Response of Bone Turnover Biochemical Markers to Extracorporeal Shock Wave Therapy in the Management of Long-Bone Nonunions

To the Editor:

Although extracorporeal shock-wave therapy (ESWT)\(^1\) has been used extensively for treatment of nonunions of bone fractures (1), this treatment fails in many patients. Plain radiography remains the gold-standard approach for monitoring nonunion healing. Biochemical bone markers, however, may provide information regarding the effects of treatment earlier than radiography.

Osteocalcin (OC) and bone-specific alkaline phosphatase (bALP) are used to monitor normal and delayed fracture healing (2). We investigated whether these biochemical bone markers can be used to distinguish between nonunions that respond to ESWT treatment and those that do not and to provide this information earlier than radiographic findings.

Study participants were 34 patients [26 men and 8 women, mean (SD) age 42.5 (5.9) years] with tibial (22 patients) or femoral (12 patients) hypertrophic nonunions. This study was approved by our local ethics committee, and all subjects gave their written informed consent. All the patients underwent the same ESWT treatment, which consisted of 4 separate ESWT sessions at 1-week intervals, with 4000 impulses per session with an energy-flux density (EFD) of 0.40 mJ/mm\(^2\), provided by an electromagnetic ESWT generator (Modulith SLK Storz Medical AG).

The nonunion healing process was assessed by clinical examination, standard anteroposterior and lateral x-ray films, and biochemical bone-marker activity. The biochemical bone markers were assessed before the ESWT treatment (baseline), during the 4 weeks of ESWT treatment (2 and 4 days after each weekly session), and 1 week and 1, 3, and 6 months after the end of ESWT treatment. The blood samples used to measure the OC and bALP were collected in the morning (between 8 and 9 am) after patients had fasted overnight. Serum was separated from whole blood and stored at $-20$ °C until measurement. OC was measured by means of a 1-step sandwich ELISA (N-MID-Osteocalcin; Roche Diagnostics) (intra- and interassay CVs $\leq 4.0\%$ and $6.5\%$, respectively). bALP was measured with the bALP test (Roche Diagnostics) (intra- and interassay CVs $\leq 5\%$). We also assigned a clinical and radiographic score (ranging from $0 = \text{no weight bearing, pain at rest, and 0\% callus formation}$ to $4 = \text{unrestricted weight-bearing walking, no pain, and >75\% callus formation}$) of nonunion healing. At the end of the study, patients were classified as either responders (score 4) or nonresponders to ESWT on the basis of the degree of nonunion healing.

Of the 34 patients, 26 healed completely (healed group), whereas the remaining 8 patients reached a score of only 2 by the end of the study (nonhealed group). We investigated possible reasons for the absence of bone tissue response in the 8 nonunions that were not healed by ESWT treatment. Of these 8 patients, 1 patient suffered an additional trauma just after the end of the treatment period. The other 7 patients had lower mean (SD) OC concentrations than the patients in the healed group [4.65

\(^{1}\) Nonstandard abbreviations: ESWT, extracorporeal shock-wave therapy; OC, osteocalcin; bALP, bone-specific alkaline phosphatase; EFD, energy-flux density.
Authors’ Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

References


Specificity of a High-Sensitivity Cardiac Troponin I Assay Using Single-Molecule-Counting Technology

To the Editor:

Cardiac troponin is the standard for diagnosis or exclusion of acute myocardial infarction. Recent guidelines recommend a cutoff value at the upper 99th percentile (99th%) of values for a reference population of healthy individuals and assay imprecision (CV) ≤10% at this cutoff (1). Many commercial assays cannot meet these criteria, and values below the 99th% cutoff appear to supply diagnostic and prognostic information (2, 3). Recently the ErennaTM cardiac troponin I (cTnI) immunoassay (Singulex) has been shown to provide sensitivity and precision that meet these goals (4), with a preliminary 99th% cutoff value at 8 ng/L and 10% CV of 1.8 ng/L (5). This novel assay uses single-molecule counting technology and has been previously described. However, we could not fully exclude the possibility that, with such sensitive limits of detect-
tion, low-level nonspecific binding (NSB) events might contribute to cTnI measurements. We explored this issue by testing potential sources of NSB events.

To test for NSB of serum or plasma constituents, cTnI was quantified in 4 specimen types from 20 healthy individuals, serum, EDTA, lithium heparin, and sodium-citrated plasma. The protocol was approved by the University of California Committee on Human Research. All 20 individuals were free of cardiac disease or symptoms (13 female, 7 male, mean age 43 years, range 23–64 years) and provided signed written consent. Blood was centrifuged within 30 min of collection, divided into aliquots, and stored frozen at −70 °C until analysis at Singulex with the Erenna system (4, 5). Values of cTnI were quantifiable in 93% of specimens (Table 1), and no trends based on age or sex were observed.

