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Falsely Undetectable Prostate-Specific Antigen (PSA) Due to Presence of an Inhibitory Serum Factor: A Case Report and Review of Pertinent Literature

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Conflict of interest: None declared

Patient: Male, 63
Final Diagnosis: Recurrent prostate cancer
Symptoms: Falsely undetectable PSA
Medication: —
Clinical Procedure: Serum dilution
Specialty: Urology

Objective: Unusual clinical course

Background: Few cases of falsely undetectable PSA due to the presence of an inhibitory serum factor have been reported in the world literature. We present a case of falsely low-to-undetectable PSA with data from a serum dilution series, the current literature on biochemical assay interference, and the implications for prostate cancer salvage treatment.

Case Report: A 63-year-old man was treated with prostatectomy for high-risk prostate cancer and was found to have a rising PSA after approximately 3 years following surgery. He subsequently transferred his care to a different health system and was found to have an undetectable PSA. He was eventually found to have an elevated PSA once again after the particular assay at this institution was changed. He thus received salvage prostate radiotherapy and androgen deprivation therapy.

Conclusions: While falsely low PSA results cannot be explained by the presence of serum heterophile antibodies, competitive antibody interference against the immunoassay reagents or anti-PSA antibodies are possible explanations for the results of the dilution experiments performed in this case study. We suggest that unexpected PSA testing results should raise concern for assay interference and warrant further clinical workup.

MeSH Keywords: Antibodies, Blocking • Immunoenzyme Techniques • Prostate-Specific Antigen • Prostatic Neoplasms

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Background

According to the American Cancer Society, there will be approximately 164 690 new cases of prostate cancer in the United States in 2018 [1]. The majority of new cases are localized and are potentially curable with definitive therapy [2]. The prostate-specific antigen (PSA) immunoassay is an important laboratory test that detects evidence of prostate cancer.

PSA is a glycoprotein enzyme that is secreted by the epithelial cells of the prostate gland. It is present in low amounts in the serum of healthy men, but it often becomes elevated in patients with prostate cancer [3]. The PSA immunoassay is a diagnostic test that measures serum PSA concentration and is used for both prostate cancer screening and for disease monitoring after treatment. In the case of disease monitoring after treatment, the earliest sign of potential prostate cancer recurrence is often a rising PSA [4].

Biochemical recurrence of prostate cancer after treatment is defined in several ways depending on the type of prior definitive treatment. However, all are based on increases of serum PSA levels. PSA testing plays an important role in determining the necessity and timing of salvage therapies. Falsely elevated or depressed PSA values interfere with the ability to accurately assess the disease status of prostate cancer. Spurious PSA levels can falsely impact treatment recommendations. There are reports that showed falsely elevated PSA due to the presence of heterophile antibodies in patient serum [5–9]. Heterophile antibodies are naturally-occurring serum antibodies that interact with foreign immunoassay reagent antibodies [5]. Falsely elevated PSA can result in more aggressive treatment and subject patients to toxicities from overtreatment. While falsely elevated PSA due to the presence of serum heterophile antibodies has been documented in numerous instances, falsely undetectable PSA due to serum antibody interference has not been well-documented. The following case report describes a patient with falsely low-to-undetectable PSA results.

