COMPLEXED PSA AND SERUM HER-2/*neu*: NEW SERUM MARKERS FOR THE MANAGEMENT OF PROSTATE AND BREAST CANCER

Rainer Neumann

European Scientific Affairs, Bayer Healthcare, Diagnostics Division, Leverkusen, Germany

Summary: Prostate cancer is the most common type of cancer in men, and breast cancer the most common malignancy in women. Based on the improvements in understanding the biology of these cancers, treatment and ongoing development of new diagnostic tools aiming towards »personalized medicine« are under clinical evaluation. This article describes the improved value of one of the immunodetectable forms of the prostate-specific antigen (PSA), the so-called »complexed PSA« (cPSA), in detecting prostate cancer, and also presents an overview of publications on the clinical value of a new immunoassay detecting the extracellular part of the HER-2 molecule in serum of breast cancer patients. Based on the publications cited, it will become obvious that complexed PSA improves the limitations of the specificity of PSA isoforms currently mainly applied to diagnose prostate cancer, and that serum HER-2/*neu* offers information about the actual HER-2 status thus adding information clinically relevant to the probability of optimizing therapy of breast cancer patients.

Key words: prostate cancer, prostate specific antigen, complexed PSA, breast cancer, HER-2/*neu*

The detection of prostate cancer by complexed PSA

Based on the characteristics of a slow growing cancer, the aim to detect prostate cancer is the detection of potentially curable cancers that are either life-threatening or reduce the quality of life.

In detecting prostate cancer, beside digital rectal examination (DRE), prostate-specific antigen (PSA) has shown to increase the number of cancer cases detected. Prostate Specific-Antigen (PSA) applied to detect prostate cancer has emerged as the best of all human tumor markers currently in use, however, the biggest problem with PSA is the lack of specificity (which means a certain rate of false-positive results), negatively impacting the acceptance of PSA testing in diagnosing prostate cancer.

To improve the specificity of PSA testing, several approaches, e.g. building the ratio of serum PSA and the prostate gland volume or PSA velocity, which is the longitudinal measurement of serum PSA, to develop the rate of increase in PSA/year, are being under clinical evaluation these days (1).

The approach most widely used to optimize prostate cancer detection stemmed from the observation of Stenman and Lilja (2–5) noting PSA in serum being present in several forms including free, uncomplexed PSA and complexed PSA, e.g. PSA complexed to α-1-antichymotrypsin.

Free PSA (fPSA) is unbound, and is associated with benign prostatic hyperplasia (BPH), and complexed PSA (cPSA) is bound with other molecules, e.g. chymotrypsin, and the proportion is higher in men with prostate cancer.

Clinically speaking, men with prostate cancer tend to have lower percent free PSA (fPSA/tPSA) values than men with BPH. Thus, the percent free PSA values have been reported since several years to act as an aid in optimizing decisions about taking biopsies to clarify a suspicious result.

In order to measure PSA, it has been recommended that all PSA assays should be equimolar in their response to free and complexed PSA, because if an assay is not an equimolar one, this will bias the outcome, and can result in unacceptable clinical performance. Furthermore, these assays should be calibrated to the World Health Organization (WHO) First International Standard for PSA (90:10). There is an interesting publication on this topic, determining which assays currently used in England fulfill these criteria (6).

Address for correspondence:

Prof Dr R. Neumann, Bayer Vital GmbH, D-51368 LEVERKUSEN
Tel: 0049 214–30–82084; Fax: 0049 214–30–51213
E-mail: rainer.neumann@bayerhealtcare.com

In 1998, a fully automated immunoassay to specifically detect complexed PSA was described by Allard and colleagues (7). In this assay, the binding site for building complexes is blocked by an antibody, and therefore only complexed PSA can be detected in a specific way. Details of the assay format are given in *Figure 1* and *2*.
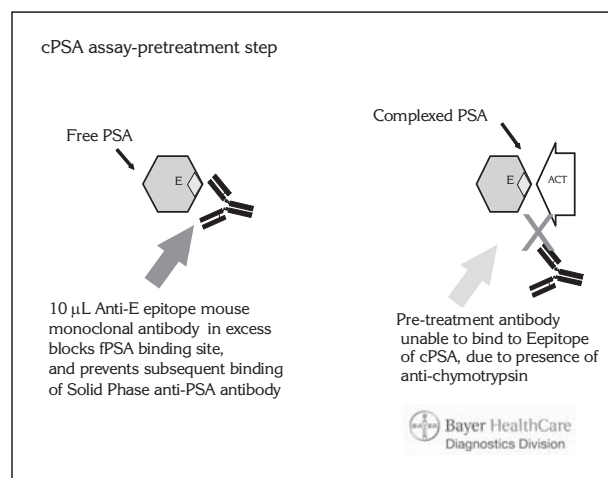

