SEED Urinalysis

Sysmex Educational Enhancement and Development
February 2012

A closer look at protein detection in microscopic urinalysis

Test strips have the advantage of being able to provide important information on renal diseases for rough orientation purposes. But they are not sensitive enough for the early diagnosis of kidney diseases. In spite of that, they have become established in everyday laboratory work, and a positive protein result is generally one of the results which attract the most attention, since a kidney disease is thus frequently discovered. In laboratory practice, a positive result for protein in the urine is therefore also often taken as reason to examine the urine more closely under a microscope or conduct follow-up tests, such as protein differentials. Most laboratories generally take a look through the microscope first. Casts indicating a renal disease cannot always be found. And sometimes casts are found in the urine without the test strips having indicated protein first. There are entirely plausible explanations behind the apparently contradictory testimony of the test strip diagnostics and microscopy, without one having to assume possibly false positive or false negative results with both analysis methods.

Potential proteins in the urine

Healthy individuals excrete up to 150 mg of protein daily. With this physiological proteinuria, about 2/3 of the proteins come from the serum. This predominantly involves albumin and microglobulin as well as a few immunoglobulins and immunoglobulin fragments. 1/3 of the proteins excreted comes from the urogenital tract. Amongst them, the Tamm-Horsfall protein can be found in dominant quantities. After passing the glomerulus, the serum proteins arrive in the primary urine and end up in the final urine as well, as long as they are not reabsorbed in the proximal tubule system. The passage through the glomerulus depends on the molecule size and electric charge, since the membrane in the glomerulus simultaneously functions as a mechanical and electrical filter. If one observes the molecular weight of the excreted proteins in the urine of healthy individuals, one will find 20 % with a low molecular weight and 40 % with a comparatively higher molecular weight. 40 % of the protein fraction consists of Tamm-Horsfall protein. Albumin is a serum protein which is synthesised in the liver. The production quantity amounts to approx. 0.2 g/kg of body weight. It is made up of 584 to 590 amino acids, the majority of which contains sulphur, and it has a molecular weight of 66 kDaltons.

Albumin is water soluble on account of the polar and charged groups on the surface of its molecular structure, which interact with the water molecules and form hydrogen bridges. The binding capacity for water amounts to 18 mL/g. As a transport protein in the body, it can reversibly bind anions and cations. 40 % of the human albumin act as transport protein in the blood vessels. Albumin is broken down in the liver and excreted via the kidneys and gastrointestinal tract.

Tamm-Horsfall protein (THP), in contrast, is produced in the tubule of the nephron, the smallest functional unit of the kidney. On average, approx. 50 mg is excreted daily. This means that THP is also present in the urine of healthy individuals, even if it is not necessarily visible in the form of casts in the sediment. The function of THP has not been conclusively proven, but several studies speculate that it protects from kidney stones and urinary tract infections. It possesses immunomodulating properties, binds to interleukins and complement, can activate neutrophil granulocytes and monocytes and activates dendritic cells via TLR4. THP is synthesised in the distal tubule. It is made up of 590 amino acids and has a molecular weight of 64 kDaltons. After being synthesised, it is transported to the luminal cell membrane via glycosylphosphatidylinositol (GPI), where it is split proteolytically and released into the lumen of the kidney tubule. It normally has no aggregated form there, since the intratubular fluid in the distal tubule has

