11.   LABORATORY STANDARDS IN THE DIAGNOSIS AND MONITORING OF THERAPY

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11.1 Abstract

Albuminuria is a powerful marker of kidney disease and predictive factor of cardiovascular disease. Early detection and treatment of albuminuria in patients with diabetic and nondiabetic kidney disease, hypertension and cardiovascular disease improves overall survival. Nephrologists and clinical chemistries should be aware of screening, monitoring and treatment of albuminuria.

11.2 Introduction

Chronic kidney disease (CKD) defined as either kidney damage or decreased kidney function for three or more months is a worldwide public health problem. It affects approximately 10% of adult population in western world (1).

CKD could be simplified classified in two major groups: diabetic and nondiabetic chronic kidney disease. (Table 11.1.) (2). The diagnosis of CKD is based on level of glomerular filtration rate (GFR) and by some of the markers of kidney damage. Differential diagnosis of CKD is based on the history, physical examination and laboratory evaluation. Proteinuria is the principal marker of kidney damage. Moreover proteinuria, i.e. albuminuria is a powerful marker of progressive kidney function decline (3). There are also some other markers of kidney damage like hematuria, abnormalities in urine sediment, abnormal findings on imaging studies, e.g. ultrasound etc.

Table 11.1. *Classification of chronic kidney disease*

Classification of chronic kidney disease
1.   Diabetic kidney disease

2. Nondiabetic kidney disease
   − glomerular disease
   − tubulointerstitial disease
   − vascular disease
   − cystic disease

In this paper we will briefly review the mechanism of proteinuria, particularly albuminuria, the clinical importance of albuminuria and clinical approach in the diagnosis of albuminuria and monitoring of the therapy.

Since 1817, when Richard Bright described proteinuria in patients with kidney disease detection of proteinuria remains one of the major indicator of kidney disease. At the late sixties of last century increased urinary albumin excretion was observed in new diabetic patients. In 1981 the term microalbuminuria was used for the first time to describe urinary albumin excretion not detected by a standard dipstick. Today it is well known that microalbuminuria and albuminuria, i.e. proteinuria are predictors of progression of renal disease and also marker and risk factor of cardiovascular disease: myocardial infarction, stroke and premature death. Sir Robert Hutchinson' s words from the beginning of 20th century are still appropriate today at the beginning of 21st century: „…the ghosts of dead patients that haunt us do not ask why we did not employ the latest fad of clinical investigation. They ask us, why did you not test my urine?" (4).

11.3 Albuminuria

A healthy adults excretes in urine less than 150 mg of protein per day. It is well known that kidney, i.e. glomerular capillary wall has high permeability to water, small solutes, low molecular proteins (< 40000 Da and radius < 30 A) but very low permeability to plasma proteins of the size of albumin (~65000 Da) and larger. Normal composition of urine is: ~40% albumin, ~10% immunoglobulin G, light chains ~5%, and ~42% other low molecular proteins. There are four mechanism of excessive (> 150 mg/24 hours) protein excretion: increased glomerular filtration (glomerular proteinuria), inadequate tubular reabsorption or increased tubular secretion (tubular proteinuria) and overflow proteinuria (5).

Albuminuria is of major interest because it is well known determinant of renal as well as cardiovascular disease.

Albumin is the most abundant plasma protein. It has diverse functions: carrier of hormones, metabolites, drugs, vitamins, ions, maintenance of the oncotic pressure and blood volume, acid-base buffer functions etc. It is well known that the size and the charge of the protein determine the amount of filtered protein (6). For many years it was thought that amount of filtered albumin is very low and that tubular reabsorption of albumin is of no clinical relevance. Recently it was recognized that mechanism regulating tubular uptake of albumin is very important and probably derangement of tubular reabsortion determine the amount of albuminuria (6). Even more increased tubular reabosption of albumin could be a cause of kidney interstitial inflammation and fibrosis. There is no secretion of albumin in tubular apparatus of the kidney, therefore glomerular filtration and tubular reabsorption of albumin determines

the amount of albuminuria. The amount of filtered albumin was detected by several techniques and despite some controversial it is clear that a significant of albumin is filtered through glomerular capillary wall (7). In proximal tubule albumin is reabsorbed by a receptor mediated endocytosis. Several receptor of albumin have been identified, but most important are megalin and cubulin (6). Why is this process important? The excess of albumin in the tubular lumen due to increased filtration through glomerular capillary wall leads to the induction of inflammation and interstitial fibrosis. Several studies in vitro has shown that in excess of albumin there is increased expression of inflammatory and fibrogenic mediators in tubular cells and it is important factor in progression in number of renal disease. Therefore, albuminuria is marker but also a pathogenic factor in progression of renal disease. The relation between albuminuria and cardiovascular disease is still poorly understood, but albuminuria is strong and independent indicator of increased cardiovascular risk, i.e. it is a marker of generalized vascular endothelial damage.

11.4 How to detect and measure albuminuria?

Urine protein testing involves a screening test to detect excess of protein, a test to detect the amount of protein and sometimes an assay to detect specific proteins. We will briefly described how to measure albuminuria because it is a central component in screening and management of patients with kidney disease and could be of great value in patients with cardiovascular disease.