Figure 1   The cPSA consists of 2 steps. In the pretreatment step outlined here, an antibody blocks the binding site of fPSA to which complex forming proteins are bound.
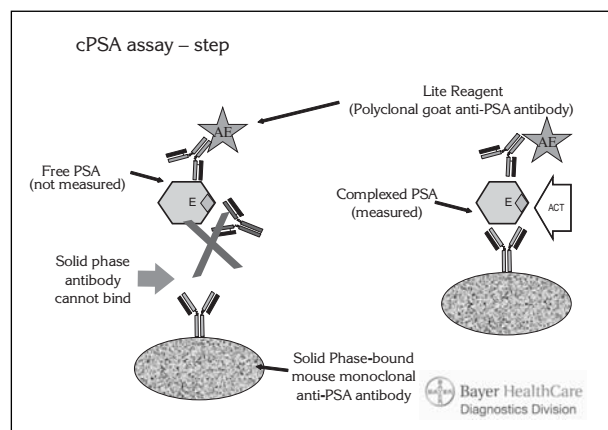

Figure 2   Within this cPSA assay step acridinium ester labeled polyclonal goat anti-PSA antibodies bind to the PSA molecules. Solid phase-bound mouse monoclonal anti-PSA antibodies added are only able to bind to complexed PSA. The signal generated after washing steps correlates to the concentration of cPSA.

Complexed PSA has several advantages over fPSA, including the stability of the cPSA molecule being higher than for fPSA, and less sensitivity to prostatic manipulation.

There are publications demonstrating cPSA being of specific clinical value at the range of 2–4 ng/mL, where, it has been reported, up to 25% of prostate cancers occur.

Over the years, there have been several reports demonstrating the improved clinical value of using cPSA as a differentiator between patients that should be referred to biopsy for the clarification of an elevated PSA result.

In 2002, a review on the publications dealing with the clinical value of complexed PSA till that time was published in Urology (8). As summary, the authors stated that cPSA is a versatile assay for screening and diagnosis of prostate cancer, prediction of the pathologic stage, and monitoring of patients with PCa. Furthermore, it was stated that cPSA is significantly more specific than tPSA at all clinically relevant sensitivities. In this review was mentioned that cPSA is as specific as % fPSA.

Based on the fact that cPSA reduces the number of unnecessary biopsies by up to 20%, application of cPSA is less costly and, based on its increased specificity, reduces the anxiety in patients reported to have an elevated PSA result (9).

In laboratory medicine we are familiar with using Receiver Operating Characteristics (ROC) curves to compare 2 assays with each other with regards to their diagnostic performance (10, 11). The ROC method results in Area Under the Curve (AUC) results providing an overall measurement which might not reflect the differences in the clinical value of the markers compared at a distinct cut-off value chosen, e.g. 4 ng/mL tPSA as cut-off value for PSA testing.

In order to characterize which of two markers is of more clinical usefulness within a restricted range, the recently introduced Discordance Analysis Characteristics (DAC) has been proven to be a better tool than ROC curves (12). Within a given range of sensitivities or specificities, this method is able to investigate only those patients who are discordantly categorized in comparison by the assay. The DAC analysis provides data, making decisions on the clinical value of two assays easier. The DAC analysis has been recently applied in a study in which data from 1624 patients were analyzed (13). Within the group of discordantly tested patients (different results for tPSA vs. cPSA), cPSA detected patients with a 2-fold higher risk for having prostate cancer and with an up to 5.5.-fold better specificity. cPSA, compared to tPSA, avoided more than 10% of unnecessary biopsies. This is in line with other publications reporting avoiding up to 20 % of unnecessary biopsies by using cPSA as a first line marker to detect prostate cancer. There are several additional retrospective and prospective studies from Europe, America, and also Asia, focussing on the clinical usefulness of cPSA and concluding that cPSA enhances prostate cancer detection (14–16).

