Radiometric assays for the measurement of PSA

A report for the co-ordinators' meeting (09-12 Dec 1997, Vienna)

Meera Venkatesh, Isotope Division, Bhabha Atomic Research Centre, India.

Prostate Specific Antigen, a serine protease enzyme, of M.W. ~ 26-33 kDa, (variations in the MW to an extent due to the various methods used for MW determination and the protein part of the molecule is believed to be ~26 kDa) is widely considered to be a very useful marker for prostate cancer. It satisfies nearly all the requirements of an ideal "Tumour Marker" and has hence attracted a lot of attention in the past decade. PSA is present in multiple forms in serum, with an appreciable fraction (~ 80%) bound to the protease inhibitor α-1-antichymotrypsin (ACT) and to a small extent to other proteins such as α-2-macroglobulin (AMG) leaving the rest in the free form. The total PSA levels have been reported to have ~ 80% sensitivity and ~ 60% specificity towards the detection of prostate cancer. The lack of specificity occurs mainly due to the high levels of t-PSA in benign prostatic hypertrophy(BPH) apart from the cancer. The concept of *free PSA* has been introduced in the recent past and the ratio of free/total PSA levels have been shown to be advantageous in the differential diagnosis of BPH from prostate cancer (specificity increased to ~80%). The f/t ratio is considered to be particularly useful in the grey zones of decision making (t-PSA levels 4-20 ng/mL).

The need for the development of assays for total and free PSA is felt due to

a. the high incidence of prostate cancers being detected currently
b. the high cost of tests (higher for free PSA assay, and the cost becomes an important parameter when a patient has to be regularly monitored after therapy) that is not affordable for many patients
c. the potential for research in the area of prostate cancer management where the PSA (total & free) assays will be of great help.

The tumour marker assays are generally immunometric for achieving high sensitivity (to detect micro-metastasis at the early stage, or for early detection in a screening procedure, when the marker is absent under normal conditions). PSA is present at < 4 ng/mL level in the normal population and for screening, a very sensitive assay may

not be needed. However for post-therapy monitoring after prostatectomy, the assay will need to be highly sensitive. An IRMA would perhaps be the best choice. In the case of PSA, the specificity of measuring the bound PSA-ACT complex and the free PSA need to be the same to get the "correct" values of PSA levels. Such "equimolar" assays would require the right combination of antibodies for the IRMA development and properly calibrated standards. It is hence planned to develop the following systems:

(i) IRMA for total-PSA with a minimum detectable limit of ~ 0.005 ng/mL and a wide standard range (0.02-100 ng/mL) with perhaps ten standards. This system could be used in two ranges, with one being super-sensitive for detection of micro-metastasis and the other for screening purposes.

(ii) IRMA for free-PSA with a sensitivity of ~ 0.005 ng/mL and a set of appropriate standards ranging from 0.01-20 ng/mL.

To achieve this the main reagents required would be

a. Antibodies:

A matched pair of specific antibodies, preferably both monoclonals would be preferred. Although many IRMAs are developed with mono/poly antibody combinations, in the case of PSA to avoid any skewedness due to the multiple form of PSA in serum, it is decided to get a matched pair of properly oriented (antigenic determinant biding sites) antibodies. However, if the cost of the antibodies is far too high, then the best possible combination to give minimum skewedness is proposed. In such a case the standards will be specially prepared to counteract the effect due to "non-equimolarity". Some commercial manufacturers who are supplying such matched pairs have been identified. A yet other point that will be considered is the cross-reaction of these antibodies against other Kallikrein molecules such as PAP. Request will be made for antibodies that have negligible cross-reaction with PAP and if possible with other kallikreins that are available.

