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The need for further characterisation of prostate-specific antigen and its use in the management of prostate cancer

M.A. BLANKENSTEIN

Prostate-specific antigen (PsA) is generally considered to be one of the most useful circulating tumour markers. Its role in the detection of prostate cancer and the management of patients suffering from this disease is proverbial. The presence in the circulation of multiple molecular forms of PsA prevents proper standardisation and hinders comparability of the results of various assays. Nevertheless, it is this molecular heterogeneity which allows for the fine tuning of possibilities to distinguish patients with benign prostatic hyperplasia from those with prostate cancer: the ratio free/total PsA is very promising in this respect. When this ratio greatly outranges the values usually obtained, this may indicate the synthesis of an aberrant PsA species with totally different properties.

Key-words: characterisation of PsA; prostate cancer; benign prostate hyperplasia; molecular heterogeneity; ratio free/total PsA

PsA is the major protein secreted from the prostate and although there is room for debate, its discovery has been attributed to Wang et al (1). Its properties rank PsA among the most useful tumour markers available today and its role in the detection of prostate cancer as well as in the follow-up of patients suffering from this disease is well established (2). In the circulation, PsA exists in multiple forms, of which the free form and a complex with α1-antichymotrypsin (ACT) are the best studied today (3,4). A quantitatively at least as important form is a complex between PsA and α_2-macroglobulin. PsA complexed to this protein, however, escapes detection by the currently available immunoassays and studies on its (patho-)physiological role and metabolic fate await the development of a specific assay. It is clear that the occurrence of an analyte in multiple forms has consequences for standardisation (5). Nevertheless, efforts are currently being undertaken to improve the identification of patients with benign disease, who can be spared the burden of sextant biopsies and other diagnostic measures applied to patients with moderately elevated PsA levels in order to detect the presence of carcinomas. In this respect, proper reference ranges have just been set for selected reagents (6).

Recent studies indicate that PsA is a member of the human kallikrein family, and it has been indicated as hK3 in this respect (7). Models for the molecular conformation of PsA and the PsA-ACT complex have been published by Villoutreix et al (8,9). Development of molecular biological techniques, in particular the reverse transcription polymerase chain reaction (rt-PCR) nowadays enables the identification of tumour cells in the circulation (10) and allows for retrospective molecular analysis of paraffin embedded material from patients who already deceased. The latter application has resulted in the identification of an aberrant PsA messenger RNA (11).

Review of our recent experience

Soon after the discovery that PsA prevails in the circulation in multiple forms experiments were started to evaluate the implications of this finding for the day-to-day practice of PsA assays in the clinical chemistry laboratory. A method was set up for the chromatographic separation of free PsA (fPsA) from the PsA which was bound to α1-antichymotrypsin (PsA-ACT) and the fractions resulting from this separation were subjected to various total PsA assays. The results indicated that all methods tested detected both forms of PsA, be it with different affinities. The free/total ratio depended on the method used and the need for the development of assays specific for the different forms of PsA was stressed (12).

As it was clear that the recognition of both PsA forms differed among different methods, it was decided to investigate the degree of the disequimolarity of the various assays. To this end, two PsA preparations were made by repeated size exclusion chromatography, one containing fPsA and one containing PsA-ACT, each at a concentration of 3 ng/ml. Mixtures of the two preparations thus contained various proportions of the two molecular forms, but the total concentration was the same. By analysing mixtures with a great variety of PsA assays the equimolarity issue raised by some of the manufacturers could be clearly mapped on the one hand, and brought back to proportions on the other hand. Some assays produce a higher signal when they react with the free form than with the complex. In patients with a disturbed ratio free-to-complex this, in theory, can cause problems. In practice, published reports on the comparison of so-called skewed vs equimolar assays invariably show an excellent correlation, to indicate that other factors in the assay design also play a role in the final result (13).

Departments of Clinical Chemistry and Endocrinology, Academic Hospital Utrecht

Correspondence to: Dr. M.A. Blankenstein, Academisch Ziekenhuis Utrecht, Huispost G02.625, Postbus 85500, 3508 GA Utrecht.

