Persistently Elevated Prostate-Specific Antigen Level After Successful Laparoscopic Radical Prostatectomy

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Clinical History

Patient
67-year-old man.

Chief Complaint
Persistently elevated prostate specific antigen (PSA) level, 4 months after successful laparoscopic radical prostatectomy.

History of Present Illness
During his annual prostate screening examination, the patient was noted to have a rise in PSA from 3.8 ng/mL in 2004 to 4.4 ng/mL in 2005, using the immunochemiluminometric assay (ICMA) TPSA assay in the ADVIA Centaur instrument (Bayer Diagnostics, Tarrytown, NY). His digital rectal exam (DRE) was unremarkable with an estimated prostate gland size of 35 g and no evidence of nodules or induration.

Past Medical History
Hypertension, well controlled with Nifedipine; no history of heart disease; no recent vaccinations; and no history of organ transplant or blood transfusions.

Family History
Prostate cancer in an older brother, who died of metastatic disease.

Social History
He is a retired professional, without occupational exposure to animals. He was born on a farm but left in 1957; has never had rodent pets, but did have dogs and cats as pets.

Physical Exam Findings
The patient was well-developed and in no acute distress. His laparoscopic incisions were well-healed. There was no palpable lymphadenopathy in the cervical, supraclavicular, or inguinal chains, and no organomegaly.

Laboratory Findings

Table 1

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA (ng/mL)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Total PSA (TPSA)</td>
<td>2.25</td>
</tr>
<tr>
<td>Prostate specific antigen (TPSA)</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Additional Diagnostic Procedures
Due to the magnitude of the rise in his PSA concentration over a 1-year interval, resulting in a PSA value >4.0 ng/mL, he underwent a 12-core transrectal prostate biopsy, which demonstrated Gleason Score 3+3 adenocarcinoma in 30% of a single core from the left side. After discussion of treatment options for his low-risk clinical grade T1c prostate cancer, he underwent an uncomplicated bilateral nerve-sparing laparoscopic radical prostatectomy. The final pathology report indicated a 42 g gland with organ-confined Gleason Score 3+3 adenocarcinoma, involving 10% of the gland (pT2cN0M0) and a positive focal margin at the left apex but no extracapsular extension. At the 3-month follow-up visit post-prostatectomy, his mean total PSA (TPSA) value from duplicate determinations was 2.25 ng/mL. Because this TPSA value was well above the functional analytical sensitivity (0.05 ng/mL) of the Beckman Coulter TPSA assay and the TPSA value expected (ie, <0.05 ng/mL) for a patient who underwent a successful radical prostatectomy, repeat TPSA testing on a new serum specimen was performed 2 weeks later at which time the patient’s mean TPSA value from duplicate determinations was 2.17 ng/mL (Table 1). He subsequently underwent computed tomography (CT) of the abdomen and pelvis and nuclear bone scan and Prostascint imaging studies. The bone scan revealed increased activity in the left ilium, suggestive of Paget’s disease. Computed tomography confirmed Paget’s disease of the ilium and no other concerning findings. Prostascint imaging revealed focal positivity in the left supraclavicular area.

Physical examination of this area was unremarkable, and follow-up contrast enhanced CT of the neck and chest demonstrated no lymphadenopathy or masses. The apparent discrepancy between the negative imaging studies, suggestive of a successful prostatectomy, and his elevated, post-prostatectomy PSA value, above 0.05 ng/mL, prompted additional laboratory testing (Table 1) of his serum samples to determine the cause of this discrepancy.

Questions

1. What are this patient’s most striking clinical and laboratory findings?
2. What is unusual about these findings?
3. How do you explain these findings?
4. What additional laboratory test could be performed to confirm your explanation of these findings?
5. What are some of the ways in which the problem that led to this patient’s spuriously increased TPSA concentration can occur?
6. What are some of the ways in which laboratories and manufacturers of diagnostic immunoassays can resolve the problem that led to this patient’s spuriously increased TPSA concentration?
7. How effective are the different approaches to identifying and/or resolving the problem that led to this patient’s spuriously increased TPSA concentration?
8. What is the incidence of the problem that led to this patient’s spuriously increased TPSA concentration?
9. Can adverse consequences occur when the problem that led to this patient’s spuriously increased TPSA concentration is unrecognized?
10. Is the problem that led to this patient’s spuriously increased TPSA concentration reversible?