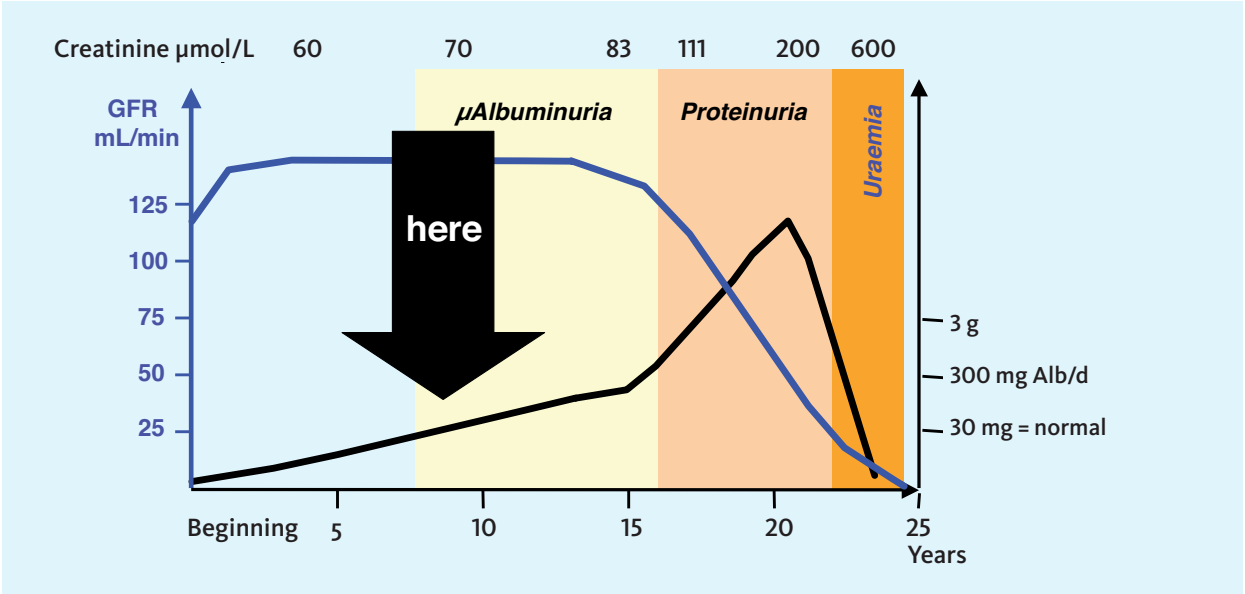
hypo-osmotic salt concentrations which inhibit THP polymerisation. THP aggregation is facilitated if the electron concentration increases, i.e. to the point that iso-osmotic salt concentrations prevail, or if the concentration of hydrogen ions increases [1].

Aspects of protein analysis in the urine

Tamm-Horsfall protein can form an undissolved protein matrix in the urine, which then becomes visible under the microscope as casts. The lumen of the tubule determines the shape in the process, so that the casts take on their typical elongated, rodlike shape during the aggregation process. If other cells are found due to pathological changes in the urine, they will become embedded in the casts' protein matrix during the aggregation process and will appear as pathological casts under the microscope. If, on the other hand, THP excretion in the distal tubule is reduced, then this is a very reliable sign of damage to the producing epithelial cells located there. But if the pH value is alkaline or the urine is diluted, it is often the case that no casts can be detected at all, even with increased THP in the urine, since the protein matrix dissolves under these conditions.

Laboratory diagnostic THP screening is mostly established in research laboratories, where RIA, radial immunodiffusion, nephelometry and ELISA techniques can be used to detect the mucoprotein. Special antibodies against the Tamm-Horsfall protein are used here. The detection techniques require a special preparation of the sample material, and thus several manual processing steps, but they make it possible to detect Tamm-Horsfall protein in dissolved form. However, in routine diagnostics with the microscope, the conspicuous THP is only visible if it is present in the form of casts. As already noted, the microscopic detection is very difficult if a basic milieu or hypoosmolar conditions prevail in the urine. The usually only small volume examined in the sediment will impair the screening, as will the well-known disadvantages of centrifugation, which can reduce the number of particles to be found in the sediment [2], [3].

The test strips usually used in routine hardly register Tamm-Horsfall protein, since it nearly does not react with the test strip's reaction field for protein at all. The reaction field primarily registers albumin, then immunoglobulin with decreasing sensitivity; haemoglobin and small-molecule proteins such as Bence-Jones protein are hardly registered. In principle, the term 'protein' for the test strip field is confusing when seen in this light, but it is firmly established for test strip analysis in laboratory diagnostics. The conventional test strips, frequently used as multi-parameter type, serve more in recognising manifest albuminuria. The test strips have a detection limit of around 150 – 300 mg/L. That is too high for timely recognition of a developing diabetic nephropathy or tubular kidney damage, as the illustration below shows. The reaction field for protein is based on a simple chemical reaction, also known as 'pH indicator error'. It shows a change in colour as the amino groups of the albumin react with the indicator. In the process, the indicator releases hydrogen ions to the free amino groups of the albumin molecule. The reaction hardly works with immunoglobulins.