Results from the European Prostate Cancer Detection Study suggest that, in patients with elevated PSA levels (4–10 ng/mL), complexed PSA is more accurate than total PSA in differentiating patients with benign and malignant diseases (15).

cPSA has recently been included as an alternative to tPSA into the guidelines of the National Comprehensive Cancer Network of the United States (17).

Based on all the publications, we can have more confidence in the results of this special PSA test for prostate cancer than in conventional testing.

Towards personalized medicine in breast cancer: the serum HER–2/*neu* test

The activation and over-expression of cellular oncogenes are considered to play an important role in the development of breast cancer, and based on improvements in the molecular understanding of the biology of cancers in general, treatment and ongoing development of diagnostic tools in the direction of »personalized medicine« continues to change towards the ultimate goal of personalized treatments which will, hopefully, promote survival. Increased emphasis is now put on less morbid treatments and improving the quality of life (18).

One important member of the oncogene family is the **H**uman **E**pidermal Growth Factor **R**eceptor known as HER-2, also commonly referred to as c-erbB-2. The HER-2/*neu* oncogene (*neu* because it was first detected in a neuroblastoma) encodes a transmembrane tyrosine kinase growth factor receptor that is expressed on the cells of epithelial origin. HER-2/*neu* exerts its effect on the cell growth through the tyrosine kinase portion of the molecule. The full-length HER-2/*neu* is composed of three domains: the internal tyrosine kinase portion, a short transmembrane section, and the extracellular ligand binding domain that is referred to as the extracellular domain (ECD). The ECD is a glycoprotein with a molecular weight between 97–110 kD and is released from the cell surface by proteolytic cleavage.

The most widely known new therapy that meets the goal of personalized medicine is a humanized monoclonal antibody directed against the external domain of the HER-2 receptor named Herceptin™. Its clinical activity has been demonstrated as a single agent in patients whose cancers overexpress HER-2. Given in combination with paclitaxel and docetaxel, in patients with metastatic disease, it achieves high rates of tumor regression with other drugs (19, 20).

Predicting response and survival in trastuzumab-based therapy is an unsolved problem. Ali et al (21) recently reported on their pooled analysis of 7 trials from first-line Herceptin™-based therapy (with or without chemotherapy) studies. In these, serial serum ECD levels were included (21). Pretreatment and post-treatment serum (16–120 days) from 307 patients and data from 236 patients on overall survival were reported. The authors concluded that patients with < 20 % decrease in serum HER-2/ levels have a decreased benefit from this kind of therapy, and patients who do not have a significant decrease in ECD levels
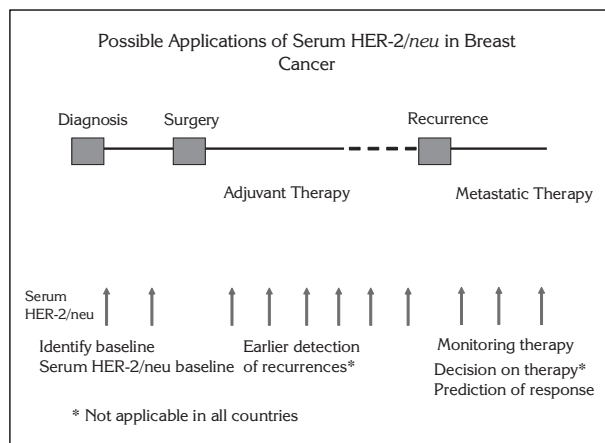

Figure 3   This figure summarizes the current status of applications of serum HER-2/*neu*. Monitoring therapy in metastatic breast cancer is today approved by FDA. Other potential applications of this serum marker have been published but not approved by FDA, and are therefore marked »Not applicable in all countries«.

should be considered for additional HER-2/*neu*-targeted therapies.

The most widely used methods to analyze the HER-2/*neu* gene and protein are the Fluorescent In Situ Hybridization (FISH), to detect gene amplifications of the HER-2 gene, and Immunohistochemistry (IHC), to detect the HER-2 protein by immunoassay for the analysis of either the full-length HER-2 molecule or the circulating ECD.

