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Elevated testosterone on immunoassay in a patient with metastatic prostate cancer following androgen deprivation therapy and bilateral orchiectomy

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ABSTRACT

We present the case of an 83-year-old man with metastatic prostate cancer who had testosterone levels reading above castration range despite appropriate medical and surgical castration. Mass spectrometry was performed to confirm presence of testosterone, but no testosterone was detected. The elevated testosterone as measured by standard immunoassay was postulated to be secondary to heterophile antibodies in the patient's serum. This report highlights the need for a high index of suspicion for interference in testosterone immunoassays when levels remain mildly elevated. Mass spectrometry may provide a more reliable method by which to detect testosterone concentration prior to escalation of care.

Introduction

Prostate cancer is the most common cancers in men, diagnosed at a rate of more than 100 new cases per 100,000 men annually in the United States.[1] Androgen deprivation therapy (ADT) is the cornerstone of treatment for metastatic prostate cancer and may be achieved by medical castration or surgical orchiectomy, with a goal of suppressing testosterone to below 50 ng/dL.[2] Commercial immunoassays are the most common method by which serum testosterone levels are measured, though these assays have limitations.[3] In this report, we describe the case of a patient with metastatic prostate cancer whose testosterone levels never reached castration range on immunoassay despite initiation of ADT, prompting bilateral orchiectomy with levels still above castration range. Mass spectrometry (MS) subsequently demonstrated that the immunoassay was inaccurate.

Case presentation

This is a report of an 83-year-old man with a history of localized prostate cancer first diagnosed in 1998 after he was found to have an elevated prostate-specific antigen (PSA) of 5 ng/mL. He was treated with radiation therapy along with 2 years of ADT. His PSA became undetectable and remained so until 2010. Between 2010 and 2014, his PSA rose to 0.8 ng/mL. No evidence of metastases was found on subsequent imaging, so he was observed for several years without treatment.

In 2019, his PSA increased to 2.8 ng/mL and a positron emission tomography (PET) scan demonstrated evidence of distant bone metastases. He was restarted on ADT along with abiraterone acetate and prednisone, but his testosterone level remained at 75–76 ng/dL on multiple checks. Therefore, in June 2020, he underwent bilateral orchiectomy with surgical pathology demonstrating bilateral atrophic testes. Again, his testosterone remained detectable at 84 ng/dL.

In December 2020, he was then referred to our institution for consideration of escalation of care in the setting of a rising PSA at 4.4 ng/mL, with total serum testosterone on immunoassay of 80 ng/dL. Mass spectrometry (MS) was performed to evaluate for ectopic testosterone production and demonstrated a result of less than 1 ng/mL, confirming castrate-resistant state of prostate cancer but also suggestive of immunoassay error. His most recent PSA was 5.05 ng/mL and he is continuing on abiraterone and prednisone.

Discussion

Here we present the case of a patient with metastatic prostate cancer, initially thought to be refractory to medical castration, who underwent bilateral orchiectomy. However, low levels of testosterone remained

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detectable on subsequent blood tests. True testosterone level was evaluated by MS, confirming appropriate surgical castration and revealing possible issues with the standard serum testosterone immunoassay. We believe the discrepancy in this patient's lab values is likely secondary to either immunoassay interference due to heterophile antibodies or known inaccuracies of such assays at low concentrations of testosterone.[3]

Radioimmunoassay and chemiluminescent immunoassay are the most common methods used to determine total serum testosterone level.[3] Both methods use anti-testosterone antibodies bound to a solid phase reactant to which the patient's serum is introduced. In radioimmunoassay, testosterone in the patient's serum displaces radioactive antigen which can be measured to determine the concentration of hormone. In chemiluminescent assays, a second anti-testosterone antibody bound to an enzyme is introduced, followed by a substrate. The enzyme metabolizes the substrate resulting in a luminescent product, and the signal is used to determine hormone concentration. The anti-testosterone antibodies are typically animal-derived, most frequently mouse IgG.[4]

