

Estimation of daily proteinuria in patients with amyloidosis by using the protein-to-creatinine ratio in random urine samples

Giampaolo Talamo,¹ Muhammad A. Mir,¹ Manoj K. Pandey,² Junjia Zhu,² Michael H. Creer,² Jozef Malysz²

¹Penn State Hershey Cancer Institute;

²Milton S. Hershey Medical Center, Hershey, PA, USA

Abstract

Measurement of daily proteinuria in patients with amyloidosis is recommended at the time of diagnosis for assessing renal involvement, and for monitoring disease activity. Renal involvement is usually defined by proteinuria >500 mg/day. We evaluated the accuracy of the random urine protein-to-creatinine ratio (Pr/Cr) in predicting 24 hour proteinuria in patient with amyloidosis. We compared results of random urine Pr/Cr ratio and concomitant 24-hour urine collections in 44 patients with amyloidosis. We found a strong correlation (Spearman's $\rho=0.874$) between the Pr/Cr ratio and the 24 hour urine protein excretion. For predicting renal involvement, the optimal cut-off point of the Pr/Cr ratio was 715 mg/g. The sensitivity and specificity for this point were 91.8% and 95.5%, respectively, and the area under the curve value was 97.4%. We conclude that the random urine Pr/Cr ratio could be useful in the screening of renal involvement in patients with amyloidosis. If validated in a prospective study, the random urine Pr/Cr ratio could replace the 24 hour urine collection for the assessment of daily proteinuria and presence of nephrotic syndrome in patients with amyloidosis.

Introduction

Amyloidosis is a very rare disease, with an annual incidence of about 1:100,000 in the U.S.¹ It constitutes a heterogeneous group of disorders, characterized by the extracellular deposition of insoluble fibrils made of misfolded proteins. More than 25 different proteins, structurally unrelated, are known to cause amyloidosis.² They are identified by their birefringent appearance when viewed with Congo red staining under a polarized microscope. The 4 most important types of systemic amyloidosis are: i) the primary form (AL amyloidosis), due to immunoglobulin (Ig) light chains; ii) the hereditary form, mostly due to mutations in

the transthyretin (*TTR*) gene (ATTR), or to much rarer mutations such as those involving the apolipoprotein AI gene (AApoAI) or the fibrinogen gene (AFib); iii) the senile form, due to wild-type TTR; and iv) the secondary type (AA amyloidosis), associated with chronic inflammatory disorders, due to the acute-phase reactant serum amyloid protein A (SAA) (AA amyloidosis). Various chronic inflammatory conditions can induce AA amyloidosis, for example rheumatoid arthritis, tuberculosis, and familial Mediterranean fever.

Amyloidosis can be a life-threatening disease, because it can cause progressive organ damage and irreversible failure. Although it may affect any organ, one of the most frequent target organs is the kidney, and clinically evident renal disease occurs in about 50-80% of cases.³⁻⁷ Typical manifestations of renal involvement are proteinuria, nephrotic syndrome (*i.e.*, concomitant proteinuria, hypoalbuminemia, and peripheral edema), renal insufficiency, and end-stage renal disease (ESRD) requiring hemodialysis. All forms of systemic amyloidosis can lead to renal involvement, including AL, ATTR, AApoAI, AFib, and AA. AL amyloidosis induces proteinuria and renal insufficiency in up to 73% and 50% of cases, respectively.⁴ ATTR amyloidosis typically does not involve the kidneys,⁸ but it can induce proteinuria and ESRD in some patients.⁹

