the hyperextension of the thumb in a patient with Ehlers–Danlos syndrome.

In summary, the suggestion that Paganini may have had Ehlers–Danlos syndrome type 3, rather than type 4, is reasonable. Clinical chemistry, had it existed during Paganini’s lifetime, might have unraveled the mystery of Paganini’s demonic violin virtuosity.

References

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Multiple Forms of Prostate-Specific Antigen in Serum Measured Differently in Equimolar- and Skewed-Response Assays

To the Editor:

Zhou et al. (1) described differences between the Ciba Corning ACS:180™ (Ciba Corning Diagnostics Corp., East Walpole, MA) and the Hybritech Tandem™-R (Hybritech, San Diego, CA) assays for measuring prostate-specific antigen (PSA) in serum. We are compelled to offer alternative explanations for their data, as follows:

1) ACS PSA is a skewed-response assay, whereas Tandem-R PSA is an equimolar-response assay. Graves (2) defines a “skewed-response assay” as one that reports quantities of free PSA (f-PSA) as greater than the same amount of PSA complexed to α1-antichymotrypsin (ACT); for ACS, this difference is about threefold. Tandem-R exemplifies an equimolar-response assay; it reports 1 ng of PSA, whether free or complexed, as 1 ng of PSA.

Equal recognition—not “maximal recognition”—of PSA forms is emerging as a desirable attribute for PSA assays (2). Equimolar assays measure the major serum PSA forms equally and report changes only when the overall PSA concentration changes. Skewed assays report changes if either the concentration or the ratio of f-PSA to PSA-ACT shifts and therefore fail to reveal what really changed. It is possible that separate assays for f-PSA and PSA-ACT will exhibit improved clinical value over current assays. The clinical utility of an assay wherein the reported value is determined by two variables, both of which vary greatly across patients, may be questioned. Patients A, B, and C reported by Zhou et al. (1) exemplify the variability of f-PSA, as do our own data. To date, we have quantified f-PSA in 653 specimens: 24% (156 of 653) of the specimens contained ≥10% f-PSA, 41% (269 of 653) contained 10–20%, 19% (125 of 653) contained 20–30%, 8% (54 of 653) contained 30–40%, 5% (19 of 653) contained 40–50%, and 5% (30 of 653) of the specimens contained ≥50% f-PSA (sample selection criterion: 0 < PSA < 20 µg/L, as reported by R. Sokoloff, 2nd Stanford Conference on International Standardization of PSA Assays, Sept. 1–2, 1994, Palo Alto, CA).

2) The ACS PSA assay displays an attenuated response for PSA-ACT relative to f-PSA; in contrast, Tandem-R and -E assays fully quantify both forms. We demonstrated these responses experimentally as follows: PSA purified from seminal fluid was incubated with ACT to allow the enzymatically cleaved form of PSA (≈60%) to complex with ACT. A control solution without ACT (100% f-PSA) was also prepared. Both solutions, containing equal amounts of total PSA, were assayed in parallel by the Tandem and ACS:180 assays and the results compared. The Tandem-E recovered nearly identical values for both control and complexed solutions, demonstrating an equimolar response; the Tandem-R PSA assay returned similar results. In contrast, the ACS assay recovered values for the complex that were 60% less than the values obtained for the control solution.

The differential responses for PSA forms that some assays display may result from polyclonal heterogeneity. The polyclonal antibody conjugates used in some commercial assays may include a subpopulation of antibodies directed against PSA epitopes that are blocked by ACT in the complex. The net result is that fewer antibody reporter molecules bind to each molecule of PSA-ACT than to f-PSA, thus leading to an attenuated response to PSA-ACT, which could be misconstrued as an exaggerated (relative) response to f-PSA. Reported PSA assay values, when influenced by the assay architecture described above, are misinterpreted when explained as “measurement of more PSA.”

3) The article by Zhou et al. (1) raises serious concerns regarding calibration of the ACS PSA assay. The authors relied on an absorptivity of 1.42 L·g⁻¹·cm⁻¹ for PSA. New data from a study in which Hybritech and Ciba-Corning participated demonstrate that a more accurate value is 1.84 L·g⁻¹·cm⁻¹ (Stamey et al., ms in preparation).

We adjusted the values in Table 1 of the Zhou article to reflect use of the 1.84 absorptivity value. The values in columns 2 and 3 and the ratios in column 6 were observed results, generated with the Tandem-E and ACS:180 PSA commercial assays, and thus were unchanged. The modified Table 1 is presented (Table 1A). The upper half of this modified table shows that Tandem-R recovered 85–93% of the added PSA, and ACS recovered three times the amount actually added. As shown in the lower portion of the modified table, Tandem-R recovered 54–56% of PSA added to serum—an expected result, given the substantial portion of PSA that complexes to α1-macroglobulin (MG), rendering the PSA nonimmunoreactive (i.e., “occult”) (3). In contrast, ACS recovered 130–140% of the added PSA. Zhou et al. failed to explain satisfactorily why ACS recovered more than 100% of PSA added, given that ~50% of PSA introduced into serum forms occult PSA-MG. Even with the uncorrected absorptivity value used in the original publication, the 100–110% recovery reported (1) is incongruent with the formation of these occult complexes.