Immunoassays may overestimate testosterone levels and are particularly unreliable at low concentrations (e.g. below 100 ng/dL), as was the case for our patient.[3] MS offers an alternative with superior reliability over a broad range of concentrations.[3] Heterophile antibodies, specifically human anti-mouse antibodies (HAMAs), are a relatively common source of interference in chemiluminescent assays. HAMAs crosslink the two mouse IgG antibodies used and produce a falsely elevated signal (Fig. 1). The prevalence of these antibodies may be as high as 30%, but as their affinity is typically weak, their effect may not be apparent unless serum testosterone concentration is low.[5]

The etiology of HAMAs is unclear, but occupational exposure to animals, prior exposure to immunotherapy and polyclonal gammopathy secondary to malignancy have all been identified as possible causes. Ramaeker et al. describe the case of a pre-menopausal woman with elevated serum testosterone levels on immunoassay.[5] Their patient was found to have a polyclonal gammopathy and was subsequently diagnosed with acute myelogenous leukemia. When immunoprecipitation was performed to remove serum IgG, repeat testosterone level was more than 90% reduced. In another case, Cheng et al. describe a post-menopausal woman with elevated testosterone on immunoassay but normal levels on MS.[4] This patient had no evidence of malignancy and further workup was deferred as the clinical picture was consistent with heterophile antibodies. The presence of HAMAs was considered to be idiopathic in this report, and this may be the case with our patient.

Given that our 83-year-old patient continued to have detectable levels of testosterone on multiple serum tests but none on MS, we suspect that the abnormal findings were secondary to heterophile antibodies or known inaccuracy of immunoassay at low concentration. It is possible that this patient could have been spared surgical castration if MS had been performed at the time of his initial evaluation. It is imperative that a high degree of suspicion is maintained for "false alarms" if low levels of testosterone are detected in the setting of medical castration before escalating care. It should be noted that MS is becoming increasingly available and affordable and should be strongly considered as a diagnostic test when testosterone levels remain mildly elevated despite appropriate medical and/or surgical castration.

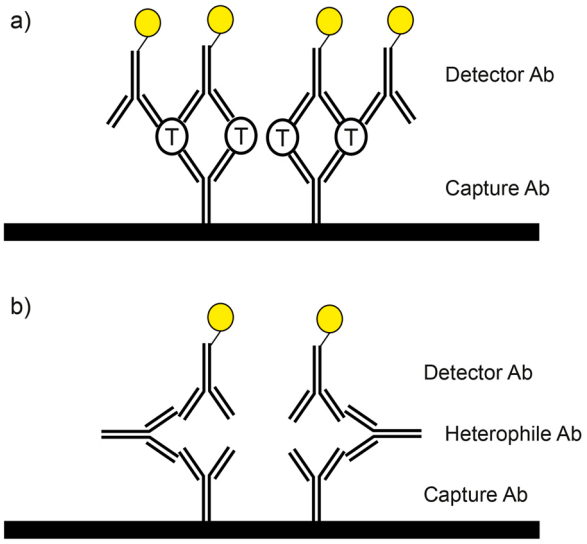

Fig. 1. a) Under normal circumstances, the target analyte is bound by both a capture antibody and a detector antibody conjugated with a signal molecule. b) Heterophile antibodies bind to the Fc regions of both capture and detector antibodies, producing a spuriously elevated signal in the absence of target analyte.

Conclusion

This is a case report of a patient with metastatic prostate cancer found to have serum testosterone concentration that was mildly elevated despite ADT and bilateral orchiectomies. Mass spectrometry demonstrated that the true hormone level was in fact consistent with adequate androgen suppression. These findings are consistent with either immunoassay interference from heterophile antibodies or known immunoassay inaccuracy at low concentrations. In patients with testosterone levels elevated on immunoassay despite initiation of ADT, mass spectrometry should be considered prior to escalation of care.

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