

# Twenty-Four-Hour Bence-Jones Protein Determinations

## Can We Ensure Accuracy?

Jennifer S. Kaplan, MD; Gary L. Horowitz, MD

• **Context.**—Light chain disease represents 15% to 20% of cases of multiple myeloma. Current guidelines recommend monitoring these patients with 24-hour urine collections.

**Objective.**—To determine the reliability of 24-hour urine collections in assessing the amount of Bence-Jones protein (BJP).

**Design.**—We included all patients from our institution from 2003 through 2008 with BJP who had more than four 24-hour urine collections. We compared BJP excretion calculated from the submitted 24-hour collection with BJP excretion calculated by normalizing the collection to that patient's mean 24-hour creatinine excretion. We also looked at differences in serial values with these 2 methods. In addition, we evaluated the feasibility of using random urine samples to determine BJP excretion.

**Results.**—A total of 14 patients with 135 24-hour urine collections met our inclusion criteria. The 24-hour urine

creatinine excretion for each patient, which should be reasonably constant, varied considerably (coefficient of variation range 12%–30%). Differences in the 2 methods of calculating BJP excretion ranged from –1588 to 2315 mg/d. Among a total of 121 serial 24-hour measurements, the differences were clinically significant in 37 (30%). Among a total of 23 random urine samples from 11 of these patients submitted within 10 days of a 24-hour collection, the estimated BJP excretion appeared to be accurate in at least 18 (78%).

**Conclusions.**—Twenty-four-hour urine collections for BJP are, in practice, often misleading. At a minimum, one should verify that the 24-hour creatinine excretion is accurate. In addition, it may be possible to use the protein/creatinine ratio from random urine samples to determine 24-hour BJP excretion.

(*Arch Pathol Lab Med.* 2011;135:1048–1051)

Current guidelines recommend that Bence-Jones proteinuria be monitored with 24-hour urine collections.<sup>1–3</sup> Implicit in this recommendation is that the collections are accurate. Without changes in lean body mass, 24-hour creatinine excretion should be relatively constant for each patient.<sup>4–6</sup> However, because of the known difficulties in collecting 24-hour urine samples, this is often not the case in practice.<sup>7</sup> Thus, we hypothesized that Bence-Jones protein (BJP) quantitation by this technique is often misleading and that, instead, one may be able to use random urine protein/creatinine ratios to provide better information.

### PATIENTS AND METHODS

#### Patient Selection

We retrieved laboratory data on all patients with Bence-Jones proteinuria who had more than four 24-hour urine collections analyzed at Beth Israel Deaconess Medical Center, Boston, Massachusetts, from 2003 through 2008. For each 24-hour collection, the data included urine volume, urine creatinine concentration (UCR), urine total protein concentration (UTP),

and proportion of BJP (%BJP). In addition, for these same patients, we found all other random urine samples on which UCR, UTP, and %BJP were reported. There were a total of 14 patients to evaluate, with 135 24-hour urine protein collections.

#### Methods

Studies were performed as a quality assurance/quality improvement effort. Patient data was identified using a hospital laboratory database to find all patients (2003–2008) with serial 24-hour BJP determinations. Urine total protein and creatinine were measured using standard spectrophotometric methods (total protein in urine/cerebrospinal fluid and creatinine Jaffé method on Hitachi 917 and P-Module analyzers, Roche Diagnostics, Indianapolis, Indiana). %BJP was determined by densitometry using HYDRASYS  $\beta$ 1- $\beta$ 2 protein electrophoresis (Sebia, Norcross, Georgia). All methods were run as specified by the respective manufacturers. All measurements were performed on fresh urine samples (whether random samples or 24-hour collections), most within 24 hours of collection but all within 72 hours of collection, stored at 4°C while awaiting analysis.<sup>2</sup>

#### Analysis

Twenty-four-hour BJP excretion was calculated in 3 different ways:

1. Submitted BJP (mg) = %BJP  $\times$  UTP (mg/dL)  $\times$  24-hour urine volume (dL)
2. Normalized BJP (mg) = %BJP  $\times$  UTP (mg/dL)/UCR (mg/dL)  $\times$  patient's mean 24-hour creatinine excretion (mg)
3. Estimated BJP (mg) = %BJP  $\times$  UTP (mg/dL)/UCR (mg/dL)  $\times$  patient's weight-based expected 24-hour creatinine excretion (mg)

Accepted for publication December 2, 2010.

