Patterns of Proteinuria: Urinary Sodium Dodecyl Sulfate Electrophoresis Versus Immunonephelometric Protein Marker Measurement Followed by Interpretation with the Knowledge-Based System MDI-LabLink, Mustapha Maachi,1,2 Soraya Fellahi,2 Axel Regeniter,3 Marie-Emilienne Diop,2 Jacqueline Capeau,1,2 Jérôme Rossert,4 and Jean-Philippe Bastard1,2 (1 INSERM Research Unit 402, Faculty of Medicine Saint-Antoine, University Pierre & Marie Curie, Paris, France; 2 Department of Biochemistry, Tenon Hospital, Paris, France; 3 Department of Laboratory Medicine, Kantonsspital Basel, University Hospital, Basel, Switzerland; 4 Department of Nephrology, Tenon Hospital, Paris, France; * address correspondence to this author at: Service de Biochimie et Hormonologie, Hôpital Tenon, 4 rue de la Chine, 75970 Paris Cedex 20, France; fax 33-1-5601-7840, e-mail mustapha.maachi@tnn.ap-hop-paris.fr)

Increased urinary total protein is a nonspecific and unreliable marker of renal function. Analysis of the pattern of proteinuria, however, can provide information regarding the pathophysiologic changes in the affected nephrons. In physiologic proteinuria, the range of daily urinary protein excretion is typically 40–80 mg/24 h with an upper limit of 150 mg/24 h. Albumin represents the main component (30–40%), whereas IgG, light chains, and IgA represent 5–10%, 5%, and 3%, respectively, of urinary proteins. The remainder consists mostly of Tamm–Horsfall protein.

Patterns of pathologic proteinuria may be classified as glomerular, tubular, prerenal, mixed, or postrenal, with glomerular patterns the most frequent. Total urinary protein excretion can exceed 2 g/day, with albumin representing the main component (~70%) and other large-molecular-weight proteins, such as transferrin and IgG, accounting for the remaining 30%. Tubular proteinuria is characterized by the dominant excretion of low-molecular-weight proteins such as α2-microglobulin (A1M) or retinol-binding protein (RBP), which correlate better with the extent of tubulo-interstitial damage than does determination of total 24-h protein concentrations (1).

Urinary total protein is frequently undetectable in predominantly tubular kidney disease, and common chemical methods also often fail to detect urinary total protein in predominantly tubular kidney disease, in which albumin usually represents <30% of the total protein content (2–8). However, some renal tubular disorders or interstitial nephritis (e.g., when caused by antibiotics and other tubulo-toxic substances) are easily treatable. Prerenal proteinuria (Bence Jones proteinuria), attributable to overproduction of light chains in monoclonal diseases, or lysozymuria in patients with leukemia and the resulting overload of tubulo-interstitial reabsorption in the kidney often lead to secondary kidney damage. Mixed proteinuria presents with glomerular and tubular protein fractions in urine, i.e., high- and low-molecular-weight proteins. Postrenal proteinuria closely resembles glomerular proteinuria but can be identified by the presence of α2-macroglobulin.

Early approaches to the evaluation of proteinuria were based on sodium dodecyl sulfate (SDS) electrophoresis (9, 10). This method, however, is time-consuming, is at best semiquantitative, and has a high analytical detection limit well above the reference interval for most protein bands (~20 mg/L), and interpretation is investigator dependent. Alternatively, urinary marker proteins can be measured quantitatively by immunoturbidimetry or nephelometry.

At least two knowledge-based systems for the further evaluation of proteinuria are available, UPES (11) and MDI-LabLink (4). MDI-LabLink, developed by Regeniter et al. (4), screens for proteinuria with few marker proteins (albumin and A1M) but uses additional marker proteins (IgG, transferrin, RBP, and β2-microglobulin) to enable comparison with the SDS-polyacrylamide gel electrophoresis (PAGE) classification system introduced by Boesken et al. (12, 13). It also evaluates the numerous formulas specific to quantitative measurement to further classify proteinuria (4, 12–16) and transforms results to a specific signature pattern (17).

