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Quantification of Urinary Light Chains

To the Editor:

Monoclonal gammopathies are usually monitored with serum and/or urine protein electrophoresis (PEL) (1). In addition, quantitative immunoglobulins are often ordered for patients with large serum M-spike. For patients with monoclonal light-chain diseases, diagnosis and monitoring can be a challenge, and free light-chain (FLC) quantification in serum has become an important additional test (2). Although there have been some conflicting reports, serum FLC should not replace urine PEL for monitoring patients with a urine M-spike (3–5). The purpose of this study was to determine if measuring urine FLCs or urine total light chains (TLCs) is useful in addition to measuring urine PEL for monitoring patients, in a manner analogous to measuring quantitative immunoglobulin as a complement to measuring serum PEL.

Sequential waste urines (n = 336) were obtained from excess samples in which a monoclonal protein was detected by urine immunofixation electrophoresis (IFE). We performed PEL assays with agarose gel electrophoresis (REP, Helena Laboratories) after we increased the protein concentration in urine samples up to 200-fold to achieve a protein concentration of 20–80 g/L. IFE assays were performed with Helena reagent sets. The FLCs and TLCs were quantified on a Dade Behring BN II nephelometer, with separate antisera for \( \kappa \) and \( \lambda \) FLCs (The Binding Site) and \( \kappa \) and \( \lambda \) TLCs (Dade Behring). The limits of quantification of the urine TLC \( \kappa \) and \( \lambda \) assays are 7 and 4 mg/L, respectively, and 1 mg/L for both \( \kappa \) and \( \lambda \) FLC. The reference range for the urine TLC \( \kappa:\lambda \) ratio was based on urine samples obtained from 54 healthy adult donors. These samples had total protein >100 mg/24 h, and we excluded 1 donor because of a highly increased TLC \( \kappa:\lambda \) ratio. The reference range for the urine FLC \( \kappa:\lambda \) ratio was based on urine samples from 91 healthy adult donors, and was the central 95% range.

Urine monoclonal protein was detected with IFE in all urine samples. The limits of quantification for both serum FLC and TLC were determined by OE. The FLCs and TLCs were quantified on the Dade Behring BN II nephelometer, with separate antisera for \( \kappa \) and \( \lambda \) FLCs (The Binding Site) and \( \kappa \) and \( \lambda \) TLCs (Dade Behring). The limits of quantification of the urine TLC \( \kappa \) and \( \lambda \) assays are 7 and 4 mg/L, respectively, and 1 mg/L for both \( \kappa \) and \( \lambda \) FLC. The reference range for the urine TLC \( \kappa:\lambda \) ratio was based on urine samples obtained from 54 healthy adult donors. These samples had total protein >100 mg/24 h, and we excluded 1 donor because of a highly increased TLC \( \kappa:\lambda \) ratio. The reference range for the urine FLC \( \kappa:\lambda \) ratio was based on urine samples from 91 healthy adult donors, and was the central 95% range.

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samples from the patient cohort; 64% of the samples had κ light chains and 36% had λ light chains. In the κ group, 132 patients (62%) had monoclonal protein quantifiable by urine PEL; an M-spike was observed in 74 (61%) of the patients with λ light chains. M-spike values were 10–8600 mg/L. To determine if quantification of the urinary FLC and TLC could aid in diagnosing monoclonal gammopathies, we determined the diagnostic sensitivities of the FLC and TLC κ:λ ratios (Table 1). The diagnostic sensitivities of the urine FLC and TLC κ:λ ratios were 80% and 70%, respectively, with no differences between κ and λ isotypes. The diagnostic sensitivity was 93% to 100% in patients with an M-spike but decreased substantially in patients with no quantifiable M-spike. Although large M-spike (>1 g/24 h) are usually associated with malignant disease, a small nonquantifiable M-spike in a patient with a monoclonal gammapathy does not rule out important clinical diseases such as multiple myeloma or primary amyloidosis. Therefore quantitative urine FLC and TLC assays do not have a role in diagnostic testing. The relatively low diagnostic sensitivity of the urine FLC assay is presumably due to the background of polyclonal FLC in the urine and the wide reference ranges for urine FLC assays. The serum FLC κ:λ ratio reference range, for example, is 0.26–1.65. The urine FLC κ:λ ratio reference range established in this study is 1–19, and the reference range has been reported elsewhere as 0.46–4.0 (2). With the use of this narrower published reference range, the diagnostic sensitivity of the urine FLC κ:λ ratio increased from 80% to 90%, but the diagnostic specificity decreased to 27%.

To determine if urine FLC or TLC may be useful to assist monitoring patients, we used linear regression to assess the relationships of light chain concentration with the quantitation of urine PEL M-spike. The correlation coefficients for the urine TLC vs the M-spike were 0.95 and 0.98 for κ and λ, respectively, and 0.90 and 0.98 for the FLC assays (Table 1). The slopes of the κ and λ TLC vs M-spike linear regression results were 0.40 and 0.60, and the slopes for κ and λ FLC were 0.07 and 0.15. These differences indicate that the standardization of these 3 assays is substantially different and reinforces the need for a traceable calibrator for the FLC assay.

