

PSA Measurement Following Prostatectomy: An Unexpected Error

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Abstract. *An unexpected and exceptional observation was made during the biological follow-up of a patient who had undergone a radical prostatectomy for a prostate adenocarcinoma. A residual PSA level was first considered as a reliable marker of relapse. Certain limits are highlighted concerning the reliability of the PSA assay, which must be interpreted along with all the other prognostic information on the tumor and possibly verified using other biological techniques.*

Elevated PSA levels, measured in the context of biological follow-up of prostate cancer following radical prostatectomy, are a reliable marker of relapse that precedes clinical relapse and can justify changes in therapy (1). The careful analysis of such biochemical relapse (date of biochemical relapse, rapidity of relapse and PSA kinetics), correlated with histological data from the surgical specimen is currently an essential aspect of patient management. Evaluation of this information is used to determine the need for second line therapy.

Clinical Case

A 65-year-old patient had undergone a radical prostatectomy in September 2004, following diagnosis of prostate adenocarcinoma, with a Gleason score 6 and with two nodules to the right and left. The prognosis was good, in terms both of pre-operative tests (clinical stage T1c, initial PSA 8 ng.mL⁻¹, two positive biopsies of a few millimeters) and post-operative histological analysis (pT2b lesion with no prostatic capsule infiltration, no perineural invasion and no seminal vesicle involvement, with a few PIN lesions, pNo two lymph nodes examined). The scintigraphy performed in June 2004 identified two areas of increased uptake at the left chondro-costal junction, attributed to a previous known trauma.

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Three months after surgery, as part of programmed follow-up, the PSA level was measured at 1.75 ng.mL⁻¹. A new specimen was assayed and the PSA level was confirmed to be 1.84 ng.mL⁻¹.

The possibility of lymph node micrometastases was considered to account for the residual PSA level since only two external iliac lymph nodes had been analyzed at the time of surgery. However a further PSA assay, undertaken at a different location determined an almost undetectable PSA level. The validity of biochemical measurements from different laboratories was therefore in question and analytical interference leading to the elevated PSA value was suspected.

Three separate assays performed on a DXi 600[®] system (Beckman Coulter Inc, Brea CA 92821, USA) showed high PSA levels in the three different specimens taken between December 2004 and December 2005. The measured values, which showed no significant variation, were 1.75, 1.84 and 1.85 ng.mL⁻¹. In contrast, PSA measurements obtained using two other different analytical systems (Kryptor[®], Brahms Inc, Hennigsdorf, Germany and PSA RIACT[®], Cis Bio[®], Saclay, France) gave a result of <0.04 ng.mL⁻¹, which corresponded quite closely to the clinical evaluation based on the known prognostic factors for this tumor.

In view of the discordance between the biological results and the clinical data, it was decided to investigate the possibility of interference (2). Interference in the Beckman Coulter[®] system could be eliminated only through dilution of the specimen or pretreatment of the specimen in a heterophilic blocking tube (HBT Scantibodies, Scantibodies Laboratories Inc, Santee CA 92071, USA). After pretreatment, the levels obtained for the initial sera dropped dramatically to 0.028 ng.mL⁻¹ and 0.08 ng.mL⁻¹, respectively.

Discussion

Numerous cases illustrating interference in immunoassays have been reported in the literature for over 20 years. Such interference can stem from many different sources and can disturb the immunological reactions during the assay. The mechanisms of interference are based on the existence in the blood specimen of either unusual molecules or molecules in an abnormally high concentration. Three main types of

immunological interference have been documented: non-specific interference attributed to the presence of autoantibodies or abnormal molecules; specific interference from substances with analogous structure (such as drug interference) and interference related to the presence of heterophilic antibodies, sometimes referred to as polyspecific antibodies or heteroantibodies, anti-immunoglobulin antibodies (anti-Ig) present in human specimens that recognize the animal immunoglobulins used in the assay and which can considerably falsify test results.

There are two methods for identifying interference. Firstly dilution is a method of identifying a difference in affinity between the antigen to be assayed and the antigen of the specimen due either to a difference in structure or to the presence of an interfering substance. Secondly pretreatment of the patient's serum in heterophilic binding tubes containing murine IgG directed against human IgM binds the potentially interfering heterophilic antibodies. Most manufacturers of immunoassay reagents use animal-based monoclonal or polyclonal antibodies (mouse, goat or rabbit for example) in their assays. In the present case, mouse antibodies were used. If a patient possesses immunoglobulins directed against these same antibodies, a bridge will form during the immunological reaction in the assay, simulating the presence of an antigen and giving a false result: falsely low in the case of competition assays, falsely elevated in the case of "sandwich" techniques as is this particular case.

In the present case, the presence of heterophilic antibodies was suspected. These heterophilic antibodies in the patient's serum could be from a variety of sources (3), including diagnostic or therapeutic treatment with immunoglobulins, prolonged contact with animals, food allergies or autoantibodies related to autoimmune disorders.

Firstly, a test to detect human anti-mouse antibodies (HAMA) was performed using the HAMA-ELISA Medac kit (Medac Diagnostika, Wedel, Germany). This gave a negative result ($<40 \text{ ng.mL}^{-1}$). Similarly, no heterophilic antibodies were found when the specimens were tested with organ sections from rats and mice.

Then tests for rheumatoid factor using two different techniques, anti-human Ig enzyme-linked immunosorbent assay (ELISA) and Waaler-Rose anti-rabbit Ig (ELISA) proved negative. Negative results were also obtained for anti-tissue autoantibodies, anti-smooth muscle autoantibodies, anti-M2 mitochondrial autoantibodies and anti-gastric parietal cell autoantibodies.

Despite all these tests, the origin of the interference in the case of this patient was not detected and still remains unexplained to this day.

To the best of our knowledge, no previous reports have been made in the literature concerning interference in PSA measurement, although the case is most probably not unique. It highlights the need for close co-operation between biologists and clinicians.

The secondary medical consequences of false-positive or false-negative biological results related to assays involving Ag-Ab reactions are not as notorious as medical errors of a therapeutic or surgical nature, even though the effects on the patient can be just as harmful (4). Sources of immunoassay interference are numerous and include the presence of autoantibodies (rheumatoid factor for example) or of heterophilic antibodies, medication or related metabolites and cross-reactivity between molecules. In the large majority of cases such interference is detected or avoided through the use of biological tests for the suspected interference, but occasionally the interference remains. A study concerning over 11,000 serum assays showed that a 4% interference rate in a conventional immunoassay could, after systematic detection of interference, be reduced to 1% or 0.06% by improving assay conditions (5).

Conclusion

For the biological follow-up of this patient, given that the nature of the interference has so far not been determined, dilution and/or pretreatment of specimens on a matrix that blocks heterophilic antibodies is recommended for any future immunoassays.

Clinicians must be aware of the potential for biological interference in Ag-Ab reactions. In the event of discordance between clinical data and biological results, dialogue between the clinician and biologist is vital. Systematic dilution of the specimen, the use of a different assay system or pretreatment of the specimen with a heterophilic blocking tube or a protein A type matrix that binds heterophilic antibodies should be considered.

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