b. Standard PSA Calibrators : The type of standards to be used in the t-PSA assay would be decided based on the kind of antibodies we can get. If the antibodies are well matched and have equimolar response towards the free and bound PSA, we plan to make standards from free PSA and validate using a commercial kit. If there is difference in the antibodies' cross-reaction towards the free PSA and PSA-ACT , then

initially we would test the extent of skewedness, although the sim at all times will be to have as error free an assay as possible. In case a skewed assay is unavoidable (mainly due to the cost) a mixture of 10-15% free-PSA and 90-85% PSA-ACT complex would be made, calibrated against a known standard (or a commercial kit) and then used. The stability of such standards on storage would be studied thoroughly as a part of assay optimisation and validation. Both PSA and PSA-ACT complex are available from reputed manufacturer. Development of an assay for PSA was already planned in our lab and as an initial step we had obtained PSA purified from human semen from an institute that was engaged in research in reproduction (their aim was to separate 'inhibin' another protein in semen, and hence we could obtain nearly pure PSA which is expected to give a very pure preparation on further purification by gel-filtration). This product will be independently tested for suitability for making the standards. In the case of free-PSA we would use free-PSA as standards. However, the undesirable effect of serum components on free-PSA stability is reported and hence we will have to find out the exact ways to make the free-PSA standards.

c. Separation System : The solid phase to be used as the separation system is an important step in the development of an IRMA, especially when a regular production and supply is considered. Although coated tubes is the most attractive from the point of users and as the latest state of art technology, our earlier experiences with coated tubes have not been too good. Good systems have been developed at a low scale of coating but we have found high imprecision, batch to batch variances etc. with the coated tubes. Moreover the antibody amount required for coating the tubes is much more than a system such as magnetic cellulose. With these points in mind, our aim is to try and make a sturdy solid phase if possible with coated beads or with magnetic cellulose. The strategy as to whether to coat with the primary antibodies or second antibodies or with avidin (to be used with biotinylated primary or second antibody) is also being considered. Second antibody coating or avidin coating of the solid phase may give us a more general reagent which could be used in many other systems. The reaction would in these cases be in a single liquid phase with better reaction rate and precision. Initially coating with both primary and second antibodies would be tried and if good binding can be still obtained, we would prefer second antibody coating. However, if not, as some of our previous experiences have been, we would go ahead

and cost with the primary antibody. Biotin-avidin system will be explored at a later stage after initial optimisation.

d Matrix supplement or free serum : Since the female serum is free of PSA we originally thought of using female serum screened for the absence of AIDS and hepatitis viruses as free serum. However, since the instability of free PSA in serum due to the presence of ACT and other binding proteins is known, we would now prefer to avoid usage of any serum and test if a buffer solution with serum components could be used. If not, we shall try and look for an animal serum substitute.

Local Production of the Reagents and Development of the Assay

Once the matched antibodies are obtained, the solid phase antibody and the tracer would be made at our lab using the routine procedues we generally follow. The tracer stability will be studied with respect to the oxidant used for iodination (Chloramine-T or Iodogen), the specific activity (3-15μCi /μg) and the storage conditions (frozen, 4 C and lyophilised at 4 C).

As mentioned earlier the possibility of purifying the PSA from seminal plasma in collaboration with another institute will be explored. The PSA thus prepared will be thoroughly calibrated using a commercial kit and if found suitable will be used in preparing the calibration standards in the assay kits.

After preparing the main reagents - namely the standard calibrators, we propose to systematically optimise the various reaction conditions and the reagent concentrations to achieve our goals of the IRMAs for free and total PSA. The initial assay system will then be fine tuned with the help of imprecision profiles. Care will be taken to eliminate the hook effect up to a dose of ~ 150 ng/mL. Alternately a two step assay with a washing step in between would be followed if the hook effect cannot be eliminated. The optimised assay would then be validated by the standard validation procedures such as parallelism testing by dilution of samples, recovery of the added standard to the samples and the external quality control analysis using authentic samples. The assay will be assessed for its analytical sensitivity, inter and intra assay variations and stability.

From the cancer institute near by, we have collected samples of known prostate cancer patients. These will be analysed by the developed method as a comparison. A more systematic method of sample collection from known prostate cancer patients would be undertaken to set up a sample bank. In this case care will be taken to collect the samples as plasma over EDTA and store the sample aliquoted at < -40 C.