From the Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts.

The authors have no relevant financial interest in the products or companies described in this article.

Presented as a poster session at the annual meeting of the American Association of Clinical Chemistry, Chicago, Illinois, July 22, 2009.

Reprints: Gary L. Horowitz, MD, Beth Israel Deaconess Medical Center, Pathology YA-309, 330 Brookline Ave, Boston, MA 02215 (e-mail: ghorowit@bidmc.harvard.edu).

### Summary of 24-Hour Urine Collections

Case No.	No. of Collections	Minimum Creatinine, mg/d	Maximum Creatinine, mg/d	Mean Creatinine, mg/d	CV, % <sup>a</sup>	Weight-Based Creatinine Excretion, mg/d <sup>b</sup>	Mean/Weight-Based Creatinine Excretion, %	Maximum Difference BJP Excretion <sup>c</sup>	Correlation Coefficient <sup>d</sup>
1	8	476	1416	1023	30	1249	82	-1184	0.89
2	9	1410	2121	1762	12	2554	69	-443	0.99
3	6	860	1352	1109	18	1563	71	-167	0.83
4	7	1113	1560	1369	12	1672	82	-208	0.72
5	8	880	2158	1553	29	1745	89	404	0.43
6	11	1131	1833	1550	13	1545	100	-509	0.69
7	5	1560	2024	1740	12	2000	87	89	0.59
8	5	458	884	616	29	695	89	-305	0.52
9	24	646	2125	1558	22	1954	80	-603	0.75
10	12	432	885	545	24	702	78	-1588	0.90
11	10	1116	1718	1434	15	1909	75	181	0.84
12	5	770	1172	941	16	859	110	294	0.98
13	6	1275	2184	1744	18	2055	85	2315	0.96
14	19	1191	3003	1929	22	2645	73	315	0.74

Abbreviations: BJP, Bence-Jones protein; CV, coefficient of variation.

SI conversion factor: To convert creatinine from milligrams to millimoles, multiply by 0.00884.

<sup>a</sup> The CV represents the variability in the observed 24-hour creatinine excretions, which in each of these patients should be relatively consistent (CV close to 0).

<sup>b</sup> Creatinine excretion per day calculated using lean body mass (15 mg/kg for women and 20 mg/kg for men).<sup>6</sup>

<sup>c</sup> For each patient, the maximum difference between BJP excretion based on the submitted collection and normalized to the patient's mean creatinine excretion.

<sup>d</sup> The correlation coefficient here represents the relationship between (submitted BJP – normalized BJP) and (submitted 24-hour creatinine – mean 24-hour creatinine). A value of 1.0 indicates that the change in BJP was completely related to changes in sample collection; a value of 0 indicates that the change in BJP was related entirely to other factors (eg, a genuine change in tumor burden).

For expected 24-hour creatinine excretion, we used the following mean values and lean body mass: 15 mg/kg for women and 20 mg/kg for men (to convert from mg/kg to mmol/kg, multiply by 0.00884).<sup>6</sup>

### RESULTS

As shown in the Table, there was considerable variability in the 24-hour urine creatinine collections in these 14 patients, with coefficients of variation ranging from 12% to 30%.

For each patient, we calculated a mean 24-hour creatinine excretion, which we thought might represent the best estimate of the patient's true 24-hour creatinine excretion, and a predicted 24-hour creatinine excretion based on that patient's gender and weight. As shown in the Table, in only 1 case were the values within 10% of each other; in most cases, the mean 24-hour collection was lower than predicted.

