

bility unlikely for the following reasons:

1) Isopropanol was detected in the patient's blood on more than one occasion during hospitalization. Specimens were drawn by different staff each time. Our current phlebotomy protocol specifically addresses the need to avoid isopropanol swab contamination. Considering the number of phlebotomists involved with the patient, it is difficult to support the contamination hypothesis.

2) Three Vacutainer Tubes received for the toxicological analyses on the patient were reasonably full. To have a blood isopropanol concentration of ~400 mg/dL due to contamination, the Vacutainer Tubes would have to be contaminated with ~60 μ L of 70% isopropanol. It is difficult to conceive how this much isopropanol could have been introduced into the specimen by contamination.

3) An experiment was conducted where blood samples from volunteers were drawn immediately after wiping their arms with isopropanol swab. When the 7- or 10-mL Vacutainer Tubes were filled with blood and the blood was analyzed for volatiles, isopropanol was not detected.

4) Of the ~30 blood specimens received daily for analysis of volatiles during the patient's hospitalization, none showed any pattern that suggested isopropanol contamination from a venipuncture site.

These observations and studies, in our opinion, make unlikely the possibility of isopropanol contamination during venipuncture of the patient.

Reference

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Was Paganini Born with Ehlers-Danlos Syndrome Phenotype 4 or 3?

To the Editor:

I read with interest the Special Report by Wolf (1) about diseases that

affected famous painters, composers, and political leaders. Papers on such paramedical subjects are very pleasant in the journal, I think, and I enjoyed this one very much. I have some criticism, however, relating to the discussion of famous composer and violinist Niccolò Paganini.

Wolf wrote: "Paganini was born with Ehlers-Danlos syndrome, a connective tissue disease causing a diffuse looseness of the connective tissue. The Ehlers-Danlos 4 phenotype, related to mutations in collagen type III on chromosome 2, results in a flexibility of all of one's joints." Indeed, Paganini is reported to have been able to bend his thumb back so far that the thumbnail touched the back of his hand. Owing to this remarkable flexibility in his wrist and finger joints, Paganini could span three octaves with little effort (2).

Ehlers-Danlos syndrome (EDS) refers to a group of connective tissue disorders, of which there are at least 10 known types. EDS 4 phenotype is the most severe form among the 10 types because of its grave consequences. In patients with EDS 4 phenotype the main symptoms are as following: thin, translucent skin with visible veins; marked bruising; and arterial, bowel, and uterine rupture. Skin and joints have normal extensibility in this form. Arterial fragility may manifest as sudden death, stroke, shock from retroperitoneal or intraabdominal bleeding, or compartmental syndromes, depending on the site of vessel rupture. Therefore, life expectancy is considerably shortened (3), whereas Paganini lived for 58 years (from 1782 to 1840). Moreover, Paganini also suffered from other severe diseases, including syphilis and pulmonary tuberculosis.

Among the EDS phenotypes, the EDS 3 phenotype is a more likely diagnosis for Paganini. In this form of EDS the major manifestation is joint hypermobility (the type name is familial hypermobility), and life expectancy is normal.

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The author of the report referred to comments:

To the Editor:

Yücel's suggestion that Paganini may have suffered from the Ehlers-Danlos phenotype 3 instead of phenotype 4, which I implied (1), has merit (2). However, because hypermobility of joints occurs in several types of Ehlers-Danlos syndrome, including types 1, 2, 3, 5, 6, 7, 8, and 10 (3), it seems more appropriate and prudent that a specific phenotype of Ehlers-Danlos syndrome not be assigned to Paganini. If Paganini was affected by Ehlers-Danlos syndrome, the contemporary clinical chemist may have been able to identify several of the phenotypes: e.g., type 4, abnormal collagen (type III) synthesis; type 6, lysyl hydroxylase deficiency; type 7, defective conversion of type 1 procollagen to collagen; type 9, abnormal copper utilization with defect in lysyl oxidase; type 10, defect in fibronectin (3). Thus, I would recommend a cautious approach since other speculations exist relevant to Paganini's demonic virtuosity.

Paganini's physician, Francesco Benati, believed that the violinist's flexibility of his left hand was inherited (4). Benati observed that there was increased elasticity of Paganini's shoulders, elbows, wrists, and upper joints of the fingers of his left hand. When Paganini played, he crossed his elbows practically one on the top of the other. Schoenfeld speculated that Paganini suffered from Marfan syndrome (5). However, he was not abnormally tall and his hands were of normal size without arachnodactyly; thus, this theory has been discounted. The distinguished writer Francois-Joseph Tetis believed that Paganini's unusual flexibility was acquired due to years of practice (6). Paganini's hyperextension of his left thumb was demonstrated in Fig. 4 of my report. Fig. 1 here demonstrates



Fig. 1. Individual with Ehlers-Danlos syndrome, demonstrating hypermobility of the thumb.

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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.

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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 α_1 -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 $\leq 10\%$ f-PSA, 41% (269 of 653) contained 10–20%, 19% (125 of 653) contained 20–30%, 8% (54 of 653) contained 30–40%, 3% (19 of 653) contained 40–50%, and 5% (30 of 653) of the specimens contained $\geq 50\%$ f-PSA (sample selection criterion: $0 < \text{PSA} < 20 \mu\text{g/L}$, 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 active portion of PSA ($\approx 60\%$) 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 mole-

cule 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 $1.42 \text{ L} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ for PSA. New data from a study in which Hybritech and Ciba-Corning participated demonstrate that a more accurate value is $1.84 \text{ L} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ (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 is presented here (Table 1). 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 α_2 -macroglobulin (MG), rendering the PSA nonimmunoreactive (i.e., "occult") (3). In contrast, ACS recovered 130–140% of the added PSA. Zhou et al. fail to explain satisfactorily why ACS recovers more PSA than was added, given that $\sim 50\%$ 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