We compared the two methods, using 138 second-morning urine samples from the Tenon Hospital nephrology department. The one-step SDS-agarose electrophoresis technique (Hydragel protéinurie® reagents; Sébia) uses unconcentrated urine and requires four or nine urine samples to fill all slots on the foil and a time period of at least 4 h.

The results of automated measurements of urinary albumin, transferrin, IgG, A1M, and RBP (immunonephelometry on an IMMAGE®; Beckman-Coulter), urinary total protein (SDS-pyrogallol red-molybdate method on a Hitachi 747®; Roche-Boehringer), and urinary creatinine (Jaffe method) were entered into the MDI-LabLink software, which also requires dipstick results for erythrocytes, leukocytes, and nitrites (Multistix® dipstick test 10 SG; Bayer) to exclude postrenally contaminated samples (14, 15).

The two methods produced identical interpretations for 105 of 138 urine samples (77%; Table 1). Differences for the entire study group (P < 0.001, McNemar paired test) were caused, in general, by the lower detection limits for the nephelometric methods (albumin, 2.0 mg/L; transferrin, 1.0 mg/L; IgG, 3.0 mg/L; A1M, 3.0 mg/L; RBP, 0.5 mg/L) and the lower, creatinine-related reference limits (albumin, <20 mg/g urinary creatinine; transferrin, <2 mg/g creatinine; IgG, <10 mg/g creatinine; A1M, <14 mg/g creatinine; RBP, <1 mg/g creatinine) of the marker approach. MDI-LabLink classified 24 samples as mixed instead of glomerular because both A1M and RBP were above the reference intervals (P < 0.001; Table 1, row 3). Two cases classified as physiologic on the SDS gel (only traces of albumin visible) were reclassified by MDI-LabLink as pathologic mixed proteinuria (Table 1, row 1), because additional tubular proteins above the reference limit (A1M and RBP) were detectable by nephelometry.

The third patient with pathologic mixed proteinuria, in row 1, presented clinically with hemolytic uremic syndrome (serum creatinine, 130 μmol/L). We found a urinary total protein of 210 mg/g of creatinine (23.8
mg/mmol of creatinine) and only a slight electrophoretic band corresponding to albumin (Fig. 1A, lane 11). By contrast, the marker protein approach revealed a mixed pattern of predominantly glomerular proteinuria as well as increased tubular marker proteins. A second urine sample 1 month later showed increased proteinuria [980 mg/g of creatinine (81.7 mg/mmol of creatinine)] associated with decreased renal function (serum creatinine, 192 μmol/L). Both electrophoresis and individual protein measurements confirmed the initial MDI-LabLink interpretation of mixed proteinuria (Fig. 1A, lane 4).

Another patient was clinically highly suspicious for drug-related tubular disease. The electrophoresis showed mixed proteinuria and a band corresponding to IgG (Table 1, row 4). In contrast, specific protein measurements and subsequent MDI-LabLink interpretation classified the urine pattern as predominantly tubular, which agreed with the clinical diagnosis and follow-up.

MDI-LabLink advised in two cases against proteinuria interpretation because low creatinine concentrations indicated a diluted sample (Table 1, row 4), and excluded one sample with a pattern of hematuria from interpretation (Table 1, row 3).

Table 1. Comparative proteinuria results in the 138 urine samples and their interpretation.