For each of the 135 samples, we calculated submitted BJP and normalized BJP. As shown in the Table, the differences in these values ranged from -1588 to 2315 mg/d (to convert from mg/d to g/d, multiply by 0.001). For each patient, we determined the correlation between these differences in 24-hour BJP and the differences in the submitted versus mean 24-hour creatinine excretion; the values ranged from 0.43 to 0.99.

We then evaluated differences in serial 24-hour BJP excretion (mg/d) by each method. We initially defined clinically significant differences as values with opposite signs (i.e., an increase by one method but a decrease by the other) or values whose magnitude was at least 2-fold different and >100 mg/d. With these criteria, of 121 events, 44 were clinically significant. We lowered this number to 37 (30% of the 121 events) by inspecting the data in the context of the patients' overall levels of 24-hour

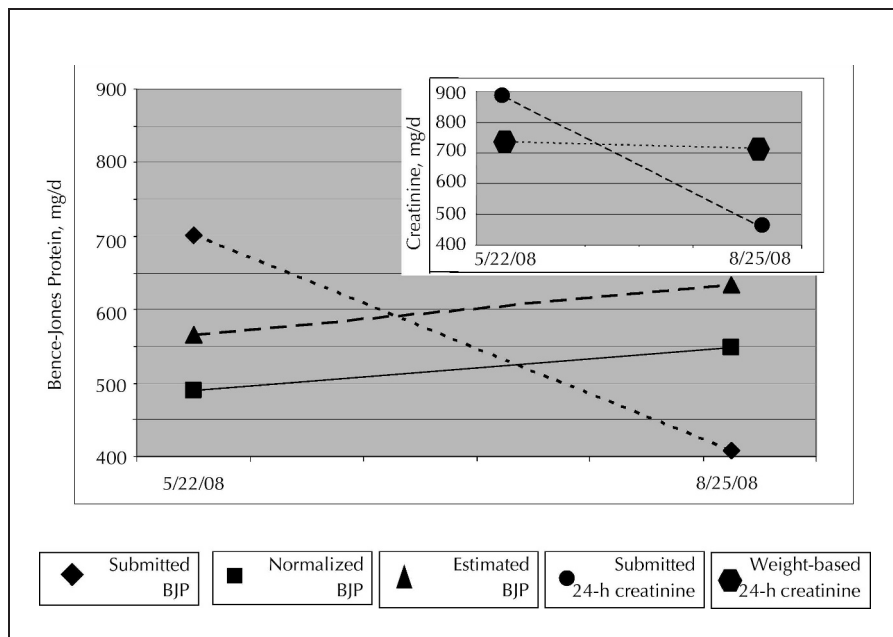
BJP and clinical scenario. A specific example is illustrated in the Figure. Three patients had no clinically significant differences; among the other 11 patients, the proportion of samples with clinically significant differences ranged from 17% to 80%.

To assess the feasibility of using random urine samples to estimate 24-hour BJP excretion, we used BJP data from random urine samples collected no more than 10 days from a 24-hour urine collection. In all, there were 23 such samples from 11 different patients. In 18 of these 23 cases (78%), the random sample gave estimated BJP results that were in excellent agreement with the clinical scenario and normalized BJP on the corresponding 24-hour sample. We defined excellent agreement in 2 ways: values whose protein to creatinine ratios were within 0.2 (10 cases) or values different by more than 0.2 but consistent with improvement or deterioration as described in clinical notes or reflected in independent laboratory data (8 cases). In the remaining 5 cases, the random samples were probably accurate, but we did not have access to enough clinical data to be certain.