Possible Answers

1. The discordance between the patient’s detectable TPSA concentration (2.25 ng/mL and 2.17 ng/mL) on 2 separate occasions (Table 1) and clinical, histopathologic, and imaging results suggests of a successful radical prostatectomy (RP).

2. Currently, the major therapeutic options for organ-confined prostate cancer include RP, radiation (external beam or brachytherapy), cryoablation, and active surveillance. One of the
Table 1  Summary of Patient’s PSA Results

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Time PP, mo</th>
<th>TPSA, ng/mL Using Reagent Lot A</th>
<th>TPSA, ng/mL Using Reagent Lot B</th>
<th>TPSA, ng/mL Using Reagent Lot C</th>
<th>TPSA, ng/mL Using Reagent Lot D</th>
<th>%FPSA</th>
</tr>
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<tbody>
<tr>
<td>-2</td>
<td>-48</td>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-1</td>
<td>-24</td>
<td>4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>2.25</td>
<td>2.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.62</td>
<td>2.05</td>
<td>341.7</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>0.65</td>
<td>2.37</td>
<td>364.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.58</td>
<td>2.17</td>
<td>370.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1R1</td>
<td></td>
<td>0.57</td>
<td>2.11</td>
<td>370.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2R1</td>
<td></td>
<td>0.35</td>
<td>2.20</td>
<td>632.8</td>
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<td></td>
</tr>
<tr>
<td>3R1</td>
<td></td>
<td>0.24</td>
<td>2.12</td>
<td>870.4</td>
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</tr>
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<td>3D1:2</td>
<td></td>
<td>2.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3D1:4</td>
<td></td>
<td></td>
<td>2.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1R2</td>
<td></td>
<td></td>
<td>2.25</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2R2</td>
<td></td>
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<td>3R2</td>
<td></td>
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</table>

**Radical Prostatectomy Performed**

- Specimen No. 3, 0.60
- Specimen No. 1, 0.65
- Specimen No. 2, 0.58
- Specimen No. 3, 0.57
- Specimen No. 3D1:2, 0.35
- Specimen No. 3D1:4, 0.24

Accepted benefits of RP is that total PSA concentration should be undetectable (ie, less than the functional sensitivity of the assay used to quantify TPSA concentration) post-operatively. Total prostate-specific antigen is an excellent tumor marker for evaluating tumor recurrence status post RP. In clinical practice, detectable TPSA after RP is considered “biochemical recurrence” and mandates consideration of metastatic work-up or adjuvant therapy. After radiation or cryoablation for organ-confined tumors, TPSA should also drop to a nadir level (eg, <0.05 ng/mL). In this case, with pathologically organ-confined, low-grade, low-stage prostate adenocarcinoma, it was extremely unexpected to have detectable TPSA values after RP. Our patient’s post-RP TPSA values were approximately 50% of his pre-operative values (Table 1). This implied the surgeon had either left half the prostate gland in the patient’s body or the laboratory TPSA results were not accurate. Based on the histopathology and post-operative abdominopelvic CT findings, it was considered unlikely that the RP surgical procedure had been unsuccessful. Therefore, the accuracy of the post-prostatectomy TPSA values was investigated further with results as shown in (Table 1). Additional unusual findings in this case include a free PSA (FPSA) concentration significantly higher (3.4- to 8.7-fold) than the TPSA concentration. Because PSA exists in two principal forms, free (FPSA) and protein bound [or “complexed” PSA (CPSA)], and the PSA assay used to quantify the TPSA in our patient’s serum samples is an equimolar-response [ie, measures both FPSA and complexed PSA (CPSA) equally, regardless of the ratio of these forms present in the patient’s serum specimen] TPSA assay, the [TPSA] = [FPSA] + [CPSA]. Moreover, in patients with prostate cancer, the [CPSA] >> [FPSA]. Therefore, the FPSA concentration cannot exceed the TPSA concentration in our patient’s serum.