The serum HER-2/*neu* from Bayer Health Care's Diagnostics Division, Tarrytown, N.Y., has been licensed by the FDA for follow-up testing and therapy monitoring in metastasing breast cancer in women who have an initial value of 15 ng/mL or greater (22).

As FISH and IHC are reflecting the status of the gene or protein at that time point when the biopsy is taken, the ECD-HER-2/*neu* assay offers the opportunity for continuous measurements of this part of the HER-2 molecule.

A comprehensive review on the value of measuring the circulating levels of the HER-2/*neu* oncoprotein in breast cancer was published in 2004 by Carney et al (23).

In summary, the serum HER-2/*neu* serum test is a suitable method for monitoring patients with metastatic breast cancer. In addition, it can also be used to detect any progression of the disease and to determine the patient's response to therapy. High serum levels of HER-2/*neu* have also been confirmed as an independent risk factor for poor prognosis.

Conclusion

This overview points out that cPSA decreases the number of unnecessary biopsies at clinically relevant cut-off values, and thereby illustrates the value of

cPSA as a first line diagnostic test to detect prostate cancer.

Serum HER-2/*neu* as a new tool in monitoring therapy of metastatic breast cancer patients is becoming more and more important as a biomarker for assessing the real-time HER-2/*neu* status of these patients.

With the emergence of new HER-2/*neu*-targeted therapies, the upcoming role of this serum marker could be in determining prognosis and selecting patients for these specific therapies, as well as in evaluating response during treatment.

Both serum markers presented in this mini review aim to provide clinically relevant information for optimizing diagnosis and treatment of prostate and breast cancer patients.

KOMPLEKSIRANI PSA I SERUM HER-2/*neu*: NOVI SERUMSKI MARKERI U LEČENJU RAKA PROSTATE I RAKA DOJKE

Rainer Neumann

European Scientific Affairs, Bayer Healthcare, Odeljenje za dijagnostiku, Leverkuzen, Nemačka

Kratak sadržaj: Rak prostate je najčešći oblik raka kod muškaraca, a rak dojke najčešće maligno oboljenje kod žena. Zahvaljujući napretku u razumevanju biologije tih kancera, lečenje i razvoj novih dijagnostičkih sredstava koji se kreću u pravcu »personalizovane medicine« trenutno su podvrgnuti kliničkom ispitivanju. Ovaj članak opisuje unapređenu ulogu jednog oblika antigena specifičnih za prostatu (*prostate-specific antigen*, PSA), takozvanog »kompleksiranog PSA« (cPSA), u otkrivanju raka prostate, i uz to predstavlja pregled publikacija na temu kliničke vrednosti novih testova koji otkrivaju izvanćelijski deo molekula HER-2 u serumu pacijentkinja sa rakom dojke. Na osnovu navedenih publikacija, pokazaće se da kompleksirani PSA ima manje ograničenu specifičnost u odnosu na druge izoforme PSA koji se trenutno uglavnom primenjuju u dijagnostikovanju raka prostate, kao i to da HER-2*neu* iz seruma daje informacije o tačnom statusu HER-2, pružajući na taj način podatke klinički važne za potencijalno unapređivanje lečenja pacijentkinja sa rakom dojke.

Ključne reči: rak prostate, antigen specifičan za prostatu (*PSA*), kompleksirani *PSA*, rak dojke, HER-2/*neu*

References

1. Berger AP, Deibl M, Steiner H, Bektic J, Pelzer A, Spranger R, Klocker H, Bartsch G, Horninger W. Longitudinal PSA changes in men with and without prostate cancer: assessment of prostate cancer risk. The Prostate 2005; 64: 240–5.

2. Stenman U-H, Hakama M, Knekt P, Aromaa A, Teppo L, Leionen J. Serum concentrations of prostate specific antigen and its complex with alpha1-antichymotrypsin before diagnosis of prostate cancer. Lancet 1994; 344: 1594–8.

3. Stenman U-H, Leinonen J, Alfthan H, Ranninkko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and alpha-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.

4. Lilja H, Christensson A, Dahlen U, Matikainen M-T, Nilsson O, Petterson P, Lovgren T. Prostate-specific antigen in serum occurs predominantly in complex with alpha-1-antichymotrypsin. Clin Chem 1991; 37: 618–25.