Urine FLC and TLC are not sensitive diagnostic tests. Urine FLC and TLC, however, do correlate with the urine PEL M-spike, with the urine TLC being in closer agreement to the PEL M-spike. The quantification of urine TLC may therefore provide a useful quality check on measurements of patient urinary M-spike values. Like serum immunoglobulin quantification, the urine TLC assays may provide redundancy in disease monitoring by M-spike measurement.

Table 1. Diagnostic sensitivity and quantitative comparison of urine FLC and TLC assays.

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<thead>
<tr>
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<th>FLC assay*</th>
<th>TLC assay*</th>
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<tbody>
<tr>
<td>Diagnostic sensitivity of κ:λ ratio (95% CI)</td>
<td></td>
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<tr>
<td>All samples (n = 336)</td>
<td>80% (75.8%, 84.3%)</td>
<td>70% (64.8%, 74.6%)</td>
</tr>
<tr>
<td>κ Immunotype (n = 214)</td>
<td>80% (74.5%, 85.1%)</td>
<td>67% (60.3%, 72.8%)</td>
</tr>
<tr>
<td>Positive for M-spike (n = 132)</td>
<td>95% (89.5%, 97.4%)</td>
<td>93% (88.3%, 95.7%)</td>
</tr>
<tr>
<td>Negative for M-spike (n = 82)</td>
<td>57% (46.5%, 67.5%)</td>
<td>24% (18.0%, 33.1%)</td>
</tr>
<tr>
<td>λ Immunotype (n = 122)</td>
<td>81% (73.3%, 87.1%)</td>
<td>75% (67.1%, 82.2%)</td>
</tr>
<tr>
<td>Positive for M-spike (n = 74)</td>
<td>100% (95.1%, 100%)</td>
<td>97% (90.7%, 99.3%)</td>
</tr>
<tr>
<td>Negative for M-spike (n = 48)</td>
<td>55% (38.3%, 65.5%)</td>
<td>43% (28.8%, 55.7%)</td>
</tr>
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</table>

Quantitation comparison to M-Spike

<table>
<thead>
<tr>
<th></th>
<th>FLC assay*</th>
<th>TLC assay*</th>
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<tbody>
<tr>
<td>κ Immunotype (n = 132)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient, R</td>
<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td>Linear regression, slope (SE)</td>
<td>0.07 (0.003)</td>
<td>0.40 (0.011)</td>
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<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>λ Immunotype (n = 74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient, R</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Linear regression, slope (SE)</td>
<td>0.15 (0.004)</td>
<td>0.60 (0.015)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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* Reference range for urine FLC κ:λ ratio: 1–19.
* Reference range for urine TLC κ:λ ratio: 0.7–6.2

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Letters to the Editor

Precision of High-Throughput Single-Nucleotide Polymorphism Genotyping with Fingernail DNA: Comparison with Blood DNA

To the Editor:

Recently, high-throughput single-nucleotide polymorphism (SNP) genotyping arrays have been used for genome-wide association study. Blood samples are commonly used for such analyses because they provide genomic DNA of high molecular weight and in high quantities. Blood sampling is often difficult, however, when patients are located a great distance from the laboratory and the sample requires careful handling for transportation. Instead, buccal swabs or urinary epithelial cells have been used as noninvasive sources of DNA (1). Fingernail clippings are also obtained noninvasively and more easily. Such samples need no special attention for transportation and can be preserved dry at room temperature for long periods; however, because fingernail clippings yield fragmented DNA that may be contaminated with certain PCR inhibitors (2), this sample type has seemed unsuitable for analyses requiring high-quality genomic DNA. In this study, we compared the precision of Affymetrix GeneChip™ array-based SNP genotyping with fingernail DNA vs blood DNA and demonstrated the usefulness of fingernail DNA in genotyping and genome copy-number analysis.

The study protocol was approved by the Ethics Committee on Human Genome and Gene Analysis, Nagasaki University, and written informed consent was obtained from every volunteer. Ten milliliters of peripheral blood and 10 fingernail clippings were obtained from 5 healthy volunteers. Blood DNA was extracted with the standard phenolchloroform method. Clipped fingernails were frozen in liquid nitrogen and crushed into fine powder with Multibead Shocker™ (Yasui Kikai). The nail powder was dissolved in a urea-containing lysis solution (2 mol/L urea, 5 g/L SDS, 10 mmol/L Tris-HCl, pH 7.5, 50 mmol/L EDTA) containing 1 g/L proteinase K and 40 mmol/L dithiothreitol and was incubated overnight at 55°C. Nail DNA was extracted by the phenol-chloroform method and suspended in 30 μL 1× Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0). Genome-wide SNP genotyping was performed with the GeneChip™ Human Mapping 250K Nsp Array according to the manufacturer’s assay manual (Affymetrix). SNP data were analyzed with GTYPE software (Affymetrix). This analysis is based on the dynamic modeling algorithm that is included in GTYPE. Genome copy-number analysis was performed with CNAG software (http://www.genome.umin.jp/) (3, 4).

The human nail plate includes soft and hard types of keratin. Hard keratin constitutes >80% of nail keratin (5) and contains large numbers of cysteine moieties linked by

References

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