Clinical Biochemistry of Renal Function

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Summary. Renal functions have been tested since ancient times. Recent advances in our knowledge and technical progress have challenged our strategy in analysing various renal functions. The example of proteinuria will be described in more detail. A quantification of proteins of different molecular size has been shown to be useful in characterizing the mechanism and medical causes of proteinuria. By analyzing urine albumin, a_1-microglobulin, immunoglobulin G and a_2-macroglobulin together with total protein, prerenal, glomerular, tubular and postrenal causes of proteinuria can be detected and differentiated by their specific urine protein patterns. Thus tubulo-interstitial diseases negative in the protein test strip procedure, are detected and clearly differentiated from other causes of proteinuria by their high a_1-microglobulin/albumin ratios. Albumin in urine has been introduce as a marker of the quality of glomerular filtration, whereas the quantity can be calculated from a single measurement of Cystatin C in Plasma/Serum. In postrenal proteinuria, a_2-macroglobulin proved to be a useful marker, when albumin excretion exceeds 100 mg/L urine. This protein exhibits plasma-like ratios to albumin in postrenal causes, whereas it is much lower in renal proteinurias. The high specificity of these new markers seems to reduce the importance of microscopic analysis of the urine sediment, challenged by the introduction of quantitative flow cytometry. The new strategy, which has been evaluated in more than 500 clinically and partly histologically proven cases of renal diseases allows to distinguish all clinically important causes of proteinuria and haematuria from analysis of a single morning spot urine sample.

Introduction

The observation of urine protein as a sign of renal disease is one of the eldest examples in medical history where individual measures are used as diagnostic symptoms. Until the 18th century the uroscopist used his eyes, nose and sometimes taste to come to a diagnostic conclusion.

From this early use of urine to establish a diagnosis, qualitative and quantitative chemical and microscopic procedures were developed in the 19th century resulting in the so called “urinalysis standard”, which is used worldwide to screen urine of patients. Several sensitive tests using acidification and boiling of urine are still in use[1], although the test strip procedure has largely replaced the qualitative chemical tests.

Regarding renal functions, glomerular filtration and renal tubular reabsorption and secretion were described as the major mechanisms of the kidney to form the final urine used as a diagnostic mirror of pathological changes.

Glomerular Filtration

Clearance procedures were used to characterize glomerular function. Using plasma creatinine as the standard marker. This marker has recently been challenged by the observations that due to unspecificity of the traditional Jaffé procedure, plasma concentration was measured too hig. Therefore the measured or calculated clearance based on these values was found to be higher than the reference inuline or isotopic clearance[2], when creatinine was measured by an enzymatic or HPLC procedure. In addition introduction of an alternative marker Cystatin C has shown, that in many cases creatinine measured in elder people seems lowered due to decreased muscle mass thus reducing the calculated clearance[3]. This together with other extrarenal causes of changes in creatinine turnover needs reconsideration of the traditional strategies in analyzing glomerular filtration in the medical laboratory.

The mechanisms of renal protein filtration may be taken as an example to show, that besides the quantity, the quality of glomerular filtration has been found to be of major diagnostic power in predicting the renal outcome of various diseases. Thus the term “microalbuminuria” covered by another speaker in this meeting, was introduced to characterize early development of diabetic nephropathy[4].

Looking at our present knowledge about the mechanism of glomerular filtration of proteins, this may be compared with a chromatographic method separating proteins according to their molecular weight

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and charge. Proteins with a molecular weight below 40 kD nearly 100% filtered, whereas proteins with a molecular weight above 80 kD do normally not pass the glomerular filter. Moreover, cationic proteins pass the molecular filter easier than anionic ones. This makes albumin an ideal marker of glomerular filtration as its excretion is increased, when both molecular charge and size permeability of the filter change. A change in molecular charge leads to a so called "selective glomerular proteinuria", whereas a change in the pore size results in an "non-selective glomerular proteinuria".
Immunoglobulin G has been recommended as marker of the latter function[5].

Tubular reabsorption.
Of the primary filtrate, more than 95% are reabsorbed in the proximal tubule. Regarding filtered proteins, however, the reabsorptive function varies according to the charge and size of molecules. Brush border proteases as well as reabsorptive and protein-degrading functions of the lysosomal system of the proximal tubule cells are involved herein[6,7]. Whereas digested proteins are reabsorbed as amino acids and released into the blood stream, larger proteins are bound to the brush border surface by a receptor like mechanism[7] reabsorbed in vesicles merging with primary lysosomes in the proximal tubule cell who split proteins and other digestible molecules to their original fragments released into the blood stream, leaving undigested fragments for secretion into urine together with the lysosomal enzymes, thus forming the normal excretion rate of lysosomal enzymes like N-acetyl-ß,D-glucosaminidase[8,9]. Depending on location and extent of dysfunction of the glomerular filter and tubular reabsorptive process, a glomerular and/or tubular proteinuria can be found.

Postrenal functions
When considering urine as a diagnostic tool to analyze renal function, postrenal contamination has to be considered. The latter is well known as a cause of haematuria by postrenal bleeding, cellular secretion as well as contamination from the outer genital tract. Considering peroteinuria based on single protein analysis, postrenal haematuria can be the major cause of contamination with proteins. These are expected to be released into urine in plasma like protein ratios[10].

