Misleading Urinary Protein Pattern in a Patient with Hypogammaglobulinemia: Effects of Mechanical Concentration of Urine, Irina V. Kaplan1 and Stanley S. Levinson2,3

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Usually urine protein electrophoresis (UPE) gives rise to distinctive patterns that indicate the source of proteinuria. However, abnormalities of endogenous protein synthesis and peculiarities attributable to mechanical concentration of the sample before analysis can cause misinterpretation. Here we describe the analysis of urine from a patient with hypogammaglobulinemia in which a probable mixed pattern appeared as pure glomerular proteinuria as a result of altered immunoglobulin synthesis.

A 72-year-old, Caucasian male patient with a 1-year history of chronic lymphocytic leukemia and a 20-year history of insulin requiring type 2 diabetes with diabetic retinopathy and neuropathy was admitted to the hospital because of syncope. Selected test results at the time of admission were as follows (reference intervals in parentheses): urea nitrogen, 0.22 g/L (0.07–0.22 g/L); creatinine, 0.01 g/L (0.006–0.014 g/L); total protein, 55 g/L (62–82 g/L); albumin, 33 g/L (35–50 g/L); IgG, 1.7 g/L (7.2–16.8 g/L); IgA, 0.1 g/L (0.69–3.8 g/L); IgM, 0.1 g/L (0.63–2.7 g/L); phosphorous, 0.022 g/L (0.025–0.048 g/L); potassium, 3.7 mmol/L (3.5–5.1 mmol/L); and glucose, 2.35 g/L (0.65–1.1 g/L, fasting). Sodium, bicarbonate, and chloride were within the health-related reference intervals. Urinalysis was remarkable for occasional squamous epithelial cells and hyaline casts, a glucose >2.5 g/L, and 6.4 g protein/24 h, with a volume of 1.5 L (reference interval, <200 mg/24 h). Hematologic studies were unremarkable except for a white blood cell count of 115 000/mm³ with 86% lymphocytes.

A 24-h urine specimen was obtained without the use of preservatives. Urinary total protein was measured using pyrogallol red-molybdate with a kit (Biotrol urine proteins, Biotrol USA) on a Cobas Fara II (Roche Diagnostics), according to the manufacturer’s directions. Immuno fixation electrophoresis (IFE) was performed using the Titan Gel Immunofix procedure and antibodies (Helena).

The patient’s serum SPE pattern (Fig. 1A) showed little staining in the γ-globulin region, which indicates hypogammaglobulinemia, consistent with the quantitative immunoglobulin results. As in the glomerular proteinuria profile (Fig. 1A), the patient’s 20-fold urine concentrate shows little staining in the γ-globulin region after UPE, but a small diffuse band of staining appears with the 100-fold concentrate (Fig. 1A). Of note, the α2 region of the 100-fold concentrate in Fig. 1A, dual bands (indicated by brackets) indicate the presence of α2-microglobulins (I, 1); however, these bands are not seen in the 20-fold concentrate. IFE (Fig. 1B) of the 100-fold concentrate revealed that the diffuse band seen after UPE of the 100-fold concentrate (Fig. 1A) was a k-Bence Jones protein (BJP). For comparison, Fig. 1C shows the UPE and urine IFE from another patient in whom multiple myeloma was ultimately diagnosed. In this case, even a 100-fold concentrate of UPE showed no banding, whereas IFE showed a band of restricted migration, stained for λ, which indicated a BJP.

