6 (IL-6) (10), monocyte chemoattractant protein-1 (MCP-1) (11) and  $\alpha$  and  $\pi$  isoenzymes of glutathione S-transferase ( $\alpha$ GST and  $\pi$ GST) (12). It should be remembered that the progressive

deterioration of the glomerular filtration rate can be best correlated with the histologic grade of renal interstitial injury (13).

### **Conclusion #5**

Because of its slow rate of progression, chronic renal injury is more difficult to study than is acute renal injury. Nevertheless, this topic is of great importance. The criteria necessary to practically study the utility of biomarkers in those with chronic renal failure are several. They include the following:

- a clinical condition with a rate of occurrence of sufficient frequency that a statistically significant enrollment of subjects is possible;
- a predictable rate of decline in renal function that allows correlation to be made between pathophysiological evidence of renal injury and changes in urinary biomarkers;
- sufficient preliminary experience to indicate that there would be a measurable effect on conventional biomarkers;
- a predictable and measurable endpoint that has clinical significance (endpoints may be the assurance that no renal injury has occurred to the ability to identify progressive renal injury).
- urinary biomarkers that estimate the magnitude of renal interstitial injury must be identified and should be incorporated into standard test batteries after appropriate confirmation.

### **Recommendation #5**

Workshop participants should identify those forms of chronic, progressive renal injury for which there is sufficient data and opportunity to develop a protocol to study the correlation between deteriorating renal function and urinary biomarkers that can be implemented. Epidemiological studies with specific target populations and sufficient statistical power should provide information on various risk factors for renal disease for use in risk reduction.

### **Conclusion #6**

There is considerable interest in defining the genetic and molecular basis of renal disease. The contribution of various urinary biomarkers in establishing the phenotype is not yet clear and mechanisms are needed for evaluating new biomarkers of susceptibility. It is possible that various susceptibility genes may be important to distinguish patients who will progress to ESRD from those who will not. As a corollary, DNA/Tissue banking has the potential to be of considerable value in the analysis of the relationship between disease processes and urinary biomarkers.

### **Recommendation #6**

The possibility of establishing a DNA/Renal tissue bank should be investigated. The goal would be to make the bank available to investigators who need standardized sam-

ples to validate either currently used or proposed urinary biomarkers. In this and other ways, an understanding of the genetic and molecular basis of renal disease should be pursued.

#### **Conclusion #7**

It seems intuitively correct that the magnitude of xenobiotic exposure that results in renal injury is lower in individuals with pre-existing renal disease. Also, it has been suggested that children are more sensitive to the effects of environmental pollutants than are adults, although little data is currently available documenting this supposition. It is hoped that the availability of newer markers that are useful in field settings will ultimately determine the public health impact of hazardous substance exposures in these high-risk subpopulations.

#### **Recommendation #7**

The contribution of exposure to nephrotoxic xenobiotics (including commonly used drugs with known or suspected nephrotoxic potential) to hasten the rate of progression of pre-existing renal insufficiency should be evaluated. Similar attention should be given to the effect of these exposures to pediatric populations and other high-risk groups.

#### **Conclusion #8**

There are a number of clinical conditions in which the time from exposure to an offending agent to the onset of readily definable injury is of a time frame that prospective, long-term longitudinal controlled studies can be performed. These studies can be directed at correlating urinary biomarkers and outcomes and can best be designed, implemented and evaluated by using the combined resources of all the assembled participants. Three clinical conditions that met the criteria for protocol development include: (i) lead (14) and cadmium exposure (15); (ii) cyclosporine (16) and tacrolimus (FK-506) (17) treatment in patients other than those receiving renal transplants; and (iii) patients who are and who have received cisplatin therapy (18).

#### **Recommendation #8**

These three clinical conditions are reasonable candidates for joint study and should be subjected to meta-analysis in order to define the natural history and rate of progression of renal insufficiency. In this way, correlations can be made between the evolution of the injury and the appearance and disappearance of conventional and proposed biomarkers. It should also be mentioned that whenever possible, a standard panel of urinary biomarkers should be incorporated in protocol for either experimental or clinical studies of acute renal failure.

### **SUMMARY**

The session concluded on a positive note with enthusiasm on the part of participants to become involved in one of the proposed joint protocols. Left to be answered was

whether or not individual urinary biomarkers can be tailored to be disease specific or will there always be a need for a panel of biomarkers to insure interpretable results?

It was agreed that proposals for three studies would be prepared. The first study will take advantage of the fact that field studies are currently being organized by both European and American groups. European scientists are working with the World Health Organization to investigate lead exposure in a region of China. At the same time, scientists in the United States are implementing a surveillance program in a population exposed to lead and other heavy metals in Kellogg, Idaho. These efforts provide an excellent opportunity for the sharing of samples and the study of a biomarkers panel that would contain both standard and candidate biomarkers. It was agreed that the parties interested in participating would alert the workshop organizers.

The second study will expand upon a protocol developed by Dr. Debroe that has as its subjects non-transplant patients being treated with cyclosporine. An additional complimentary study of tacrolimus (FK-506) nephrotoxicity will also be developed. This protocol will be designed to follow the loss of renal function with a urinary biomarkers panel.

The third study will follow the lead of Dr. Safirstein who urged the consideration of Cisplatin nephrotoxicity as a singular model for analyzing the usefulness of various biomarkers as measures of both acute and chronic nephrotoxicity.

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