<table>
<thead>
<tr>
<th>Type of proteinuria</th>
<th>Hydragel SDS electrophoresis</th>
<th>MDI-LabLink</th>
<th>n</th>
<th>Percentage</th>
<th>Comments</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiologic</td>
<td>10</td>
<td>Physiologic</td>
<td>7</td>
<td>70</td>
<td>Identical interpretation</td>
<td>Not significant</td>
</tr>
<tr>
<td>Mixed glomerular/tubular</td>
<td></td>
<td></td>
<td>3</td>
<td>30</td>
<td>False negative by electrophoresis; lower detection limit of immunonephelometry</td>
<td></td>
</tr>
<tr>
<td>Tubular</td>
<td>10</td>
<td>Tubular</td>
<td>8</td>
<td>80</td>
<td>Identical interpretation</td>
<td>Not significant</td>
</tr>
<tr>
<td>Mixed tubular/glomerular</td>
<td></td>
<td></td>
<td>2</td>
<td>20</td>
<td>False negative by electrophoresis; lower detection limit of immunonephelometry</td>
<td></td>
</tr>
<tr>
<td>Glomerular</td>
<td>44</td>
<td>Glomerular</td>
<td>19</td>
<td>43</td>
<td>Identical interpretation</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mixed glomerular/tubular</td>
<td></td>
<td></td>
<td>24</td>
<td>55</td>
<td>False negative by electrophoresis; lower detection limit of immunonephelometry</td>
<td></td>
</tr>
<tr>
<td>Excluded</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>Excluded because of hematuria</td>
<td>Not significant</td>
</tr>
<tr>
<td>Mixed glomerular</td>
<td>58</td>
<td>Mixed glomerular</td>
<td>55</td>
<td>95</td>
<td>Identical interpretation</td>
<td>Not significant</td>
</tr>
<tr>
<td>Tubular</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>Band migrating at the IgG position</td>
<td></td>
</tr>
<tr>
<td>Excluded</td>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td>Excluded because of low urinary creatinine concentration</td>
<td></td>
</tr>
<tr>
<td>Mixed tubular</td>
<td>16</td>
<td>Mixed tubular</td>
<td>16</td>
<td>100</td>
<td>Identical interpretation</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

The excretion pattern of glomerular proteinuria has diagnostic and prognostic significance. Transferrin and IgG, two glomerular markers with different molecular weights, distinguish selective from unselective proteinuria. Selectivity based on the urinary transferrin/IgG ratio uses a defined cutoff (moderately selective, 1–1.5; selective, >1.5) and correlates well with the clearance index of Cameron and Blandford (18) without the additional need to collect a separate serum sample (4). The selectivity by electrophoresis, in contrast, is evaluated on the presence or absence of the IgG band, which is not always easily detectable and which may not be seen by all investigators.

SDS electrophoresis identifies proteins above their detection limits with a molecular weight marker protein (Fig. 1A, lanes 1 and 6, glomerular proteins with Mr >66 000 and tubular marker proteins with Mr <66 000). The appearance on the gel depends partly on the affinity of the staining solution for protein and therefore has no clearly defined cutoff. The supplied molecular weight markers from the manufacturer can also have a different reactivity than urinary proteins. Therefore, sight-reading of the foils remains subjective. Prerenal proteinuria (Bence Jones proteinuria), however, is suspected when additional bands are visualized at either Mr 25 000, which corresponds to light chain monomers, or Mr 50 000 for light chain dimers.

Although the primary focus of MDI-LabLink is the quantification of glomerular or tubular damage, it suggested immunofixation in most cases of Bence Jones proteinuria (7 of 10 cases of the study group) seen on the gel by routinely calculating the difference between total protein content and the sum of all measured specific proteins (19). The software is also able to evaluate nephelometric results for total or free light chains to calculate the k/λ ratio, a complementary or even alternative method to detect Bence Jones proteinuria (20), and urinary immunofixation results can be entered to enable a complete workup of proteinuria, which was not investigated in this study. In a case of light chain myeloma (λ) identified by urinary immunofixation (Fig. 1A, lanes 9 and 10), electrophoresis of the urine sample showed a broad intense band corresponding to light chains but masked other low-molecular-weight proteins such as...
A1M because of comigration. By contrast, individual protein measurements showed a high concentration of A1M at 241 mg/g of creatinine (27.2 mg/mmol of creatinine). In this situation, only individual protein measurements can distinguish the specific protein variations and provide a more accurate assessment of proteinuria.