5. Lilja H, Cockett ATK, Abrahamsson P-A. Prostate-specific antigen predominantly forms a complex with alpha-1-antichymotrypsin in blood. Cancer 1991; 70: 230–4.

6. Roddam AW, Rimmer J, Nickerson C, Ward A. Prostate-specific antigen: bias and molarity of commercial assays for PSA in use in England. Ann Clin Biochem 2006; 43: 35–48.

7. Allard WJ, Zhou Z, Yeung KK. Novel immunoassay for the measurement of complexed prostate-specific antigen in serum. Clin Chem 1998; 44: 1216–23.

8. Parsons JK, Partin AW. Applying complexed prostate-specific antigen to clinical practice. Urology 2004; 63: 815–8.

9. Babaian RJ, Naya Y, Cheli C, Fritsche HA. The detection and potential economic value of complexed prostate – specific antigen as a first line test. J Urol 2006; 175: 897–901.

10. Hanley JA, McNeil BJ. Meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982; 143: 29–36.

11. Obuchowski N, Lieber ML, Wians FH. ROC curves in clinical chemistry: uses, misuses, and possible solutions. Clin Chem 2004; 50: 1118–25.

12. Keller T, Butz H, Lein M, Kwiatkowski M, Semjonow A, Luboldt HJ, Hammerer P, Stephan C, Jung K. Discordance analysis characteristics as a new method to compare the diagnostic accuracy of tests: example of complexed versus total prostate-specific antigen. Clin Chem 2005; 51: 532–9.

13. Jung K, Lein M, Butz H, Stephan C, Loening SA, Keller T. New insights into the diagnostic accuracy of complexed and total prostate specific antigen using discordance analysis characteristics. J Urol 2006; 175: 1275–80.

14. Okihara K, Ukimura O, Nakamura T, Ushijima S, Mizutani Y, Kawauchi A, Naya Y, Kjima M, Mik T. Complexed PSA improves prostate cancer detection: results from a multicentre Japanese clinical trial. Urology 2006; 67: 328–32.

15. Djavan B, Remzi M, Zlotta AR, Ravery V, Hammerer P, Reissigl A, Dobronski P, Kaisary A, Marberger M. Complexed prostate-specific antigen, complexed prostate-specific antigen density of total and transition zone. Complexed/total prostate-specific antigen ratio, density of total and transition zone prostate-specific antigen: results of the prospective multicentre European trial. Urology 2002; 60: 4–9.

16. Herrmann W, Stöckle M, Sand-Hill M, Huebner U, Herrmann M, Obeid R, Wullich B, Loch T, Geisel J. The measurement of complexed prostate-specific antigen has a better performance than total prostate-specific antigen. Clin Chem Lab Med 2004; 24: 1051–7.

17. National Comprehensive Cancer Network: Clinical Practice Guidelines in Oncology – v.1.2004: Prostate Cancer Early Detection. Version 1.2004.

18. Chatterjee SK, Zetter BR. Cancer biomarkers: knowing the present and predicting the future. Future Oncology 2005; 1: 37–50.

19. Plosker GL, Kean SJ. Trastuzumab. A review of its use in the management of HER-2 positive metastatic and early-stage breast cancer. Drugs 2006; 66: 449–75.

20. Nahta R, Esteva FJ. Herceptin: mechanisms of action and resistance. Cancer Lett 2006; 232: 123–38.

21. Ali SM, Esteva FJ, Fornier M, Harris L, Kostler WJ, Lotz JP, Luftner D, Pichon MF, Tse C, Lipton A. Serum HER-2/neu change predicts clinical outcome to trastuzumab-based therapies. J Clin Oncol 42nd Annual Meeting. Vol 24 (185 Part 1) Abstract # 500).

22. Payne C, Allard JW, Anderson-Mauser L, Humphreys JD, Tenney D, Morris D. Automated assay for HER-2/neu in serum. Clin Chem 2000; 462: 175–82.

23. Carney WP, Neumann R, Lipton A, Leitzel K, Ali S, Price CP. Monitoring the circulating levels of the HER-2/neu oncoprotein in breast cancer. Clin Breast Cancer 2004; 5: 105–16.