3. Based on the information provided in (Table 1), the most likely cause of this patient’s spuriously elevated post-RP TPSA values is the presence in his serum of human anti-mouse antibodies (HAMA). Evidence in support of this conclusion includes: (1) markedly different TPSA values using different lots of TPSA reagents; (2) lack of proportional results with dilution [ie, Specimen No. 3, (Table 1)]; and (3) the ability of a HAMA blocking reagent (HBR), when added to the patient’s serum, to adsorb HAMA and yield TPSA results significantly different from an aliquot of the same serum sample to which the HBR was not added [ie, HBR Reagent Lot “Z”, (Table 1)]. Note that not all HBRs were equally effective in inactivating the HAMA present in our patient’s serum.

4. Direct measurement of HAMA in the patient’s serum sample. Human anti-mouse antibodies measurement by immunoradiometric assay (IRMA) (Specialty Labs, Santa Monica, CA) of our patient’s serum yielded a value of 440 ng/mL, a value ~6-fold higher than the upper limit (74 ng/mL) of the reference range. The fact that this upper limit is relatively high, compared to the likely functional analytical sensitivity of the HAMA IRMA, suggests that most individuals have a certain amount of HAMA in their serum.

5. Circulating anti-animal antibodies, such as HAMA, can arise in humans from 2 sources: iatrogenic and non-iatrogenic. Examples of iatrogenic-induced causes of anti-animal Ab production in humans include: administration of a diagnostic or pharmaceutical agent derived from an animal source, blood transfusion, vaccination, administration of unconventional agents derived from animal sources, and organ transplant patients who receive mouse antibodies (eg, anti-CD3, anti-CD4, or anti-IL-2R) for immunophylaxis. Examples of non-iatrogenic-induced causes of anti-animal Ab production in humans include: placental transfer to a fetus, occupations involving animal husbandry, pets, and the presence of disease (eg, idiopathic cardiomyopathy) associated with a high incidence of human anti-animal Ab production. We were not able to determine the cause of our patient’s high HAMA level.

6. The principal procedures available to laboratory professionals in identifying and/or resolving HAMA interference in
immunoassay testing are: (1) diluting the patient’s serum sample, testing each dilution for the analyte of interest, and observing whether or not the analyte results are linear or non-linear compared to the analyte concentration in an undiluted serum sample (Note: Non-linear results suggest the presence of HAMA; however, 50% of HAMAs are likely to dilute linearly); (2) re-testing of the patient’s serum sample for the analyte of interest using a different immunoassay method. Human anti-mouse antibody interference may affect one immunoassay method to a lesser extent than other immunoassay methods (eg, 2-site immunometric assays are especially prone to HAMA interference); (3) using a commercially available HBR to remove HAMA interference; or (4) testing for the presence of HAMA in the patient’s serum using a HAMA-specific immunoassay method (Note: Unfortunately, there is no universally applicable immunoassay that will measure all different types of HAMA and measure them equally as well in all patient’s serum samples). Approaches by manufacturers of diagnostic immunoassay methods to limit HAMA interference in their assays include: (1) use of the Fab or F(ab)’, fragment instead of intact immunoglobulin (Ig) as the capture and signal antibodies in 2-site immunoassays (Note: This approach eliminates interference from anti-animal antibodies with specificity for the Fc portion of an IgG antibody reagent); (2) use of chimeric monoclonal reagent antibodies in lieu of monoclonal antibodies derived from mouse spleen cell-human cancer cell hybridomas; and (3) use of monoclonal reagent antibodies derived from chicken cells such that these antibodies do not have specificity for mouse antibodies. [Note: Such a strategy may simply transfer the problem from HAMA to HACA (human anti-chicken antibodies; however, HACA probably occur less frequently in human serum than HAMA)]. Reagents used in several modern automated immunoassays routinely include nonimmune HBRS. While this strategy has reduced the number of false positive immunoassay results, it is not 100% effective, as demonstrated in this case study. Other physical and chemical techniques used by laboratory professionals and/or manufacturers to remove heterophile Ab interference include ultracentrifugation, precipitation with trichloroacetic acid, and pretreatment of serum with agents such as polyethylene glycol and detergents. However, none of these techniques is universally applicable for all analytes, these techniques are cumbersome, and their reliability is unknown. Moreover, another difficulty is that the problem of solving HAMA interference is a moving target (ie, reagent lots for immunoassays vary over time and HBRS are not equally effective in removing all HAMA interference). In our patient, TPSA testing with reagent lots C, D, and E (all from Beckman Coulter) and reagent lot E with 3 different HBRS gave different results. These findings suggest that the HAMA in our patient’s serum had different affinities for the murine antibodies (Abs) contained in these different reagent lots.