People who are under physical exertion, orthostasis, emotional stress or thermal stress (heat/cold) exhibit interindividual fluctuations in albumin concentration. But also diseases such as cardiac insufficiency, blood sugar imbalances or even operative interventions may cause a reversible albuminuria. One fifth of all women exhibits values of a benign albuminuria during pregnancy involving transient results, and a concentration of 300 mg/L is normally not exceeded [4]. Other causes of a transient albuminuria may be inflammatory diseases such as urinary tract infections or acute febrile diseases. If an ascending urinary tract infection involving the kidneys is not present, pathological casts will not generally be found in the examples listed, meaning that albuminuria, and thus a positive test result for protein, can indeed be found on an isolated basis.

Results of previous studies clearly show that screening with test strips yields a positive result for protein in about 17 % of the samples. But a serious urinary tract disease could only be found in 2 % of these patients [5]. This means that the test strip method has the aforementioned drawback that it will only show a positive test strip result if albumin concentrations are relatively high. This makes it unsuitable for the early detection of beginning kidney damage, such as with diabetics. Another drawback is that casts which are actually pathological can only be found in a small percentage of patients with a positive test strip result. About 50 % of the early stages of kidney damage are not detected with either the test strip or sediment microscopy. In addition to tubular kidney damage, it primarily involves microalbuminurias which remain undetected because the analytical sensitivity of the test strips ranges from 150 to 300 mg/L. Also, with easily treatable early forms of diabetes, the GFR (glomerular filtration rate) is markedly increased and not decreased. In such cases, more sensitive methods must be used to detect the microalbuminuria, since even with the urinary sediment actual pathological particles are only detected in less than 20 % of those samples with proven proteinuria.

Conversely, though, it can also happen that the protein result on the test strip comes up negative, but pathological casts are found in the sediment, such as with tubulointerstitial kidney damage, in which small-molecule proteins are increasingly released into the tubule lumen. These tubular proteinurias are not registered by the test strips and make up approx. half of all kidney diseases. In laboratory diagnostics, they can be detected by screening the α1-microglobulin and β2-microglobulin, since in such cases these small-molecule proteins are reabsorbed in the tubule in diminished numbers. More Tamm-Horsfall protein is also excreted in aggregated condition as a result of tubular damage. So it is entirely possible for pathological casts, such as leukocyte casts, to appear in the event of interstitial and tubular diseases; the protein screening, be it via test strips or total protein determination, generally shows results in the normal spectrum. A further example is acute kidney failure, in which pathological casts are also flushed out, without protein being detected with the test strips.

Protein analysis in the scope of routine urinalysis

The procedure of screening urine for pathological components is medically established, but for decades, it has neither been challenged nor changed. Initially, the samples are analysed with test strips. A urine sediment test is frequently only conducted if one of the parameters, such as protein, white blood cells or red blood cells, reacts positively ('test strip sieve'). The following manual sediment will probably miss numerous pathological particles on account of methodical error (centrifugation, manually pouring out the supernatant and manual assessment) [2], [6]. The progression of a glomerular nephropathy, such as one caused by diabetes, can certainly be stopped if it is detected early. Microalbuminuria detection using monoclonal antibodies is suitable for early detection, but it is more expensive than the test strips [7]. The worldwide increase of chronic kidney diseases is a major reason why the question of a different screening approach (even a more expensive one) will probably be raised more and more often. This way, it would be possible not only to maintain the health of those affected and improve their quality of life, but also reduce the socio-economic costs caused by chronic kidney diseases which can be predicted on the long term. Test strip diagnostics became established because of its simple handling, cost-effective testing potential and the rapid availability of the test results in the scope of screening. Urine test strips are often supplemented by automated wet-chemistry protein determination (i. e. the pyrogallol red method or benzethonium chloride), which has better analytical sensitivity (up to 40 mg/L), meaning that possible kidney damage is more likely to be detected. Albumin and α1-microglobulin can be measured in an automated

way (nephelometry, turbidimetry), especially for patients with an increased risk of developing kidney damage. Patients at risk, such as those with established cases of hypertension or diabetes in the family medical history, have a particularly high risk of developing renal damage. If this is detected promptly, it can also be treated promptly and with greater success [8].

