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Detection of Complexes between Prostate-Specific Antigen and Protease Inhibitors in Plasma

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Featured Article: Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and α_1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51:222–6.[2]

Prostate-specific antigen (PSA)[3] had been in clinical use for several years when we encountered a problem with 2 samples that did not give expected results upon dilution. To explore this finding, we subjected the samples to gel filtration and found that a major part of immunoreactive PSA had a molecular size of about 90 kD rather than the expected size of 30 kD. Because PSA is a protease, we assumed that the 90-kD component was a complex between PSA and a protease inhibitor. We therefore developed assays for possible complexes using a PSA antibody for capture and, as tracer, antibodies to α_1-protease inhibitor, α_1-antichymotrypsin (ACT), inter-α-trypsin inhibitor, and α_2-macroglobulin. PSA complexes with all these inhibitors were detected, but the PSA–ACT complex was by far the most abundant one. At comparable PSA concentrations, that of PSA–ACT was higher in sera from patients with prostate cancer than in those with benign prostatic hyperplasia (BPH). Thus the diagnostic validity was improved by calculating the proportion of PSA–ACT relative to total PSA.

Our 1991 paper in *Cancer Research* was the first to report findings demonstrating that most of PSA in circulation consists of a complex with ACT. Our findings were soon confirmed in an independent study by Lilja et al. *(1)*. However, the huge excess in plasma of free ACT, part of which was nonspecifically adsorbed to the solid phase, caused a variable background that hampered assay performance. The problem was reduced, but not eliminated, by measuring PSA–ACT and total PSA simultaneously with a double-label assay, by correcting for the nonspecific background measured separately in each sample, and by using a monoclonal antibody to the PSA–ACT complex *(2)*.

The reason for devoting so much effort to the accurate measurement of PSA–ACT was that it is the most cancer-specific form of PSA. Other PSA complexes may account for up to 10% of total PSA, but contrary to PSA–ACT, the proportions of PSA- α_1-protease inhibitor and PSA-α_2-macroglobulin are higher in BPH than in cancer. Thus, measurement of all complexed forms of PSA together is inferior to measurement of PSA–ACT. Likewise, the proportion of free PSA (%fPSA) is theoretically inferior to PSA–ACT, but measurement of %fPSA is metrologically more favorable and has therefore become the preferred method *(3)*. The impact of %fPSA on the probability of finding a prostate cancer on biopsy is considerable, e.g., at a total PSA concentration of 4 μg/L, the probability of cancer increases from 3% to 39% when the %fPSA decreases from 35% to 7%. Even when total PSA is 10 μg/L, the probability is below 15% when %fPSA is above 25% *(4)*. The risk of prostate cancer detection within the next 7 years, on the other hand, is 33% when total PSA is 2–3 μg/L and %fPSA is below 10% *(5)*. These examples show that measurement of the 2 major forms of PSA can be used both to improve early detection of aggressive disease and to avoid unnecessary biopsies.

PSA is considered the most useful tumor marker, but the use of PSA is not without problems. The widespread use of PSA for opportunistic screening has dramatically increased prostate cancer incidence, but it has also caused extensive overdiagnosis; about half of the men with a positive biopsy would never develop symptoms of prostate cancer during their lifetime. Ideally, these men should not have undergone biopsy. The need to do a biopsy should be based on risk calculation rather than fixed cutoff values for PSA. In particular, elderly men with BPH and a moderately increased PSA in combination with a high %fPSA have a relatively low risk *(5)*. Furthermore, the need to perform a biopsy in these individuals is low because a high %fPSA is associated with a low risk of aggressive cancer *(6)*.

Risk calculation algorithms that use total and free PSA, prostate volume, and findings of digital rectal ex-

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[2] This paper has been cited more that 780 times since publication.
[3] Nonstandard abbreviations: PSA, prostate-specific antigen; ACT, α_1-antichymotrypsin; BPH, benign prostatic hyperplasia; %fPSA, proportion of free PSA.

amination are freely available *(4)*. The algorithms should be modified to include estimates of probability to gain insight regarding overall and disease-free survival. Use of such algorithms improves the chances of finding cancers that can be cured while avoiding detection of tumors that do not threaten the health and life of the patient. These goals can be achieved at minimal additional costs by using currently available markers and clinical findings more efficiently.

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