7. Because each of the approaches to identifying and resolving HAMA interference have limitations and none are perfect, a multi-pronged approach involving all of the laboratory strategies indicated above is optimal. One explanation for the lack of a “perfect” HBR is the polyspecific nature of heterophile Abs, such that while many of them can be blocked, others can not. This is because some HAMA are characterized by low-affinity binding, whereas others have higher affinity. Their heterogeneity is explained by the current hypothesis that HAMA are naturally occurring Abs that arise either by chance or as idio
type-specific antibodies. The HAMA may represent non-specific, weakly-binding antibodies produced by the humoral immune system in the normal Ab production process. In this model, the naïve B cell population generates 10^4-10^6 different Abs that passively survey the internal milieu for antigens. Once exposed to an antigen, clonal expansion of specific B cells leads to Ab-producing plasma cells and memory B cells. Thus, HAMA simply arise by chance in a given individual’s repertoire of 10^4-10^6 naïve Abs. By contrast, HAMA may represent “idiotypic Abs.” Idiotypic Abs are those that bind to the idiotope (Id), a site within the variable region of immunoglobulins that binds other Abs. It is thought that such Id-(anti-Id) interactions regulate humoral immunity. Thus, while idiotypic Abs are normal regulatory elements of humoral immunity, they can corrupt clinical immunoassays by also acting as interfering HAMA. In the clinical chemistry literature, the problem of HAMA interference in immunoassay testing has been long recognized. Usually, HAMA interference leads to spuriously elevated analyte values, but falsely decreased values can occur, albeit less commonly. The mechanism by which HAMA may produce falsely decreased or increased results in 2-site immunometric assays is shown in Figure 1. Finding an easy solution to detect and eliminate HAMA interference in immunoassay testing has been elusive.

8. The incidence of HAMA interference, and spuriously low or high analyte results from this interference, in clinical immunoassays is unknown. Estimates of this interference vary from <1% to as high as 80%. Even if the lowest estimate is correct, the incidence may be rising, however, because of the widespread use of automated 2-site immunometric assays employing monoclonal, mouse spleen cell-derived antibodies and the increasing use of conventional and unconventional agents derived from animal sources (eg, mice) for diagnostic or therapeutic medical purposes. In a recent study published by the Mayo Clinic, 500