Case Report

The patient was a 63-year-old man without significant risk factors for prostate cancer and without other medical comorbidities, who presented to the University of Minnesota Medical Center (UMMC) with an asymptomatic PSA of 3.6 ng/mL with a 14% free PSA. A digital rectal examination revealed a small right-sided nodule and he was referred to a urologist. The patient subsequently underwent a prostate biopsy, which revealed Gleason grade 4+4 cancer from the right apex. The patient had an unremarkable bone scan and CT scan of abdomen and pelvis. He did not have a pre-operative MRI. His clinical stage was therefore cT2a N0 M0. He subsequently underwent a prostatectomy in 2006. The pathology from the prostatectomy showed Gleason grade 4+4 disease measuring 0.7 cm. There was no evidence of angiolymphatic invasion or seminal vesicle invasion. The surgical margin was involved but there was no extra-capsular extension. There was no metastasis seen in 7 lymph nodes removed. Pathologic staging was therefore pT2 N0 M0. Adjuvant radiotherapy was considered but not administered. Since the prostatectomy in 2006, the post-operative PSAs (obtained approximately every 4 months at UMMC) remained undetectable (<0.10 ng/mL) until 2009, when it was noted to be 0.13 ng/mL. A repeat PSA 1 month later was undetectable. The patient's serial PSAs remained undetectable until 2011, when the PSA rose to 0.39 ng/mL. The PSA was repeated 1 month later at UMMC and it was 1.04 ng/mL. The patient was recommended to receive androgen deprivation therapy along with salvage radiotherapy. However, the patient sought a second opinion at another institution that used a different diagnostic system for PSA testing, and the patient's PSA was undetectable (<0.10 ng/mL). The discrepancy between the 2 assays used at UMMC (Ortho Clinical Diagnostics VITROS 5600 total PSA assay) and the outside facility (Roche Cobas 6000 total PSA assay) was hypothesized to be due to the presence of an inhibitory factor in the patient serum. A series of dilution studies were performed to test this theory. In this dilution study, the patient's serum PSA was measured simultaneously on both the UMMC (Ortho Clinical Diagnostics - Assay A) and outside institution (Roche-Assay B) PSA assays. At the time of the dilution study, the patient's serum PSA measured 0.99 ng/mL by Assay A and <0.10 by Assay B. Next, the patient's serum was diluted 1: 2 and 1: 5 with known PSA values of reference serum that was 8.8 ng/mL (Assay A) and 9.3 ng/mL (Assay B). In addition, the patient's serum was also diluted 1: 2 and 1: 5 using a reference serum that measured <0.10 ng/mL on both assays. The diluted samples and the respective PSA results are illustrated in Table 1.

As illustrated in the dilution study (Table 1), 1: 2 dilution assay of 1 part patient serum and 1 part reference serum with measurable PSA (8.8 ng/mL) reduced the total PSA measured to 4.1 ng/mL on Assay A. This value is slightly less than the expected value of 4.9 ng/mL ([0.99+8.8]/2). However, the result of this same dilution was significantly lower in Assay B (1.9 ng/mL). This suggested the presence of an inhibitory factor in the patient's serum resulting in an abnormally low PSA of 1.9 ng/mL instead of the expected 4.7 ng/mL (9.3/2). Likewise, a 1: 5 dilution of 1 part patient serum and 4 parts reference serum reduced the total PSA measured to 7.3 ng/nL on Assay A. This value was very close to the expected value of 7.2 ng/mL ([0.99+8.8*4]/5). However, the measured PSA result after a 1: 5 dilution on Assay B was 6.3 ng/mL, which is once again lower than the predicted value of 7.4 ng/mL (9.3/5). This indicates that the inhibitory serum factor continued to interfere with Assay B, even at low concentrations.

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Table 1. PSA dilution assay.

Sample	UMMC Ortho clinical Diagnostics Vitros 5600 Assay A (ng/mL)	Outside Institution Roche Cobas 6000 Assay B (ng/mL)	Comment
Patient serum, undiluted	0.99	<0.10	
Reference serum with known *undetectable PSA*	<0.10	<0.10	
Reference serum with known *detectable PSA*	8.8	9.3	
Patient serum diluted 1: 2 using reference serum with undetectable PSA	0.49	<0.10	Half of the value of 0.99 as expected on Assay A
Patient serum diluted 1: 5 using reference serum with *undetectable PSA*	0.21	<0.10	Approximately 1/5 of the value of 0.99 as expected on Assay A
Patient serum diluted 1: 2 using reference serum with *detectable PSA*	4.1	1.9	Interference seen in Assay B caused by the patient's serum
Patient serum diluted 1: 5 using reference serum with *detectable PSA*	7.3	6.3	Interference seen in Assay B caused by the patient's serum

The patient chose to be followed at the outside institution without salvage therapy, where the serial PSAs remained undetectable until the fall of 2014 when the PSA became detectable at 1.3 ng/mL, corresponding to the time when the outside institution changed their institutional PSA assay. It subsequently rose to 3.0 in the spring of 2015. The patient was seen again at UMMC in 2015 for a follow-up, and the PSA at the time showed a similar value of 2.88 ng/mL. The patient subsequently received combined androgen deprivation and salvage radiotherapy. The patient's medication list included 1 generic over-the-counter multivitamin per day for many years prior to the initial diagnosis of the prostate cancer, as well as during the follow-up period after prostatectomy. The patient's multivitamin contained 300 µg of biotin.