4) Skewed-response assays that use calibrators modeled after serum will report concentrations inaccurately in specimens that differ substantially from calibrators. Zhou et al. state that “ACS-emulation calibrators” were prepared by adding purified PSA to serum. PSA added to serum distributes across PSA-ACT, PSA-MG, and f-PSA in proportions differing substantially from the distribution of endogenous serum PSA (Chen et al., ms in preparation). Along with van Straalen et al. (4), we speculate that this phenomenon may be partially due to different clearance rates for PSA forms in vivo. Because each form will display a particular molar response in a given skewed-response assay, the
Table 1. Corrected values for uncomplexed and complexed PSA
determined by ACS:180 and Tandem-R (TD-R) assays.

<table>
<thead>
<tr>
<th></th>
<th>Analytical*</th>
<th>ACS</th>
<th>TD-R</th>
<th>ACS/anal</th>
<th>TD-R/anal</th>
<th>ACS/TD-R</th>
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<tr>
<td></td>
<td>(Col 1)</td>
<td>(Col 2)</td>
<td>(Col 3)</td>
<td>(Col 4)</td>
<td>(Col 5)</td>
<td>(Col 6)</td>
</tr>
<tr>
<td>Uncomplexed PSA in HSA/PBS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal 1</td>
<td>77.2 (100)</td>
<td>230.2</td>
<td>65.4</td>
<td>3.0 (2.3)</td>
<td>0.85 (0.65)</td>
<td>3.5</td>
</tr>
<tr>
<td>Cal 2</td>
<td>38.6 (50)</td>
<td>109.5</td>
<td>35.1</td>
<td>2.8 (2.2)</td>
<td>0.91 (0.70)</td>
<td>3.1</td>
</tr>
<tr>
<td>Cal 3</td>
<td>19.3 (25)</td>
<td>63</td>
<td>18</td>
<td>3.3 (2.5)</td>
<td>0.93 (0.72)</td>
<td>3.5</td>
</tr>
<tr>
<td>Serum-complexed PSA</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal 1</td>
<td>77.2 (100)</td>
<td>100.7</td>
<td>41.5</td>
<td>1.3 (1.0)</td>
<td>0.54 (0.42)</td>
<td>2.4</td>
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<tr>
<td>Cal 2</td>
<td>38.6 (50)</td>
<td>54</td>
<td>21.2</td>
<td>1.4 (1.1)</td>
<td>0.55 (0.42)</td>
<td>2.5</td>
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<tr>
<td>Cal 3</td>
<td>19.3 (25)</td>
<td>27.8</td>
<td>10.8</td>
<td>1.4 (1.1)</td>
<td>0.56 (0.43)</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* Determined spectrophotometrically, and corrected for absorptivity of 1.84 L·g⁻¹·cm⁻¹. This correction affected ratios determined in columns 4 and 5.

References

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The authors of the article referred to respond:

To the Editor:
In response to Strobel et al., we offer the following observations:
Except for the Ciba Corning ACS™ PSA assay (1, 2), there is no published documentation as to how other PSA assays, including the Hybritech® Tandem® assay, were originally standardized and quantitated. Until recently, no authorized PSA reference materials of defined concentration were available with which manufacturers could uniformly standardize various PSA assays. Meetings sponsored by the National Committee for Clinical Laboratory Standards (NCCLS), the American Cancer Society, the World Health Organization (WHO), and two Stanford Conferences on International Standardization of PSA Assays have been convened to develop appropriate reference materials and consensus for standardizing the >15 PSA assays currently available worldwide. Ciba Corning is an active member in these national and international efforts.

As an outcome of this process, all assays, including Tandum, will require a value change to conform to standardization based on true mass value determined by purely analytical techniques. As first made public at the first Stanford Conference in December 1992, both the Hybritech Tandem and Yang Procheck® assays were initially standardized to uncomplexed (i.e., PSA). This quantitation was performed with a colorimetric Lowry protein assay with bovine serum albumin (BSA) as standard (3, 4). This arbitrary method of standardization is used when large quantities of the pure protein of interest are unavailable. A recent comparative study of Lowry protein calibration curves for purified preparations of PSA and BSA showed a 23% difference in absorbance at 650 nm per microgram of protein (3).

As an improved approach to providing true analytical standardization, Graves et al. (5) in 1990 published an absorptivity at 280 nm of 1.42 for a 1 g/L solution of free PSA based on a precise gravimetric determination of purified, lyophilized PSA from seminal fluid. It is this value that Ciba Corning currently uses to quantify PSA. While Strobel and associates offer their opinion that the Hybritech assay is the reference assay to which all assay values should be adjusted, we ask how this can be done without the availability of a physical reference material or at least documentation in sufficient detail to allow reproducible preparation and quantitation of this putative reference material? For uniform PSA assay standardization, it is poor manufacturing practice to standardize an assay based solely on the results of running patients’ samples in a competitor’s kit.

To conform to basic principles of as-