![Figure 1. Mechanisms of heterophile antibody (eg, HAMA) interference in 2-site immunometric assays leading to (A) no HAMA interference, (B) falsely high (or positive) values, or (C) falsely low (or negative) values for the antigen (AG), or analyte, being measured. Yellow asterisk indicates the production of a signal based on the nature of the ligand attached to the detection antibody (AB). Various ligands used in these assays include substances capable of chemiluminescence, fluorescence, emission of radioactivity, or substrate conversion by enzyme-linked AB to a product that can be quantified based on the direct relationship between the quantity of AG captured by the capture AB bound to the capture support and the amount of signal produced when the detection AB binds to a different epitope (antigenic site) of the AG being quantified than the capture AB. The nature of the ligands attached to the signal AB define the type of immunometric assay as either an immunochemiluminometric assay (ICMA), immunofluorometric assay (IFMA), immunoradiometric assay (IRMA), or immunoenzymetric assay (IEMA), respectively.](labmedicine.com)
serum samples were tested using 8 automated immunoassays for tumor markers. They found that the incidence of heterophile Ab interference ranged from 0.2% (alpha-fetoprotein) to 3.7% (calcitonin). The other tumor markers in this report were gastrin, CA 125, CA 15-3, human chorionic gonadotropin (hCG), TPSA, and FPSA. In an older study that tested free thyroxine and thyrotropin, the incidence of heterophilic interference was 7 out of 21,000 samples (0.033%). The wide range (0.033% to 3.7%) of published values for the incidence of heterophile Ab interference in immunoassay methods for a variety of analytes can be explained by differences in the analytes tested, immunoassay design characteristics (eg, the animal species used to obtain cells for producing reagent monoclonal/polyclonal antibodies), and reagent lots that contain or do not contain HAMA blocking agents.

9. Yes. HAMA can potentially interfere with any clinical immunoassay that uses murine antibodies. In a widely-publicized case, a false elevation in hCG due to HAMA led to unnecessary hysterectomy and lung resection in a 22-year-old woman. In a similar case, a 49-year-old man with a TPSA concentration of 3.3 ng/mL and Gleason Score 6 prostate cancer underwent unnecessary surgery followed by adjuvant radiation and androgen ablation for 22 months before HAMA was suspected as the cause of his elevated TPSA concentration. Fortunately, our patient was not given unnecessary adjuvant therapy, but his true TPSA will likely never be known due to the high HAMA level in his serum. Based on the 0.2% to 3.7% incidence of HAMA interference in immunoassays for tumor markers reported in the Mayo Clinic study, it is alarming to imagine the extent of the potential psychologic distress and under- or overtreatment of patients with spurious analyte results due to HAMA interference. The present case and those referred to above underscore the importance of teamwork between clinicians and laboratory staff when laboratory results are not consistent with clinical findings or expectations. Clinicians should be aware of heterophilic Ab interference in immunoassay testing and if the laboratory result for a test performed by immunoassay is incongruous with the patient’s clinical status, consideration of HAMA is warranted.

10. In our patient, it is unlikely that his serum HAMA interference in our TPSA immunoassay can be completely eliminated. Three HAMA blocking agents were unsuccessful in completely eliminating his HAMA interference using the TPSA immunoassay in the ACCESS instrument. Use of HBRs X, Y, and Z, provided TPSA values that were 8.0%, 14.3%, and 77.7%, respectively, lower than the HBR-untreated serum sample (Table 1); however, none of the TPSA values on the HBR-treated samples were <0.05 ng/mL. However, it is possible that using a TPSA assay from a different manufacturer (eg, Bayer TPSA assay in the ADVIA Centaur instrument) could provide an accurate TPSA value on our patient’s serum. When we referred all 3 of our patient’s serum samples for testing using the Bayer TPSA assay in the ADVIA Centaur instrument, all values obtained on all 3 of these samples were ≤0.05 ng/mL (Table 1), confirming that our patient’s prostatectomy was successful, and when coupled with our other laboratory data, that HAMA was the most likely cause of his spuriously increased TPSA values using the TPSA reagents and assay in the Beckman Coulter ACCESS instrument. Unlike the Beckman Coulter ACCESS TPSA assay, which uses both mouse monoclonal capture and detection antibodies (see Figure 1), the Bayer Centaur TPSA assay uses a mouse monoclonal capture antibody and a goat polyclonal detection antibody. 1

Keywords: prostate cancer, radical prostatectomy, human anti-mouse antibodies, interference, 2-site immunoassay, blocking reagent

3. Wiens FH Jr. The role of prostate-specific antigen testing in the diagnosis, treatment, and follow-up of patients with adenocarcinoma of the prostate. ASCP Check Sample, Clinical Chemistry 1997;57:77-103.