values <15.0 kIU/L, and 1 for anti-protein C IgM (28.4 kIU/L; normal values <18 kIU/L) autoantibodies, and 2 were negative for all anti-phospholipid antibodies investigated by the ELISA method. Thus, the new SCT assay was positive in 87% of those who had a positive result by our LA test and was negative in 93% of those whose results were negative by our LA assay.

All of these patients had histories of thromboembolic disease (4 with venous thrombosis and 2 with arterial thrombosis). These findings can suggest, at least in some patients, a role of SCT as an independent risk factor for thrombosis. Furthermore, the presence of SCT positivity in patients with thromboembolic events reduces, at least in part, the number of patients who are otherwise seronegative for anti-phospholipid autoantibodies. More data and prospective studies are needed to confirm this hypothesis.

References


Immuonochemical Quantification of Free Light Chains in Urine, Ileana Herzum,* Hartald Renz, and Hans Günther Wahl (Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Marburg, Germany;* address correspondence to this author at: Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, 35033 Marburg, Germany; e-mail herzumi@med.uni-marburg.de)

Quantitative measurements of plasma and urinary paraprotein concentrations play a major role in the monitoring of patients with multiple myeloma. The concentrations are routinely estimated from the size of the M-spike on protein electrophoresis (PEL) or by automated immunologic assays for IgG, IgA, IgM, IgE, or IgD. In the case of light chain myeloma and intact immunoglobulin myeloma with predominant light chain production, light chain concentrations could, until recently, be measured only by the size of the urinary light chain M-spike on PEL or by the measurement of the total (free and bound) light chain concentrations.

A latex-enhanced assay (Freelite; The Binding Site, Ltd.) measuring free light chains (FLCs) in serum and urine has recently become available for the BNII (Dade Behring) analyzer. The Myeloma Management Guidelines (1) recommend the Freelite test for serial monitoring of the FLCs in serum, but periodic 24-h urine collection is still required for Bence Jones proteinuria (BJP) and total urinary protein (TUP) quantification. Depending on the glomerular and tubular function, serum and urine FLC concentrations may not change to the same degree (2), so that monitoring of serum FLC alone is questionable for revealing the actual degree of disease in patients with BJP and tubular dysfunction.

We evaluated the analytical performance of the immunochemical test for serum and urine with the BNII analyzer. The test uses antibodies that specifically recognize an epitope of the common region of κ and λ light chains that is “hidden” when the light chains are attached to the immunoglobulin heavy chain (3).

Intra- (within) and interassay (day-to-day total) imprecision (CV) was determined with control material and with patient samples containing high and low concentrations of polyclonal or monoclonal FLCs (Table 1). The high CV observed for the serum sample with a high monoclonal λ FLC concentration may reflect the variable

![SCT confirm ratios in individual patients.](image-url)
degree of polymerization, which is common at high FLC concentrations. This phenomenon has been described previously, most notably for \( \lambda \) FLCs (4). The linearity of urine samples from patients with BJP, evaluated as the correlation coefficient of the linear regression line of the measured vs expected values in serial linear dilutions, was good for \( \kappa \) FLC (9 dilutions; range, 118–865 mg/L; slope, 0.885; intercept, 81 mg/L; \( r = 0.90 \)) and \( \lambda \) FLC (17 dilutions; range, 17–14 900 mg/L; \( r = 0.97 \)). Linearity of TUP measured simultaneously by the benzethonium chloride method (Roche Diagnostics) was very good for the sample with \( \kappa \) FLC (9 dilutions; range, 0.03–0.58 g/L; slope, 1.0145; intercept, \(-0.06 \text{ g/L}; r = 0.995 \)) and \( \lambda \) FLC (17 dilutions; range, 0–3.01 g/L; slope, 0.945; intercept, \(-0.01 \text{ g/L}; r = 0.96 \)). The linearity of serum samples was also determined for both FLCs: \( \kappa \) (9 dilutions; range, 88–984 mg/L; slope, 1.0754; intercept, 151.4 mg/L; \( r = 0.9984 \)) and \( \lambda \) (8 dilutions; range, 384–6600 mg/L; slope, 1.022; intercept, \(-50.29 \text{ mg/L}; r = 0.9912 \)). Because we observed extremely high concentrations of FLCs, much higher than the TUP, in some urine samples with BJP, we studied the reliability of the Freelite test in urine.