The New Diagnostic Concept

To evaluate this theoretical concept, we have examined the following markers in more than 500 urines of patients whose diagnoses were clinically and partly histologically established. The quality of glomerular filtration was screened by measuring albumin. Total protein was determined for plausibility, with a protein gap indicating prerenal proteinuria[11,12]. When a protein gap between total protein and the measured single proteins of more than 60 % is observed, a prerenal cause like Bence-Jones proteinuria is suspected. Of the various proteins freely filtered, but reabsorbed in the proximal tubule, α_1-microglobulin was chosen as a marker of tubular protein reabsorption, because it is easily measurable, stable in urine and sensitive in detecting tubular dysfunctions[12,13]. Other possible markers used and discussed are β_2-microglobulin, retinol-binding protein and Cystatin C. In contrast to α_1-microglobulin, however, these proteins seem of lower diagnostic applicability because of their prerenal influences (retinol binding protein), instability in urine at physiological pH (β_2-microglobulin) or very low levels in normal urine (Cystatin C). To separate postrenal proteinurias, α_2-macroglobulin has proven to be useful, as this macroprotein (720 kD) is nearly not filtered by the kidney but occurs in plasma ratios to albumin in postrenal bleeding[14].
All proteins can be measured nephelometrically and turbidimetrically on routine analyzers by using commercially available antibodies[12,15].

Clinical results based on the new strategy

When comparing results of α_1-microglobulin versus albumin in primary glomerular diseases, the tubular marker increases slowly above its normal excretion rate with increasing progression of the glomerular diseases. When albuminuria exceeds 3g/g (339 g/mol) creatinine, an increase in α_1-microglobulin is always found[16]. When various forms of glomerular nephropathy were separated, it turned out that the cases found on the lower margin of the glomerular group represented more selective forms like minimal change nephropathies, whereas rapidly progressing forms were found on the upper layer [17]. An equation describing the minimal α_1-microglobulin excretion in glomerular diseases with nephrotic proteinuria helps to distinguish between a tubular proteinuria caused by the exhaustion of the tubular reabsorptive function (tubular "overload proteinuria") and a tubular proteinuria indicating an interstitial involvement in the primarily glomerular diseases. In contrast, tubulo-interstitial nephropathies showed a completely different urine protein pattern. Although exhibiting similar albuminurias, α_1-microglobulin now was the leading marker clearly separating this group from the glomerulopathies. Interestingly, a third group appearing between the two previous ones defines diseases with secondary renal dysfunction due to diabetes mellitus or hypertension[16,18]. This finding supports the idea that these renal diseases represent mixed types of tubulo-interstitial and glomerular dysfunction[18]. Interestingly, the ratio of α_1-microglobulin to albumin seems to correlate to the progression of the glomerulopathies opening the possibility that a predictive information may be derived from measuring these proteins[19].

When we tried to separate from these renal diseases postrenal causes of proteinuria, α_2-macroglobulin was found to be a useful marker. When comparing urological cases with postrenal bleeding to glomerular causes of hematuria, it turned out that α_2-macroglobulin can clearly separate these cases when albuminuria exceeds 100 mg/L [8].

Regarding the technical aspects of this strategy, morning spot urine provided the same results as 24h urine collection, making the 24h collection of urine unnecessary[20]. An optimal interpretative result can be obtained by combining graphics, tables and text results. A computer program which is now in daily use in our laboratory to support the interpretation of various protein ratios was developed and evaluated by Ivandic et al[16,20].

From our experience in applying urine protein pattern analysis in clinical routine, the following strategy can be recommended (Table 1). When screening urine for exclusion of renal dysfunction, albumin and α_1-microglobulin should be measured as glomerular and tubular markers respectively, in addition to total protein. The ratio of total albumin to protein can provide information on the presence of prerenal causes of proteinuria such as Bence-Jones-proteinuria. In addition, leucocyte esterase and hemoglobin pseudoperoxidase are the most sensitive indicators of inflammatory and haematuric diseases in urine. When all these tests are in the normal range, renal dysfunction can be excluded with much higher certainty than in screening programs using test strip and sediment microscopy[12]. Regarding the quantification of glomerular clearance, we recommend to measure Cystatin C in a plasma/serum sample and calculate the clearance by the formula given by Chantrel et al[21]: GFR = 88/Cystatin C (mg/L).

A positive result for proteinuria, leukocyturia or hematuria has to be differentiated by measuring specific markers. IgG excretion in relation to albuminuria can differentiate unselective from selective glomerular proteinurias. However, tubular causes of IgGuria are to be considered. Here, α_1-microprotein can be used as a sensitive marker. The tubular enzyme N-acetyl-β, D-glucosaminidase, on the other hand, can help in separating chronic tubulo- interstitial disorders from acute tubulo-toxic diseases. Likewise, leukocyturia and haematuria can either be differentiated by phase contrast microscopy[22], if not supported by new markers of inflammatory stimulation.

By applying these new techniques, the information derived from urinalysis can be increased several fold, thus improving the quality of laboratory medicine.

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