Some nephrology centers combine a more sensitive SDS-PAGE with silver staining and obtain an improved analytical reference limit down to or even below the concentration of 1 mg/L per band. Assessment of proteinuria of renal origin can be further optimized to include tubular function when SDS-PAGE is combined with the additional measurement of β2-microglobulin after pretreatment of patients with mercaptoethanol to alkalinize the urine (21). This approach, however, is dependent on individual experience and not suitable for the routine workup of a large number of patients.

Nephelometric measurement is more sensitive and specific and is standardized among manufacturers (18); in addition, automated analysis requires only a few minutes. The MDI-LabLink software uses creatinine values to adjust untimed urine samples so that results are comparable to 24-h measurements (14, 22–24). It excludes samples with hematuria and urinary tract infections closely resembling glomerular proteinuria from interpretation, detects most causes of prerenal proteinuria, and calculates glomerular selectivity. Most importantly (25, 26), it quantifies the amount of renal damage and converts the different reference intervals of the urinary marker proteins to a graphic result signature pattern (Fig. 1B), which provides instant understanding of a complex biochemical situation (17). We found this to be an investigator-independent and standardized approach superior in evaluating proteinuria and have replaced SDS electrophoresis with the protein marker approach in our laboratory. Use of MDI-LabLink could potentially enable evaluation of
proteinuria with specific marker proteins in routine laboratories, in which nephelometric instrumentation is frequently available but manpower is limited and the expert knowledge required to interpret proteinuria is lacking.

References

10. Boesken WH, Rohrbach R, Schollmeyer P. Vergleich von Histologie und Laboratorien, in Which nephelometric instrumentation is frequently available but manpower is limited and the expert knowledge required to interpret proteinuria is lacking.

Rapid Detection of Cystic Fibrosis Transmembrane Conductance Regulator Gene IVS8 5T Variant by Real-Time PCR, Eniko Kámyör,* Beáta Cskay, and Zsolt Holló (Genodia Molecular Diagnostics Ltd., Bajaki Ferenc 1-3, 1211 Budapest, Hungary; * author for correspondence: fax 36-1-4270350, e-mail kamyor.eniko@genodia.hu)

Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasian populations and is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. The CFTR gene encodes a transmembrane protein that forms a cAMP-regulated chloride channel. Wide variations in disease manifestations are observed among affected CF patients, and a multitude of disease-causing mutations have been found in the CFTR gene (1). In 97% of men with CF, bilateral congenital absence of the vas deferens (CBAVD) blocks the transport of spermatozoa from testicular structures to the distal genital tract, causing azoospermia (2). Infertility attributable to CBAVD does not necessarily coincide with other manifestations of CF. CBAVD accounts for ~1–2% of all male infertility and at least 6% of the cases of obstructive azoospermia.

Isolated CBAVD patients carry either a CF mutation (ΔF508 in 16–83% of cases and R117H in 6–29% of cases) and/or a 5T variant in intron 8 (12–47% of patients), supporting the hypothesis that CBAVD represents a mild, primary genital form of CF (2–7). Three length variations of a polythymidine (polyT) tract within the splice acceptor site in intron 8 of the CFTR gene (GenBank accession no. M55106) have been associated with variable efficiency of exon 9 splicing (8). On the basis of the increased frequency (compared with the general population) of the five-thymidine (5T) variant [vs seven or nine thymidines (7T or 9T)] in CBAVD patients, the 5T variant was classified as a CBAVD mutation (7).

Currently available but time-consuming methods for 5T/7T/9T genotyping include PCR amplification followed by acrylamide gel electrophoresis (9, 10), detection of mRNA length (2), restriction endonuclease digestion (7), single-strand conformation polymorphism analysis and direct sequencing (11), capillary zone electrophoresis (12), and dot-blot hybridization (12).

We have developed a much faster, sensitive, single-step method to detect the IVS8 5T variant in CBAVD patients. Our method is based on the LightCycler technology (Roche) using rapid PCR followed by analysis of the melting behavior of fluorescently labeled hybridization probes (13). The sensor probe covers the 5T/7T/9T poly-