### COMMENT

Multiple myeloma is a plasma cell dyscrasia, representing 1% of all cancers and slightly more than 10% of all hematologic malignancies in the United States.<sup>8</sup> The incidence is greater than 20 000 cases per year in the United States, and it leads to an estimated 10 580 deaths per year.<sup>8</sup> A significant minority of patients (15%–20%)<sup>9,10</sup> have light chain multiple myeloma, a form of the disease in which only immunoglobulin light chains are produced. The vast majority of these patients excrete these monoclonal light chains in the urine; this has traditionally been termed Bence-Jones proteinuria.<sup>11,12</sup> The College of American Pathologists' guidelines for detecting and quantifying

Between the serial collections from this patient on May 22, 2008, and August 25, 2008, the submitted Bence-Jones protein excretion decreased substantially (from 702 to 408 mg/d), but the normalized BJP excretion increased slightly (from 489 to 548 mg/d), as did the estimated BJP excretion (from 566 to 633 mg/d). As shown in the inset this is because the 24-hour creatinine excretion decreased from 884 to 459 mg, despite the fact that the patient's weight-based expected 24-hour creatinine excretion did not change appreciably (from 736 to 714 mg).



BJP are based on a 24-hour urine specimen.<sup>1-3</sup> More specifically, the guidelines suggest measurement of total 24-hour protein, electrophoresis and immunofixation of concentrated urine to detect BJP, and densitometric determination of the ratio of the BJP peak to the total protein.<sup>3</sup>

It is well known, however, that 24-hour urine collections can be quite inconsistent.<sup>13</sup> In the absence of significant changes in lean body mass, 24-hour creatinine excretion should be reasonably constant in any given patient.<sup>4-7</sup> Thus, the total 24-hour creatinine excretion serves as a good indicator of the reliability of the 24-hour collection. In our study, where weight changes were minimal, the variability of this parameter was considerable, with coefficients of variation ranging from 12% to 30%.

We then sought to correct for these apparent sample collection errors in 2 different ways: using the mean of each patient's 24-hour collections and using a gender- and weight-based prediction equation. Our results showed that the mean values were, in general, significantly less than the predicted values. Whether this represents consistent undercollection of 24-hour urine samples or limitations in the weight-based formula is not clear.

By calculating 24-hour BJP excretion in 2 different ways (as submitted versus normalized to the mean 24-hour creatinine value for each patient), we sought to determine how much of the variability in that parameter could be explained simply by inconsistencies in the collection of the 24-hour urine. Our results indicate that the differences in magnitude of 24-hour BJP by these 2 methods can be considerable (-1588 to 2315 mg/d). Furthermore, there was a strong correlation between these differences and the differences in the 24-hour creatinine values. In other words, differences in 24-hour BJP excretion might not be related to changes in the underlying disease but rather to inaccuracies in the collection process.

It has been demonstrated that the protein to creatinine ratio on random urine samples can function as a screen for proteinuria in certain clinical contexts, and several studies suggest random urine samples could potentially replace

the cumbersome 24-hour collections.<sup>14,15</sup> We could find only 1 article in the literature that addressed detection and monitoring of Bence-Jones proteinuria in random versus 24-hour collections.<sup>16</sup> This study did not address whether random urine samples could be used to reliably estimate 24-hour BJP excretion. Recently, Abraham et al<sup>9</sup> suggested that serum free light chain assays might replace 24-hour urine collections for BJP, stating that, in individual patients, serum free light chain concentrations "correlate linearly on a log-log scale with changes" in the latter. Subsequent studies on this finding have come to conflicting conclusions.<sup>17,18</sup>

In the present study, although we were not able to compare individual random urine samples with the same 24-hour urine collection, we were able to find 23 random urine samples collected within a 10-day window of a 24-hour urine collection. The 24-hour BJP excretion estimated from these samples was in excellent agreement with the normalized value in at least 18 (78%) of the cases; in no case was the estimate clearly incorrect.

Our data validate that 24-hour collections are inconsistent in practice and can lead to mistakes in quantifying BJP. At a minimum, using a weight-based or mean 24-hour creatinine excretion for each patient to calculate BJP excretion may be a better way to quantify BJP than using the unreliable 24-hour collections. Furthermore, our limited data suggest that a random urine sample for protein to creatinine ratio, which would be a simpler method for both the patient and the laboratory, may be just as accurate as, if not better than, a 24-hour sample. This possibility should be verified in patients by calculating BJP through both random urine quantifications and 24-hour urine collection.