Discussion

Heterophile antibodies present in the serum are a well-established source of falsely elevated PSA. Heterophile antibodies are immunoglobulins (Igs) that bind to 1 or more animal Igs [5]. Heterophile antibodies usually are naturally occurring, but occasionally result from contact with animals [8]. The prevalence of heterophile antibodies in the general population is unknown, and studies report a range from 3.4% [10] to 40% [11] in patient samples. The immunoenzymatic PSA assays use a solid-phase anti-PSA animal Ig, which binds to one site of the PSA molecule, while a second Ig labeled with a quantifiable tracer (electrochemiluminescent) binds to the PSA molecule at a separate distant site. Both the labeled tracer and the solid-phase antibody are derived from animals immunized against human PSA. In a typical case, PSA is bound by both the solid-phase Ig and the tracer Ig, and the amount of bound tracer is proportional to the PSA level. In the case of falsely elevated PSA due to heterophile antibodies, the heterophile antibody itself acts as excess PSA by binding both the solid-phase Ig and the tracer Ig, resulting in a falsely elevated result. However, an analogous mechanism has not been proposed for a falsely low or undetectable PSA. This may involve antibody binding to the same site as either the solid-phase Ig or the tracer Ig on the PSA molecule itself, but not both as in the case of heterophile antibodies. This consequently blocks the interaction of the PSA molecule with the assay reagents via competitive inhibition and thus is expected to result in a lower than expected PSA concentration [12].

It is important to consider that there are other mechanisms for discrepancies between different immunoassays in addition to antibody interference. PSA in serum is most often complexed with alpha 1-antichymotrypsin. However, 12–15% of prostate cancer patients demonstrate predominantly uncomplexed or free PSA [13]. Commercial immunoassays show variations in reactivity to the uncomplexed form of PSA [14], and it has also been suggested that the use of a thrombin tube for serum collection can result in a falsely negative PSA [15]. However, neither of these scenarios are consistent with the results of the above dilution studies, which suggests the presence of an inhibitory factor in the patient serum. Of the possible sources of immunoassay interference, the presence of human anti-animal antibodies (HAAA) is the most likely in this scenario. These are high-affinity antibodies produced against a specific animal IgG or IgM [16]. Human anti-mouse antibodies (HAMA) are most common, but anti-animal antibodies against cattle, goat, rabbit, and sheep antibodies are also possible and are present in

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30–40% of patient samples [17]. HAMA have been shown to interfere with numerous analytes, including cardiac marker assays thyroid function tests, drugs, and tumor markers such as CA-125 [12]. Furthermore, anti-PSA antibodies have been shown to exist in patients with prostate cancer and BPH. By blocking specific PSA epitopes, immune complexes with the PSA molecule can result in a falsely negative result [18]. Other serum factors that have been shown to interfere with immunoassays include complement, lysozyme, and paraprotein [12]. One additional source of potential immunoassay interference is the biotin contained in our patient's multivitamin supplement. Biotin has been shown to interfere with the results of streptavidin-based immunoassays [19]. Both the Ortho Clinical Diagnostics and Roche assays are streptavidin-based and biotin has been shown to potentially interfere with both assays [19,20]. In addition, our patient was taking a daily multivitamin with a significantly lower value of biotin (300 µg/day) than the level reported to potentially cause erroneous results (10 mg/day) [19]. While it is possible that one of the previously described non-antibody factors was responsible to the falsely decreased PSA results of our patient, the lack of inhibitory effect on the UMMC assay suggests a highly specific process that may be consistent with an antibody-mediated effect. Additionally, the undiluted patient serum was also tested using a Siemens Centaur analyzer, which yielded total PSA level in concordance with the Ortho Clinical Diagnostics result and a separate Roche analyzer at another institution, which also yielded a falsely low result. These results support the hypothesis that there is a serum factor interfering specifically with the Roche assay but not with other similar assays.

More recently, there has been interest in the utility of reverse-transcription polymerase chain reaction (RT-PCR) for the detection of circulating tumor cells (CTCs) in prostate cancer patients. Such RT-PCR assays do not quantify levels of circulating PSA, but rather test for the presence of PSA mRNA in peripheral blood samples [21]. RT-PCR for the detection of PSA mRNA is a potentially useful predictor of biochemical-free survival in both the pre-operative and post-operative settings [22]. In addition, it is not subject to the same sources of assay interference as are the previously discussed immunoassays. However, the use of RT-PCR results for guiding adjuvant treatment recommendations in the absence of accurate serum PSA levels remains unclear.

Conclusions

The prevalence of falsely undetectable PSA is unknown. There are a number of potential sources for interference with immunohistochemical assays for PSA. In select patients who are at high risk for prostate cancer recurrence after primary therapy such as surgery, it is imperative that clinicians interpret PSA results in the context of all available clinical data and consider retesting serum using a different PSA assay when a spurious result is suspected.

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Conflicts of interest

None.

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