Sysmex UF-Series

As an additional pillar of routine urinary diagnostics on the part of microscopy, an analytical system which determines exact white blood cell and red blood cell figures can not only reduce the amount of work in the laboratory, it can also improve the clinical statement. The Sysmex UF-1000i and UF-500i urine flow cytometers classify up to 65,000 particles in native urine whilst completely standardising the initial analysis steps, which are otherwise usually manual. The known sources of error caused by centrifuging the urine and the statistical imprecision of the count results deriving from the uneven distribution of the particles in the microscope preparation were eliminated by hydrodynamically focussing the particles in the non-centrifuged urine after conducting a special fluorescence staining procedure and classifying them at high speed. Regardless of the procedure used in routine, urinalysis remains a combination of multiple laboratory diagnostic measures to this very day. Each method detects different components and this has its validity. As with other laboratory results, it is to be kept in mind that a single result is never interpreted on its own (in this case referring to the protein detected), but as part of a combination of results, with special attention to the aforementioned, but sometimes neglected, background information.

We thank Dr. Axel Regeniter for his expert advice during the composition of this article.

References

[1] ***Kobayashi K. and Fukuoka S. (2001):*** *Conditions for Solubilization of Tamm-Horsfall Protein/Uromodulin in Human Urine and Establishment of a Sensitive and Accurate Enzyme-Linked Immunosorbent Assay (ELISA) Method. Archives of Biochemistry and Biophysics, 388 (1): 113.*

[2] ***Ishii T., Hara T., Nakayama A., Matsumoto H. (2003):*** *Examination of Remaining Cells by UF-100, Fully Automated Urine Cell Analyser in the Supernatant after Centrifugation. Sysmex J Int 13: 53.*

[3] ***Kouri T., György A. , Rowan R. M. (2003):*** *ISLH Recommended Reference Procedure for the Enumeration of Particles in Urine. Laboratory Hematology 9: 58.*

[4] ***Scherberich, J. (1998):*** *Nichtinvasive Diagnostik von Nierenerkrankungen, Differentielle klinische Bewertung der Proteinurieformen. Der Bayerische Internist 18: 80.*

[5] ***Woolhandler S., Pels R.J., Bor D.H., Himmelstein D.U., Lawrence R.S. (1989):*** *Dipstick urinalysis screening of asymptomatic adults for urinary tract disorders: I. Hematuria and Proteinuria. JAMA 262: 1214.*

[6] ***Hannemann-Pohl K. and Kampf S.C. (1999):*** *Automation of Urine Sediment Examination: a Comparison of the Sysmex UF-100 Automated Flow Cytometer with Routine Manual Diagnosis (Microscopy, Test Strips, and Bacterial Culture). Clin Chem Lab Med 37(7): 753.*

[7] ***Mogensen C. E. (2003):*** *Microalbuminuria and hypertension with focus on type 1 and type 2 diabetes. Journal of Internal Medicine 254: 45*

[8] ***Regeniter A., Freidank H., Dickenmann M., Boesken, Werner H. , W.H. Siede:*** *Evaluation of proteinuria and GFR to diagnose and classify kidney disease: Systematic review and proof of concept. European Journal of Internal Medicine 20(6): 556.*

Sysmex Europe GmbH
Bornbarch 1, 22848 Norderstedt, Germany, Phone +49 40 52726-0 · Fax +49 40 52726-100 · info@sysmex-europe.com · **www.sysmex-europe.com**
Please find your local Sysmex representative address under www.sysmex-europe.com