We measured FLCs and TUP in 105 urine samples (87 patients) on which immunofixation electrophoresis (IFE; NEBIA) had been performed. We measured TUP by the benzethonium chloride and biuret method with the Hitachi 917 analyzer and by the modified biuret and pyrocatechol violet dry-chemistry method with the Vitros 250 analyzer (Johnson & Johnson). Urine IFE showed 63 samples with monoclonal bands; 20 were \( \kappa \) FLC, 15 were \( \lambda \) FLC, 21 were intact immunoglobulins plus \( \lambda \) (n = 10) or \( \kappa \) (n = 11) FLCs, and 7 were intact immunoglobulins without FLC.

The FLC concentration ranges were 1–4800 mg/L for \( \kappa \) and 1–14 200 mg/L for \( \lambda \). The lowest FLC concentration with an associated monoclonal band by IFE was 4 mg/L. Considering a \( \kappa/\lambda \) ratio outside the interval 1.271–1:0.25 (3) to be abnormal, we identified the FLC type of the BJP, as shown by the monoclonal bands in the urine IFE, with a sensitivity of 87% and a specificity of 53%.

We determined the imprecision (CV) of the TUP methods, using the same urine sample with \( \lambda \) BJP. The CVs were 7.2% (0.06 g/L) for the benzethonium chloride, 12% (0.56 g/L) for the biuret, 4.2% (0.48 g/L) for the modified biuret, and 6.7% (0.05 g/L) for the pyrocatechol violet method. The total urinary FLC concentration exceeded the benzethonium chloride TUP in 54 of 105 cases, the biuret TUP in 26 of 105 cases, the modified biuret TUP in 17 of 105 cases, and the pyrocatechol violet TUP in 46 of 105 cases, with maximum differences of 11, 9.2, 7.8, and 14 g/L, respectively.

To assess recovery of FLC by TUP methods, we measured FLC and TUP in a normal urine sample without bands by IFE (\( \kappa = 17.60 \text{ mg/L}; \lambda = 7.44 \text{ mg/L} \)) to which we had added purified \( \kappa \) and \( \lambda \) light chains. The solutions of purified material were provided and quantified by radial immunodiffusion by The Binding Site. The final concentrations of \( \kappa \) and \( \lambda \) FLCs added were 1240 and 930 mg/L, respectively. The linearity for the urine sample with added FLCs, evaluated as the linear regression line of the measured vs expected values in serial linear dilutions, was good for both \( \kappa \) (5 dilutions; range, 816–9530 mg/L; \( R = 0.945 \)) and \( \lambda \) (6 dilutions; range, 834–1600 mg/L; \( R = 0.9441 \)). TUP measurements of the samples showed good linearity for all methods. Recovery of the purified FLCs, however, differed among the 4 TUP methods. For \( \kappa \), the TUP results were 0.12, 3.9, 2.39, and 0.39 g/L for the benzethonium chloride, biuret, modified biuret, and pyrocatechol violet, respectively, and for \( \lambda \), the TUP results were 0.61, 3.2, 2.65, and 0.57 g/L, respectively.

Previous authors have emphasized the difficulty of measuring clones of FLCs, as their structures are heterogeneous and can be modified through pH, polymerization, and oligomerization (5–8). Both of the routinely used methods for monitoring BJP, urine PEL and TUP, are unspecific for FLCs and have several drawbacks. Urine PEL is time-consuming and insensitive, requires previous concentration, and is subject to interference from other small urinary proteins in a tubular proteinuria pattern, which frequently occurs in such patients (9–11). The TUP methods show variable, partial recovery of the FLCs (12, 13). The Freelite assay provides specific quantification of BJP and has acceptable analytical performance.