Although the definitive diagnosis of renal involvement by amyloidosis is established by a renal biopsy, this method is impractical, at least for screening purposes. Some authors have proposed to define renal involvement by the presence of urinary protein excretion exceeding 1 g/24 hours,¹⁰ but this threshold is arbitrary, as the degree of proteinuria varies in each case, and it may range from an asymptomatic and barely detectable laboratory phenomenon to a massive proteinuria (as high as 30 g/day), complicated by profound hypoalbuminemia and severe peripheral edema.⁸ Whatever the threshold used, the degree of proteinuria is traditionally calculated from 24-hour urine collections.¹⁰⁻¹³ Unfortunately, this method is problematic both for patients and physicians: first, patients can find it cumbersome to collect all urine excreted in 24 hours. In fact, several studies have reported high rates of incorrect collection.¹⁴⁻¹⁶ In one of them, more than 20% of the 24 hour samples were discarded because they were found to be incomplete.¹⁶ Due to the difficulties involved in obtaining a complete sample, results are often inaccurate and unreliable.^{17,18} Finally, laboratory manipulation of specimens is costly and relatively time-consuming.¹⁹⁻²¹

The use of the random (*spot*) protein-to-creatinine (Pr/Cr) ratio has proven to be a valid alternative in estimating the daily proteinuria.^{22,23} Variations in the concentration of excreted protein throughout the day are due to

Correspondence: Giampaolo Talamo, Penn State Hershey Cancer Institute, 500 University Drive, Hershey, PA 17033, USA.
Tel.: +1.717.5318678 - Fax: +1.717.5311656
E-mail: gtalamo@hmc.psu.edu

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fluctuations in the amount of water excreted, which can vary based on several factors, including diet, degree of physical activity, and body temperature. Since the excretion of creatinine remains reasonably constant throughout the day, the Pr/Cr ratio can correct for these variations. Several studies have validated its use as a predictor of 24 hour urine protein excretion.^{16,17,20,24-29} Since 2002, the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines state that the Pr/Cr ratio in an untimed urine specimen should replace protein excretion in a 24 hour collection as the preferred method for detecting and monitoring proteinuria.³⁰ The Pr/Cr ratio has been shown to be a valid estimator of daily protein excretion in a variety of conditions associated with significant proteinuria, including chronic kidney disease,²⁰ systemic lupus erythematosus (SLE),¹⁶ and diabetes mellitus (DM).^{26,27} We published the first study to address the use of the Pr/Cr ratio in patients with multiple myeloma (MM), and found that random urine specimens provided quantification of daily proteinuria in those patients as accurate as the 24-hour urine collection correlation (Spearman's $\rho=0.81$).³¹ The aim of this study was to evaluate the correlation between the urine Pr/Cr ratio in randomly collected, untimed urine samples with proteinuria meas-

ured by a 24 hour urine collection in patients with amyloidosis. The ability of the Pr/Cr ratio to predict various threshold levels of protein excretion was also assessed.

Materials and Methods

We retrospectively reviewed data from 82 consecutive patients with systemic amyloidosis followed at our Institute in 2010-2013. We excluded from the analysis patients with localized amyloidosis. Renal involvement was defined as a positive renal biopsy or as a protein excretion >1000 mg in a 24-hour urine collection (in the absence of DM, SLE, hypertensive nephropathy, or any other disease causing proteinuria). The urine Pr/Cr ratio was expressed in mg/g units (mg protein: g creatinine). We correlated the results of Pr/Cr ratio in random urine and total proteins from a 24-hour urine collection, using specimens collected on the same day. Urine samples were obtained within 4 weeks from the diagnosis of amyloidosis. Random and 24 hour urine total protein were measured by reaction with a pyrocatechol violet-molybdate complex, using the Vitros Upro Slide method. Urine creatinine was measured using the Vitros Crea slide method, which utilizes an enzymatic assay. All tests were done on the Vitros 5,1 FS, or Vitros 4600 Chemistry System (Ortho-Clinical Diagnostics, Rochester, NY, USA).