For each donor, the mean value assessed for each specimen type fell within 2 SDs of the mean, indicating no significant differences between specimen types (95% CI). For 1 donor increased cTnI occurred in all specimen types. Grub’s test results (P < 0.05) indicated that this donor was a statistical outlier, and all data from this donor were excluded. No further clinical data on this individual are available at this time. The combined adjusted mean (SD) value for cTnI was 2.78 (1.93) ng/L.

To test capture antibody NSB, we obtained 8 additional serum samples from healthy blood bank donors (4 male, 4 female, mean age 25.5 years, range 22–30 years). Frozen serum was assayed as described previously (4, 5), with the following changes. Microparticles (MPs) were coated with one of the following nonspecific capture antibodies, amyloid-β-42 (Covance), macrophage inflammatory protein-1 α (R&D Systems), granulocyte–colony-stimulating factor (R&D Systems), or prostate-specific antigen (BiosPacific). As a positive control MPs were coated with a cTnI capture antibody (R&D Systems), and as a negative control MPs were left uncoated (blank MPs). Additional experimental details are available on request. Across all 28 donors, cTnI was detectable in all (limit of detection 0.2 ng/L) and quantifiable in 93% (lower limit of quantitation 1 ng/L) of specimens (Table 1). Serum and plasma from only 1 volunteer (5%) were quantifiable with a nonspecific antibody, which displayed 55% cross-reactivity with amyloid-β-42 (1.26 ng/L) compared to cTnI (2.36 ng/L).

### Table 1. cTnI concentrations across specimen types and capture antibody cross-reactivity in healthy human specimens analyzed with the Singulex MP-based cTnI assay.

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Mean (SD), ng/L</th>
<th>Range, ng/L</th>
<th>Outlier, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>2.90 (1.90)</td>
<td>0.86–9.27</td>
<td>24.74</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium heparin</td>
<td>2.93 (2.11)</td>
<td>1.12–10.47</td>
<td>20.86</td>
</tr>
<tr>
<td>EDTA</td>
<td>2.66 (1.86)</td>
<td>0.70–9.16</td>
<td>22.89</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>2.66 (1.89)</td>
<td>0.67–9.29</td>
<td>20.98</td>
</tr>
<tr>
<td>Combined</td>
<td>2.78 (1.93)</td>
<td>0.84–9.55</td>
<td>22.37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MP capture antibody</th>
<th>Total samples, n</th>
<th>ND (&lt;LoD, 0.2 ng/L), n (%)</th>
<th>DNQ (≥0.2 ng/L), n (%)</th>
<th>Quantifiable (≥LoQ, 1.0 ng/L), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnI</td>
<td>28</td>
<td>0 (0)</td>
<td>2 (7)</td>
<td>26 (93)</td>
</tr>
<tr>
<td>Blank</td>
<td>28</td>
<td>26 (93)</td>
<td>2 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amyloid-β-42</td>
<td>20</td>
<td>18 (90)</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Macrophage inflammatory protein-1 α</td>
<td>8</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Prostate-specific antigen</td>
<td>8</td>
<td>7 (88)</td>
<td>1 (13)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Granulocyte–colony-stimulating factor</td>
<td>8</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* This serum sample from a 64-year-old woman was identified as an outlier and removed.

b ND, nondetectable; LoD, limit of detection; DNQ, detectable but nonquantifiable; LoQ, lower limit of quantitation.

c One serum sample displayed 55% cross-reactivity with amyloid-β-42 (1.26 ng/L) compared to cTnI (2.36 ng/L).
prostate-specific antigen (13%). No NSB was observed with granulocyte–colony-stimulating factor.

Analyte NSB events were tested with standard curves and linear regression analysis of cTnI standard (NIST) and nonspecific skeletal troponin (Hytest) assayed over 0.1–100 ng/L. Back-interpolated standard curves of the NIST standard cTnI ($y = 1.053x – 0.229; R^2 = 0.999$) correlated poorly with skeletal troponin ($y = 0.000x + 0.156; R^2 = 0.001$), indicating an absence of NSB when skeletal troponin was used as the analyte. Interassay precision was determined by independent assay of 2 control sera (14 runs over 4 days), and showed interassay imprecisions (CVs) of 7% and 10%, with mean cTnI measurements of 8.3 and 2.2 ng/L respectively.

These data support the specificity of this novel single-molecule assay for quantification of cTnI in apparently healthy persons. Even in individuals with documented NSB, binding was below the limit of quantitation in all but 1 case (Table 1), in which it accounted for 55% of the cTnI signal, a value that would not have moved this specimen out of the normal range ($4, 5$). As more sensitive detection techniques are developed, such studies will be essential to confirm the specificity of detection. Cross-reactivity studies that evaluate troponin orthologs (i.e., skeletal muscle troponin I and troponin T) and other NSB events (matrix, antibodies, and MPs) are critical for the validation of these assays. These data, taken together with those reported previously ($4, 5$) provide strong evidence that this particular cTnI assay maintains the specificity of cTnI detection. Similar data will be necessary for other “high-sensitivity” troponin I assays.

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**References**


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