### Table 1. Imprecision of the Freelite assay on the BN II analyzer.

<table>
<thead>
<tr>
<th>FLC</th>
<th>Intraassay imprecision</th>
<th>Interassay imprecision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 10)</td>
<td>3.3% (20 mg/L)</td>
<td>2.7% (20 mg/L)</td>
</tr>
<tr>
<td></td>
<td>6.1% (42 mg/L)</td>
<td>6.1% (42 mg/L)</td>
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<tr>
<td>Patient samples</td>
<td></td>
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<tr>
<td>Polyclonal FLCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (n = 10)</td>
<td>2.8% (95 mg/L)</td>
<td>2.0% (93 mg/L)</td>
</tr>
<tr>
<td>Urine (n = 10)</td>
<td>6.0% (90 mg/L)</td>
<td>2.1% (37 mg/L)</td>
</tr>
<tr>
<td>Monoclonal FLCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (n = 10)</td>
<td>2.7% (121 mg/L)</td>
<td>13% (1090 mg/L)</td>
</tr>
<tr>
<td>Urine (n = 10)</td>
<td>4.3% (172 mg/L)</td>
<td>8.0% (4490 mg/L)</td>
</tr>
<tr>
<td></td>
<td>4.3% (15 mg/L)</td>
<td>4.4% (31 mg/L)</td>
</tr>
<tr>
<td></td>
<td>9.2% (1.7 mg/L)</td>
<td>12% (1.2 mg/L)</td>
</tr>
</tbody>
</table>
Because the diagnostic performance is poor, monoclonality needs to be confirmed by IFE (14).

We conclude that monitoring of renal involvement and BJP in patients with FLC myeloma can be improved by measuring both TUP and FLC in urine. Monitoring of the TUP concentration should be performed with the same assay.

We thank I. Pietrek and R. Heinz for excellent technical support and The Binding Site, Ltd., for the purified light chains.

References


DOI: 10.1373/clinchem.2004.045435

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The cardiac hormone B-type natriuretic peptide (BNP) is synthesized in myocytes as a prepro 134-amino acid residue molecule. The 108-residue proBNP mature form of the hormone is proteolytically cleaved to a biologically active form of 32 amino acids (residues 77–108) and an N-terminal fragment (residues 1–76; NT-proBNP) with an as yet undefined biological function (1). Clinically, both BNP and NT-proBNP have shown great promise as secreted, bloodborne diagnostic markers of left ventricle dysfunction. Measurement of each is based on immunoassays; it therefore is likely that changes in the molecular form, e.g., posttranslational modifications, further proteolytic processing, and an oligomeric state for either analyte, could affect their measurements (2–5). These types of confounding molecular issues are likely for many analytes, with the myocyte damage marker cardiac troponin I one of the better studied. In this case, the commercial assays use antibodies directed to different epitopes, making “universal” calibration and determination of absolute analyte concentration difficult (6).

A previous report has indicated that NT-proBNP exists as a coiled-coil trimer, based on size-exclusion HPLC (SE-HPLC) of human, plasma-extracted material and a computer algorithm that predicts coiled-coils (7). I reinvestigated this claim on synthetic NT-proBNP, using the physicochemical techniques of analytical sedimentation, equilibrium ultra centrifugation, and circular dichroism (CD), and demonstrate that NT-proBNP is a monomer and not a trimer.

NT-proBNP was produced by solid-phase peptide synthesis (AnaSpec, Inc.) and obtained as a gift from DadeBehring (Newark, DE). I used N-terminal sequencing and mass spectrometry as quality assurance procedures. Edman sequencing (8) was performed by Midwest Analytical, Inc. on 2 Coomassie-stained Immobilon-PSQ (Sigma) NT-proBNP–containing membrane sections. The first 52 residues were positively identified, with no preview, before the signal was not discernable from background (data not shown). Matrix-assisted laser desorption/ionization mass spectrometry (8) gave an experimental mass of 8457.0 compared with a calculated value of 8457.6 (data not shown). Lyophilized NT-proBNP was dissolved in and exhaustively dialyzed vs phosphate-buffered saline (pH 7.2) at 4 °C. Analyte concentration was estimated gravimetrically and based on a molar absorptivity at 280 nm (ε280) of 0.82 L·g⁻¹·cm⁻¹; the 2 different techniques yielded better than 95% agreement.

The synthetic peptide in a neutral pH physiologic salt solution was run at 0.5 mL/min on SE-HPLC, and the resulting elution profile is plotted in Fig. 1A as A214nm vs time in minutes. The chromatographic process is monitored at 214 nm, which measures “peptide bond” absorbance; there thus is no bias in analyte detection under these analysis conditions. This is in contrast to the antibody-based, postcolumn analysis of plasma-extracted peptide (7), where detection is strictly a function of antibody reactivity. Furthermore, it is unclear what effect, if any, the C18 solid-phase plasma extraction procedure used in that study has on the molecular state of NT-proBNP before chromatography. Fig. 1A shows NT-proBNP eluting well before cytochrome C (12.4 kDa) and