Statistical analysis was performed using the program SAS® software, version 9.3 (SAS Institute, Cary, NC, USA) and R Programming Language, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). We calculated the Spearman correlation coefficient between the 24 hour urine total protein and random urine Pr/Cr. Receiver operating characteristics (ROC) curves were constructed to test the ability of the random Pr/Cr ratio to predict the 24 hour urine protein excretion at 500, 1000, 3500, and 5000 mg/day. These thresholds were chosen arbitrarily, to reflect different levels of clinical significance: <300 for normal proteinuria, 500 and 1000 as commonly accepted thresholds to define renal involvement, and >3500 for nephrotic-range proteinuria.

Results

Our sample included 82 patients. Median age at diagnosis was 66 years, and 50 (61%) were male. Patients had the following types of systemic amyloidosis: AL (63 pts, 76.8%), ATTR (15 pts, 18.3%), AA (3 pts, 3.7%), and keratin-type (1 pt, 1.2%). AL amyloidosis was of lambda type in 39 patients (61.9%), and kappa type in the rest of them. Tissue biopsy was obtained in

all patients, and tandem mass spectrometry was requested and available in 12 of them, when the type of amyloid was not immediately evident. Renal biopsy was performed in 30 patients. Renal involvement was observed in 44 of 82 (54%) patients. Renal insufficiency, defined as a glomerular filtration rate (GFR) <60 mL/min, was present in 27 patients, and 3 of them required hemodialysis.

The median 24 hour urine total protein was 3279 mg/day (IQR 713-6,983, range 80-24,648), and the median random urine Pr/Cr ratio was 3159 mg/g (IQR 364-7,893, range 30-76,000). Paired 24 hour urine total protein measurements and random urine Pr/Cr ratios were available for analysis in 44 patients. There was strong correlation between the random urine Pr/Cr ratio and the 24 hour urine total proteins (Spearman's $\rho=0.874$, $P<0.001$). A scatter plot of the 24 hour urine total protein and random urine Pr/Cr ratio is shown in Figure 1. The data were log-transformed for better display of the linear relationship between the two variables. ROC curves were constructed to test the ability of the random Pr/Cr ratio to predict 24 hour urine protein excretion at 500, 1000, 3500, and 5000 mg/day, thresholds chosen arbitrarily but reflective of a different clinical significance. (total proteins <300 mg/day can be considered clinically insignificant, while a daily proteinuria >3500 mg/day defines the nephrotic range). The sensitivity and specificity of the discriminant cutoff values at different amounts of protein excretion is shown in Table 1, along with the area under the various ROC curves.

Using the 24-hour urine total protein level for predicting renal involvement, the optimal cut-off point in our sample was 685 mg (or 6.53 on the log-scale). This provided a sensitivity and specificity of 95.5% and 100%, respectively, with an area under the curve (AUC) value of 99.6%. For the Pr/Cr ratio, the optimal cut-off value for predicting renal involvement was 715 mg/g (or 6.57 on the log-scale), which provided a sensitivity and specificity of 91.8% and 95.5%, respectively (AUC 97.4%) (Figure 2).

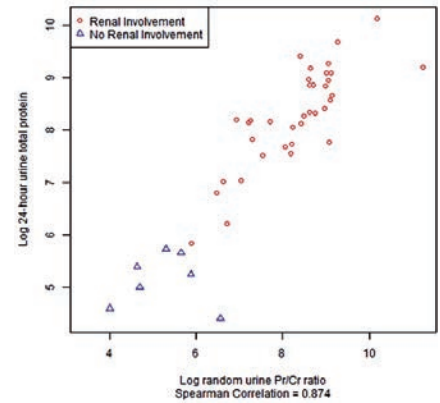


Figure 1. Scatter plot of 24-hour urine total proteins and random urine Pr/Cr ratio in patients with amyloidosis. Data was log-transformed for better display of the linear relationship between the two variables.