The findings in the patient in Fig. 1A can be compared with typical patterns (also Fig. 1A). In glomerular disease (Fig. 1A, third lane from top), larger proteins pass more freely than usual through the leaky glomerulus so that >3 g of protein in 24 h passes. This excreted protein is largely albumin (the most abundant protein in plasma),

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without small globulins, which are reabsorbed by the tubules, but with larger proteins other than albumin seen when the threshold for tubular reabsorption is exceeded. In pure tubular proteinuria, the small proteins, usually found in the glomerular filtrate (e.g., polyclonal free light chains and α₂-microglobulins), are not reabsorbed because of renal tubular failure; they appear in the urine, giving the tubular pattern seen in Fig. 1A. The mixed pattern (Fig. 1A, top lane) is seen with combined glomerular and tubular damage. The overflow pattern (Fig. 1A, fourth lane from top) occurs when the BJP concentration exceeds the tubular capacity for reabsorption, and does not necessarily imply major renal damage.

Although the physiology and pathophysiology that produce the typical patterns shown in Fig. 1A are well characterized, peculiarities in the synthesis of serum proteins and methodological manipulations that effect interpretation have not been as well defined and can lead to misinterpretations. Both of these features are illustrated in the present case.

Hypogammaglobulinemia is not uncommon as chronic lymphocytic leukemia progresses. In many cases, BJp is also associated with this syndrome. In the present case, it was assumed by the clinical staff that the nephropathy was most likely of diabetic origin, which most commonly leads to increased glomerular permeability and the development of nephrotic syndrome (3). The initial UPE with a 20-fold concentrate (Fig. 1A) along with 6.4 g protein/day seemed to justify this view because the UPE pattern resembled a pure glomerular pattern. However, because the serum electrophoresis showed hypogammaglobulinemia (Fig. 1A), the urine was reexamined at 100-fold concentrate, and double bands were seen in the α₂-globulin region, indicating a tubular component (1, 2). Other evidence suggesting a tubular component included the low serum potassium and phosphorous, and urea nitrogen and creatinine within the health-related reference intervals. These are all generally increased in pure glomerular failure, but serum potassium and phosphorous are low in cases of failure of tubular reabsorption (4).

Epithelial cells with hyaline casts are also seen in the urinalysis with tubular damage. Most likely, the correct interpretation for this case is mixed proteinuria because of both glomerular and tubular disease.

These patterns highlight the effect of mechanical concentration on the interpretation. With a mixed UPE pattern containing a substantial glomerular component, it may be difficult to see all tubular constituents in a more

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**Fig. 1.** UPE and IFE analysis of serum and urine from patient with hypogammaglobulinemia (A and B) and patient with multiple myeloma (C).

(A), patient’s serum and urine electrophoretic profiles compared with typical urinary protein profiles seen in disease. U × 20 and U × 100 indicate approximate degree of mechanical concentration of patient’s urine. Brackets indicate α₂-microglobulin, which appears as dual bands. All comparison patterns (Tubular, Glomerular, Mixed, and Overflow) were from urines concentrated 100-fold. (B), IFE of patient’s urine for 100-fold concentrate. G, IgG; A, IgA; κ, κ free and bound light chains; λ, λ free and bound light chains; Fκ, free κ chain; Fλ, free λ chain. (C), comparison example of UPE and IFE of 100-fold concentrate from another patient, demonstrating no band after UPE, but a band with IFE. G, IgG; A, IgA; M, IgM; κ, κ light chain; λ, λ light chain. This patient’s urine contained 150 mg/day of protein.
dilute specimen. Usually, the intense contribution from polyclonal free light chains is sufficient to enable the correct interpretation in the absence of visible α2-microglobulins; however, in the present case this was not true because of a lack of polyclonal immunoglobulin synthesis. This case illustrates the importance of comparing serum and urine results whenever possible.