It is important to know that albumin excretion could be, and usually is increased after exercise, after a meal and in young people erect posture can also increase albumin excretion (4, 8). There is day-to-day variation in albumin excretion , and what is very important, there is a circadian rhythm of urinary albumin excretion. Therefore, measurement of 24-hour urine albumin excretion is the "gold standard" to assess albuminuria. Unfortunately, collecting urine during 24 hours is time-consuming and inconvenient, it is also subject to error due to inaccurate timing and incompleteness. It is widely accepted to use dipstick test to detect protein, i.e. albumin in the urine. The test is semiqantitative and is insensitive to detect small amounts of albumin, i.e. < 30 mg/dl. The test has specificity of > 95% but very low sensitivity ~40%. It can give false-positive results (concentrated urine, hematuria, contrast agents etc) and false - negative results (dilute urine). Dipsticks test for microalbuminuria (very low level of albumin in urine) are also available, with good sensitivity of 88% and a specificity of 80%. At present various antibody - based methods are used to measure urinary albumin (RIA, ELISA, nephelometry etc). Recently, a new method, i.e. high-performance liquid chromatography (HPLC) was developed. By this method immunoreactive and immunononreactive albumin could be measured (8, 9, 10). It is beyond the scope of this lecture to evaluate these techniques.

From clinical point of view more important is which sample of urine should be collected and how should be albuminuria expressed.

There is no doubt that the reference method to measure urinary albumin excretion is a 24-hour urine collection. But it is impractical, and we need more simple and less costly methods, et least in screening and in epidemiological studies. There are another reliable methods in evaluation of albuminuria: timed overnight collections,

spot urine, i.e. first morning samples and random samples. Last two methods are untimed and results are expressed as albumin concentration or as albumin-creatinine ratio (Table 11.2.) To avoid influence of circadian variation, physical activity and hydration status the best sample is first-morning sample. More studies have been published and suggest that expression albuminuria as albumin-creatinine ratio is acceptable method in evaluation of albuminuria with good correlation with gold standard, i.e. 24-hour albumin excretion. Creatinine excretion in the urine depends on muscle mass, i.e. on gender, therefore we need different definitions for albuminuria for women and men. (Table 11.2.) (8, 9).

Table11.2. *Classification of urinary albumin excretion*

Method of urine collection	Normal	Microalbuminuria	Macroalbuminuria
24h urine (mg/24h)	< 15	30 to < 300	> 300
Overnight urine (μg/min)	< 10	20 to < 200	> 200
Spot urine			
– albumin (mg/L)	< 10	20 to < 200	> 200
– albumin/creatinin ratio			
male (mg/mmol)	< 1.25	2.5 to < 25	> 25
female (mg/mmol)	< 1.75	3.5 to < 35	> 35
male (mg/g)	< 10	20 to < 200	> 200
female (mg/g)	< 15	30 to < 300	> 200

11.5 When and how often to evalaute albuminuria?

Once again albuminuria is an early sign of progressive kidney and cardiovascular disease in persons with and without diabetes (8, 11, 12). Unfortunately, despite many data of clinical value of screening and treatment of albuminuria, many diabetic and much more non diabetics are not screened for albuminuria. Any clinical screening program should fulfil some criteria, e.g. the disease for which the screening test should be used is an important health problem, the course of the disease is well described, the disease could be detectable in an early phase, there is suitable test to indicate the early phase of the disease, etc. Early detection of albuminuria in diabetics but also in general population fulfils these criteria.

It is now imperative to test for albuminuria in every day practice in persons with increased risk for chronic kidney disease, persons with increased risk for cardiovascular disease and to monitor therapy. There is no doubt that screening albuminuria is of great value in diabetics. Besides them it is reasonable to screen for albuminuria in individuals with obesity, hyperlipidemia, metabolic syndrome and with hypertension could (13, 14). At this moment we do not have enough date to start with screening in general population. We need to have in our mind that many individuals are not aware that they have diabetes or hypertension but they have albuminuria. Moreover in the PREVEND study has been shown that albuminuria gradually increases with increasing blood pleasure or plasma glucose level even within normal range. In other words, persons with higher but normal range of blood pressure or

glucose level are et risk to have albuminuria, i.e. it means that albuminuria may precede manifest hypertension or diabetes.

Another important issue is treatment of albuminuria. A lot of studies (IRMA, BENEDICT, PREVEND IT etc.) have shown that lowering of albuminuria by either an angiotensin-converting enzyme (ACE) inhibitors or an angiotensin II receptor blocker (ARB) are associated with a better renal and cardiovascular outcome (15, 16, 17, 18). In fact there is suggestion by some authors that albuminuria reduction should be a clinical treatment target, like blood pressure changes in hypertension or glucose level in diabetes. Some observational studies showed that reduction of albuminuria strongly predict improve cardiovascular and kidney outcomes and that this prediction could be largely dissociated from blood pressure changes. Unfortunately we do not have enough randomized controlled trials to support albuminuria as an independent therapeutic target. Currently renoprotective drugs (ACE or ARB drugs) are primarily antihypertensive drug and reduction of albuminuria is "side effect".

At the end, how often should be albuminuria tested? First, every positive result should be repeated in next two weeks. Authors opinion is that if both tests are positive treatment to lower albuminuria should be started in diabetics, individuals with hypertension and cardiovascular disease. If only one test is positive it should be repeated after three months (4, 8). During treatment once per year detection of albuminuria could be performed (author's opinion).

11.6 Conclusion

There is a lot of evidence that screening for albuminuria should be carried out in individuals with diabetes, but also in individuals with hypertension and cardiovascular disease. At this moment we need more date to support screening for albuminuria in general population.

At the end, the author did not use in this review the term microalbuminuria. As it is stated in article by Ruggenenti P and Remuzzi G, it is time that term microalbuminuria should be eliminated from our lexicon as there are data to suggest that albuminuria in "normal" range carries significant risk of cardiovascular risk. In other words there are no "cut off" values of normoalbuminuria and microalbuminuria (19). In addition in urine could be find the intact molecule but also albumin fragment. Therefore the term microalbuminuria was not used in this article.

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