## CONCLUSION

Variability in 24-hour urine collections is not infrequent and can cause clinically significant errors in estimates of 24-hour BJP excretion. At a minimum, one should verify accuracy of 24-hour urine collection by checking 24-hour creatinine excretion before using it to estimate 24-hour BJP excretion in the traditional way. It appears likely that one can

use protein to creatinine ratios from random urine samples to estimate 24-hour BJP excretion. Although there is no evidence to suggest variations in BJP excretion, validation of constant protein to creatinine ratios in random urine samples throughout 24-hour periods in patients with BJP would confirm the accuracy of using any random sample.

#### References

1. Graziani M, Merlini G, Petrini C. Guidelines for analysis of Bence Jones protein. *Clin Chem Lab Med.* 2003;41(3):338–346.
2. Kyle RA. Sequence of testing for monoclonal gammopathies: serum and urine assays. *Arch Pathol Lab Med.* 1999;123(2):114–118.
3. Keren DF. Procedures for the evaluation of monoclonal immunoglobulins. *Arch Pathol Lab Med.* 1999;123(2):126–132.
4. Forbes GB, Bruining GJ. Urinary creatinine excretion and lean body mass. *Am J Clin Nutr.* 1976;29(12): 1359–1366.
5. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem.* 1992;38(10):1933–1953.
6. Lamb EJ, Price CP. Creatinine, urea, and uric acid. In: Burtis CA, Ashwood ER, Bruns DR, eds. *Tietz Fundamentals of Clinical Chemistry.* St. Louis, MO: Saunders; 2008:363–372.
7. Côté A-M, Firoz T, Mattman A, Lam EM, von Dadelszen P, Magee LA. The 24-hour urine collection: gold standard of historical practice. *Am J Obstet Gynecol.* 2008;199(6):625.e1–625.e6
8. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin.* 2009;59(4):225–249.
9. Abraham RS, Clark RJ, Bryant SC, et al. Correlation of serum immunoglobulin free light chain quantification with urinary Bence Jones protein in light chain myeloma. *Clin Chem.* 2002;48(4): 655–657.
10. Magrangeas F, Cormier M-L, Descamps G, et al. Light-chain only multiple myeloma is due to the absence of functional (productive) rearrangement of the IgH gene at the DNA level. *Blood.* 2004;103(10): 3869–3875.
11. Jones HB. On a new substance occurring in the urine of a patient with mollifies ossium. *Philos Trans R Soc Lond.* 1848;188:55.
12. Edelman GM, Gally JA. The nature of Bence-Jones proteins: chemical similarities to polypeptide chains of myeloma globulins and normal gamma-globulins. *J Exp Med.* 1962;116(2):207–227.
13. Price CP, Newall RG, Boyd JC. Use of protein:creatinine ratio measurements on random urine samples for prediction of significant proteinuria: a systematic review. *Clin Chem.* 2005;51(9):1577–1586.
14. Ginsberg JM, Chang BS, Matarese RA, Garella S. Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med.* 1983;309(25): 1543–1546.
15. Lemann J, Doumas BT. Proteinuria in health and disease assessed by measuring the urinary protein/creatinine ratio. *Clin Chem.* 1987;33(2):297–299.
16. Brigden ML, Neal ED, McNeely MD, Hoag GN. The optimum urine collections for the detection and monitoring of Bence Jones proteinuria. *Am J Clin Pathol.* 1990;93(5):689–693.
17. Bradwell AR, Carr-Smith HD, Mead GP, Harvey TC, Drayson MT. Serum test for assessment of patients with Bence Jones myeloma. *Lancet.* 2003; 361(9536):489–491.
18. Tate JR, Mollee P, Dimeski G, Carter AC, Gill D. Analytical performance of serum free light-chain assay during monitoring of patients with monoclonal light-chain diseases. *Clin Chim Acta.* 2007;376(1–2): 30–36.