A second misinterpretation that may emerge from inadequate concentration of urine is failure to identify a BJ. In the case described here, although nothing was observed in the γ region of the 20-fold concentrate with UPE, a diffuse region of staining was seen in the 100-fold concentrate. After IFE, this diffuse staining clearly appears as a band of restricted migration, which stained as a κ-BJ (Fig. 1B). The problem of screening for BJPs with UPE alone is further accentuated by the patterns shown in Fig. 1C, from a second patient, where nothing is seen after UPE of a 100-fold concentrate, but a κ-BJ is seen clearly after IFE of the same sample. In the case shown in Fig. 1C, the total protein was found to be 150 mg/24 h, an amount that is within the reference interval. Most laboratories quantify urine protein with a precipitation method, such as trichloroacetic or sulfosalicylic acid, or with a protein binding dye such as Coomassie blue or Ponceau S. The degree to which these methods measure free light chains is unclear. Very few laboratories use the biuret method, which reacts with the peptide bonds in proteins, measuring all proteins more equally. As a result, except for biuret method, these methods cannot always be relied on to provide useful information regarding quantification of free light chains. Furthermore, the detection limit of all of these methods, including the biuret, are higher than that of IFE (5). Although disagreement remains as to which combination of UPE and IFE provides the most efficient and cost-effective screening approach to detecting BPs (6), the cases presented here show that even with a 100-fold concentration, UPE in the absence of IFE is an inadequate screen for low concentrations of BJ in samples.

Some commercial manufacturers of kits indicate that the urine sample should be concentrated to a defined protein concentration, which in our experience often turns out to be only a 10- to 20-fold concentration for UPE or IFE. The importance of greater mechanical concentration is emphasized in this study. In our opinion, the best solution to these problems is to concentrate specimens 80- to 160-fold and to perform IFE as well as UPE on all specimens for which identification of BJPs is important. An alternative method for screening urine samples by using κ/λ ratios in conjunction with UPE has been described (7). We have found that this alternative approach may reduce the more tedious IFE by ~30%.

References


Identification of a Pharmacologically Active Metabolite of Mycophenolic Acid in Plasma of Transplant Recipients Treated with Mycophenolate Mofetil, Ekkehard Schütz, Maria Shipkova, Victor W. Armstrong, Eberhard Wieland, and Michael Oellerich (Abteilung Klinische Chemie, Georg-August-University, D-37075 Göttingen, Germany; * address correspondence to this author at: Abteilung Klinische Chemie, Zentrum Innere Medizin, Georg-August-Universität, Robert Koch Strasse 40, D-37075 Göttingen, Germany; fax 49 (0)391-398551, e-mail eschuetz@med.uni-goettingen.de)

Mycophenolic acid (MPA), the active moiety of mycophenolate mofetil (MMF), is an antiproliferative agent that acts by inhibition of inosine monophosphate dehydrogenase type II (IMPDH-II), a key enzyme in the de novo purine biosynthetic pathway (1, 2). Several studies have documented that MMF is effective in the treatment of refractory rejection in renal, heart, and liver transplant recipients (2). The major pathway for elimination of MPA involves glucuronidation (3) at the phenolic hydroxyl group to form mycophenolate 7-O-glucuronide (7-O-MPAG). Modification of this phenolic hydroxyl residue leads to a loss of pharmacological activity toward IMPDH-II (4, 5).

Most studies on the pharmacokinetics of MPA have utilized HPLC procedures (6, 7) to measure both MPA and MPAG. Recently, the first immunoassay became available for the quantification of MPA ( Emit-MPA, Dade Behring). 7-O- MPAG does not cross-react with this assay. Comparison of plasma MPA concentrations from clinical samples determined with HPLC showed an overestimation in relation to those obtained with the Emit of up to 100%, with an average of 35% in a group of 37 kidney recipients, which accounts for a mean overestimation of 20% for the calculated areas under the concentration-time curve (8). Through a modification of our HPLC procedure (7), we were able to identify two putative MPA metabolites, M-1 and M-2, from the plasma of transplant recipients, of which M-2 was found to cross-react in the immunoassay (8). Recently, we showed that in clinical samples from heart, kidney, and liver recipients, the relative amounts of M-2 correlate with the bias between MPA values determined with HPLC and the immunoassay (9). In pharmacokinetic studies, it was shown that the areas under the concentration-time curve for both M-1