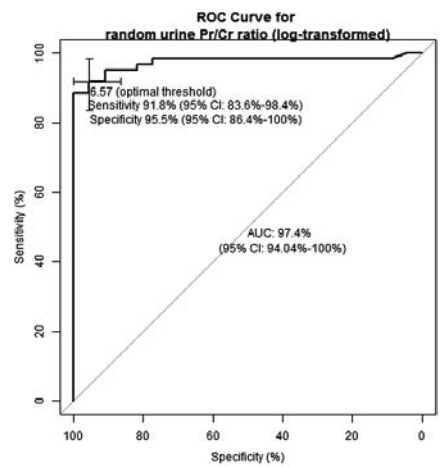


Figure 2. Optimal cut-off point of urine Pr/Cr ratio to predict renal involvement in amyloidosis. In the log-scale, 6.57 corresponds to about 715 mg/g of urine Pr/Cr ratio.

Table 1. Discriminant random urine protein/creatinine ratios that predict proteinuria at ≥ 500 , ≥ 1000 , ≥ 3500 , and ≥ 5000 mg/day.

24 hours urine total proteins (mg/day)	Discriminant random urine Pr/Cr ratio (mg/g)	Sensitivity (95% CI)	Specificity (95% CI)	Area under ROC curve (95% CI)
≥ 500	735	97.2% (91.7-100%)	100%	99.7% (98.7-100%)
≥ 1000	915	97.1% (88.2-100%)	100%	99.7% (98.9-100%)
≥ 3500	4105	86.4% (72.7-100%)	90.9% (77.3-100%)	91.5% (82.8-100%)
≥ 5000	5100	93.3% (80.0-100%)	86.2% (72.4-96.6%)	94.5% (88.4-100%)

Discussion

The screening for renal involvement in patients with amyloidosis is traditionally done by a collection of a 24-hour urine specimen.¹³ However, this test is cumbersome and often inaccurate. In this study, we analyzed the application of the Pr/Cr ratio in the random urine samples of patients with amyloidosis, because this test has been validated in other clinicopathologic conditions, it accurately reflect the 24-hour urine protein loss, and it is the method recommended by the KDOQI guidelines.³⁰ The Pr/Cr ratio is the preferred method to estimate proteinuria in veterinary medicine, because the 24-hour collection is impractical in animals. In fact, it has already been used to detect renal involvement in sheep with amyloidosis.³² In dogs, the 24-hour urine protein excretion, obtained placing the animals in metabolism cages, was found to be highly correlated with the urine Pr/Cr ratio in random samples ($r=0.97$).³³

The 24 hour collection is a commonly used test in the screening and monitoring of patients with MM, and it is recommended by the International Myeloma Working Group both at the time of diagnosis and periodically during follow-up, in order to assess response to treatment and monitor disease activity.^{34,35} In the last decade, the introduction of the quantitative serum free light chains (FLC) assay has diminished the importance of the 24-hour urine collection, because of excellent correlation between FLC and levels of Bence-Jones proteins.³⁶ However, the 24-hour urine collection cannot be replaced by the FLC assay in amyloidosis, because most proteins excreted in the urine consist of albumin and not Bence-Jones, FLCs levels can be misleading, and a normal serum FLC level does not rule out significant proteinuria.³⁷

We previously reported the use of the urine Pr/Cr ratio in patients with MM.³¹ In this study, we address the use of this test for the quantification of daily protein excretion in patients with amyloidosis. We believe that the Pr/Cr ratio could be used not only for the screening, but also for the monitoring of amyloid-associated proteinuria. Although organ damage is permanent in many patients with amyloidosis, the proteinuria can decrease during disease remission. This has been observed both in AL amyloidosis successfully treated with high-dose chemotherapy and stem cell transplantation,^{10,38} and in AA amyloidosis, after the underlying chronic inflammatory disorder becomes inactive.^{39,40}

Conclusions

Because of its accuracy and markedly increased convenience for patients and physi-

cians, the random Pr/Cr ratio can potentially replace the 24 hour urine collection. At least, it could be useful in patients who do not want or cannot provide a 24-hour urine collection, because of noncompliance, dementia, urine incontinence, or any other reason.

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