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Immunoassays for fPsA became available and these were also tested with the mixtures of fPsA and PsA-ACT as indicated in the previous paragraph. Also their application to distinguish prostate cancer (PC) patients from patients with benign prostatic hyperplasia (BPH) was evaluated. The ratio free/total PsA was found to distinguish these patients better than either component of the ratio alone. Unfortunately there was still some overlap in the results of the two groups indicating that biopsies will remain necessary (14,15).
The development of more sensitive PsA assays allowed studies on the specificity of PsA. From the literature, it became clear that PsA is no longer to be considered as specific for prostate tissue (hence the small "s" in PsA throughout this paper). This was clearly shown in a collaborative study with Dr. J.L.P. van Duijnhoven (Elisabeth Hospital Tilburg, presently Elkerliek Hospital Helmond) with one of the ultra-sensitive assays, where we could confirm the presence of PsA in breast cancer cytosols and its relationship to the concentration of oestrogen receptors (16). Moreover, in collaboration with Prof. L. Gooren and Dr. H. Asscheman from the Free University we could establish that the extraprostatic PsA is also under androgen control (17). For application in urological oncology, however, this finding is considered to be of minor importance.
Recently a patient was identified who suffered from prostate cancer but whose serum showed remarkably different results in different PsA assays (18). Following chromatography of the serum and comparison of the results of the different assays for free and total PsA applied to the specimens obtained from this patient, it was found that an extremely high proportion of the PsA was free (>50%) and the hypothesis was formulated that the PsA produced by the patient failed to bind to PsA because of a molecular defect. This effect would lead to a three-dimensional structure in which one or more of the epitopes recognised by the assays giving low results would be lost. Preliminary molecular biological characterisation of archival material from this patient indeed revealed the presence of an mRNA which lacked 123 nucleotides in Exon3. The corresponding protein would miss 41 amino acids, but, since the deletion was an integer number of triplets, would remain otherwise unaffected (11). To our knowledge this is the first case of an aberrant PsA species described in the literature. Our future research in this intriguing area is aimed at evaluation to which extent this and other aberrant PsA species prevail in the population and to study the (patho)-physiological and molecular consequences of the findings. In the following section our plans for such an investigation are outlined.

Further investigation
The survey of the prevalence of aberrant PsA species and their molecular biological characterisation will be carried out along the following lines:

Investigation of the prevalence of the present mutation in prostate and other tissues
To this end, surgical specimens of benign and malignant prostate tissue and a variety of other tissues, including breast, will be analysed by reverse transcription PCR with probes specific for the mutation identified. With this approach, mRNA encoding wild type PsA is also detected. When it turns out that expression of this aberrant mRNA is not restricted to our index patient but has a more general prevalence, attempts will be made to quantify the PCR to such an extent that the ratio wild-type/mutant mRNA can be established. Internal standardisation of the PCR will be the first option in this respect.

Characterisation of the protein translated from the mRNA
The mutant found will be translated into protein in a cell free system and the properties of the resulting protein will be established. First, its complex formation with ACT will be established to test the hypothesis that the lack of binding to ACT can account for the high proportion of free PsA observed in the index patient. Secondly, interaction of the protein with antibodies used in the different PsA assays will be studied to show whether the low results in some assays can be attributed to differences in specificity of the antibodies

Occurrence of the mature protein in vivo
In view of the extreme sensitivity of the rt-PCR technique it is imperative to establish whether the protein encoded for by the aberrant mRNA is also translated in vivo. It is anticipated that it will be virtually impossible to find an antibody with sufficient specificity to allow for a direct immunoassay. A western blot assay in which the wild-type and the mutant are separated prior to immunodetection will be established to circumvent this problem. This approach will also enable detection of other mutant proteins should these occur.

Determination of factors which determine the wild-type:mutant ratio
In the likely event that the aberrant protein turns out to be synthesised in addition to the wild-type protein, it becomes necessary to establish why in our index patient and, depending on the results obtained during the execution of the project, possibly in others as well the expression of the aberrant protein is preferred over expression of the wild type. This question may be best answered with the aid of a prostate cancer model system, either in cell culture or in serial transplantation in nude mice.

Prevalence of other aberrant mRNA's
It is not unlikely that other aberrant mRNA's are found in the course of this project. If so, these will be subjected to the same evaluation protocol as described in the preceding paragraphs. Active searching for this will be done with the use of rt-PCR, not in a random fashion, but upon indication by the urologist who finds the PsA level in the serum of particular patients to be discordant with the clinical results or by the clinical chemist who observes discrepancies when comparing PsA assays or finds apparently higher free than total PsA results.

All colleagues are encouraged to contribute to this investigation by scouting for specimens suitable for further analysis, either because the free PsA concentration exceeds the measured total PsA, or because the PsA result is largely discordant with the clinical observations.

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