Clinical Chemistry 54:7
1248–1249 (2008)

Citation Classic

Testing New PSA Subforms to Enhance the Accuracy of Predicting Cancer Risk and Disease Outcome in Prostate Cancer

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Featured Article: Lilja H, Christensson A, Dahlen U, et al. Prostate-Specific Antigen in Serum Occurs Predominantly in Complex with α1-Antichymotrypsin. Clin Chem 1991;37:1618–25.[2]

Prostate-specific antigen (PSA),[3] a kallikrein-like serine protease, is one of the most abundant proteins secreted by the prostate into seminal fluid *(1)*. In the early 1990s, when the study reported in the paper presented here was initiated, serum testing for PSA was coming into widespread use as a means of testing for prostate cancer. Nevertheless, several aspects of PSA in serum remained puzzling; notably, different assays yielded up to 4-fold differences in measured serum PSA concentrations, despite standardization against PSA from seminal fluid.

Before our investigation, PSA in seminal fluid was known to occur as active monomeric peptidase *(1)*, but the nature of PSA in blood remained unclear. We speculated that release of catalytic PSA into an abundance of protease inhibitors in blood could prompt PSA to form inhibitor complexes, similar to the way the digestive enzyme trypsin from the pancreas forms enzyme-inhibitor complexes with α_1-antitrypsin in blood. A search for interactions between PSA and a panel of common protease inhibitors in serum allowed us to show that in vitro, catalytic PSA was able to form stable complexes with certain of these inhibitors, such as α_1-antichymotrypsin *(2)*. We hypothesized that this finding could have an important influence on immunodetection of PSA in blood. We searched for monoclonal antibodies that would enable recognition of PSA bound to α_1-antichymotrypsin, because such antibodies could be critical reagents in immunoassays that could comprehensively characterize and accurately measure the concentrations of all major forms of PSA occurring in serum in vivo. The 1991 report in *Clinical Chemistry* featured here resulted from these efforts.

Our collaborative work with researchers in Finland led to the generation of a set of anti-PSA monoclonal antibodies with novel binding characteristics. One clone recognized PSA isolated from seminal fluid but not PSA when it had formed the complex with α_1-antichymotrypsin, because the critical epitope became inaccessible in this complex. We also found monoclonals that independently recognized both free and complexed PSA in a roughly equimolar manner. Using these antibodies (along with a polyclonal to α_1-antichymotrypsin), we developed the first assays specific for the major PSA forms in serum, including an assay that uniquely recognized the free uncomplexed PSA form and an assay for total PSA that in an approximately equimolar manner measured the sum of free PSA and PSA in complex with α_1-antichymotrypsin.

We applied these assays to analyze sera from 64 men who were suspected to have prostate cancer. By use of gel filtration chromatography, we found the majority of PSA immunoreactivity (median, 86%) to be in complexes of the size expected for a 1:1 molar ratio PSA:α_1-antichymotrypsin. Only a minority of the PSA in serum was recognized in the assay for free PSA, and this immunoreactivity was shown by gel filtration to correspond to the size expected for free PSA. The presence of these different forms of PSA in serum provided an explanation for the variability previously observed for the serum concentration of PSA; presumably, the assays that yielded low values used antibodies with inefficient recognition of complexed PSA.

This study provided the basis for later work demonstrating that both free and complexed PSA are increased in serum of men with prostate cancer, but the ratio of free to total PSA is lower in men with vs men without cancer *(3, 4)*. This ratio can help to differentiate prostate cancer from benign prostate conditions. The assays for total PSA and free PSA, with minor modifications, became commercial assays and are in widespread clinical use today.

The frequent citation of this paper is a reflection of the continued interest in assays of PSA subforms in serum as a means of improving screening for prostate cancer. Although PSA is a highly useful marker—one of the most

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[2] This paper has been cited nearly 600 times since publication.
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Received January 31, 2008; accepted March 20, 2008.
Previously published online at DOI: 10.1373/clinchem.2007.101204
[3] Nonstandard abbreviation: PSA, prostate-specific antigen.

valuable of all cancer markers—it is not perfect. PSA screening can lead to unnecessary biopsies in men with benign prostate conditions, and to overdiagnosis and overtreatment of clinically insignificant prostate cancers. A second problem is that the current standard threshold for pursuing a diagnosis of prostate cancer (4 μg/L) misses some significant cancers *(5)*. To address these problems, investigators continue to test new subforms of PSA and to refine established PSA testing methods *(6)*.

Grant/Funding Support: H. Lilja was supported by the grants: P50-CA92629 SPORE Pilot Project 7 from the National Cancer Institute, U24 RFA CA 07-112 NIH Clinical Proteomic Technology Assessment for Cancer, Swedish Cancer Society project no. 3555, Swedish Research Council (Medicine) project no: 20095, European Union 6th Framework contract LSHC-CT-2004-503011 (P-Mark), FP6-2004-Mobility-11 contract 040569. He also received support from Fundacion Federico SA.
Financial Disclosures: H. Lilja holds patents for free PSA and hK2 assays.
Acknowledgment: Janet Novak of Helix Editing contributed substantial amount of editing to the manuscript and was paid for her work by Memorial Sloan-Kettering Cancer Center.

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Editor's Comment

The figure below is from the original article featured here. In their study Lilja and colleagues used three monoclonal antibodies with epitope specificities against noncomplexed PSA (△), PSA complexed to α_1-antichymotrypsin (×), or both (□), plus a polyclonal antibody directed against α_1-antichymotrypsin. By combining gel filtration chromatography of patient sera and PSA immunoreactivity experiments these authors identified a larger peak corresponding to a mass of 80–90 kDa and a smaller peak corresponding to a mass of 25–40 kDa. These results provided evidence that PSA complexed to α_1-antichymotrypsin constitutes the predominant form *in vivo*, with free noncomplexed PSA being a minor fraction in serum.