Title: The Use of Prostate-Specific Antigen in Prostate Cancer Diagnostics

Introduction:

Prostate-specific antigen (PSA) is a serine protease produced in the prostate and secreted into ejaculate and blood. PSA functions to break up major gel forming proteins in ejaculate. The production of PSA is largely regulated by the androgen-dependent activation of the androgen receptor on prostate cells. Both normal and malignant prostate epithelial cells produce PSA. In normal prostates most of the PSA is secreted into the seminal vesicles and only a small amount leaks into circulation. However, in prostate cancer the epithelial cells have an abnormal architecture and more PSA is released into circulation. Thus, the PSA level in serum is a sensitive marker for prostate cancer. Problems with this diagnostic method arise, however, when PSA serum levels are also elevated in benign prostatic hyperplasia (BPH). The majority of elevated PSA levels in men over the age of 50 are due to BPH, though generally serum PSA levels increase with age regardless of tumor presence. In the circulatory system, several forms of PSA are present. Active PSA readily forms complexes with protease inhibitors such as α_1-antichymotrypsin (PSA-ACT) and α_2-macroglobulin (PSA-A2M). The half-lives of these three forms of PSA range from several minutes for PSA-A2M, one to two hours for free, active PSA, and two to three days for PSA-ACT. Active PSA is cleared in the kidneys while the larger complexed PSA-A2M molecules are eliminated in the liver. PSA-ACT can be cleared through either organ. PSA is also present in two inactive, uncomplexed forms. ProPSA is a precursor molecule that is normally cleaved in the prostatic lumen by human kallikrein-2 (Hk2) from a 244-residue protein to the active 237-residue active form. The other form, nicked PSA, is a molecule formed from the proteolysis of active PSA.

In men with high PSA levels, the ratio of uncomplexed PSA to total PSA (PSA ratio) in the serum can be used to distinguish between prostatic cancer and BPH. This ratio can be helpful in detecting prostate cancer in the grey zone, which is defined as a PSA level between 4 $\mu g/mL$ and 10 $\mu g/mL$. At a PSA level of 6 $\mu g/mL$, for example, the probability of finding prostate cancer by biopsy is about 0.45 at a PSA ratio of 0.1, but decreases to 0.11 at a PSA ratio of 0.3. A difference in the type of free PSA found in the serum may be the explanation for the divergence seen in PSA ratios in BPH and prostatic cancer. The proportion of nicked PSA or proPSA escaping into the circulation may be higher in prostatic cancer than in BPH. This would lead to higher levels of free PSA since the inactive variations of PSA do not form complexes with the common protease inhibitors. Alternately, the activation rate of proPSA to active PSA may be higher in prostate cancer than in BPH.

Research Question: Serum PSA levels are used as a prostate cancer detection marker, but the current method is imperfect. In this project, I will develop mathematical models for the different forms of free and complexed PSA, investigate the model solutions and use statistical analysis to compare the mathematical models with available experimental data.

Methodology:

Data for this project will be provided by Dr. Kristen Swanson and Dr. Lawrence True with the University of Washington Department of Pathology. Dr. True collects data from severe compromised immunodeficient (SCID) mice. Xenografts of aggressive cases of prostatic cancer are implanted subcutaneously into the mice, and blood samples are taken from the tail in order to test serum PSA levels. Specifically, sterile samples of prostatic tumor tissue (20-40 mg) are implanted and allowed to grow for up to nine weeks or until the death of the mouse. Both androgen dependent and androgen independent cases are used, the androgen independent cases being those with castrated mice. Dr. Swanson works with human data, particularly from patients undergoing radical prostatectomies, though biopsied tissue is also used. Blood serum samples are taken from each patient so PSA levels can be analyzed. Enzyme-Linked ImmunoSorbet Assay (ELISA) techniques are used to detect PSA levels in the serum. ELISA is easily able to detect all forms of free PSA as well as the PSA-ACT complex. The PSA-A2M complex is more difficult to detect using antibodies against PSA since A2M engulfs most of the PSA molecules it complexes with, leaving little surface area for the antibodies to attach to. PSA concentrations of as low as 0.05 $\mu g/mL$ can accurately be detected using ELISA.

The data obtained from Dr. Swanson and Dr. True will be used to develop a better model of PSA dynamics in order to decrease the "grey zone" of PSA values and hopefully improve diagnostic techniques. A system comprised of four differential equations will be used to model each of the major forms of PSA. These equations are:

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\begin{aligned}
P' &= \beta_a V(t) - \delta_1 P + \lambda_1 M_1 - \delta_2 P + \lambda_2 M_2 - \gamma_1 P \\
Q' &= \beta_i V(t) - \gamma_2 Q \\
M_1' &= \delta_1 P - \lambda_1 M_1 - g(M_1) \\
M_2' &= \delta_2 P - \lambda_2 M_2 - \gamma_4 M_2
\end{aligned}
$$

where the concentrations of free and active PSA, free and inactive PSA, the PSA-ACT complex, and the PSA-A2M complex are represented by P, Q, M_1 and M_2, respectively. In this system V(t) is the volume of the tumor or prostate, depending on the data source (tumor volume for mice, prostate volume for humans). β_a and β_i are the production rates of active and inactive PSA, δ_1 and δ_2 are the association rates of PSA with ACT and A2M, and λ_1 and λ_2 represent the dissociation rates of these complexes. The various γ terms signify the clearance rates of each form of PSA. The term $g(M_1)$ is the elimination rate of the PSA-ACT complex and is dependent on the concentration of the complex. At high concentrations, the elimination rate is essentially constant because the clearance pathway is relatively inefficient. At lower complex concentrations, however, the clearance rate looks very similar to that for the PSA-A2M complex. Lower concentrations of PSA-ACT are generally found only in patients who have undergone radical prostatectomies or chemical castration in which the production of PSA has been halted.

Solutions to these equations will be found for several cases. Multiple cases for the degradation rate of PSA-ACT must be considered. The differences in solutions using only one complex formation term (M, in these equations) compared with using both complex formation terms will also be studied. This system of equations can be solved using a steady-state assumption. Biologically this is reasonable since the concentrations

of each form of PSA in the body should be relatively stable. If the solutions to this system with a steady state assumption to not match the data from Dr. Swanson and Dr. True, more accurate solutions can be found by solving the system as differential equations. Computer programs such as Maple and MatLab will be used visualize the solutions using three dimensional graphs and contour plots, as graphical visualizations will make the solutions to various cases easier to compare and analyze.

Expected Results:

We expect that one specific form of either free or complexed PSA will be more prevalent in patients with prostate cancer than in patients with healthy prostates or BPH. With one specific form of PSA in abundance, we hope to find PSA ratios that improve upon the probability of returning positive biopsy results when compared with the ratio methods currently in use.

The mathematical model developed in order to incorporate the various PSA forms can reduce the "grey zone" of PSA ratio values in which about half of patients undergoing biopsies returned positive results. This can then further reduce the number of unnecessary biopsies performed. It may also aid in detecting prostate cancer at earlier stages in order to make less radical treatments a plausible option for many patients.

Annotated Bibliography:

1. Björk T, Ljungverg B, Piironen T, Abrahamsson PR, Pettersson K, Cockett ATK, Lilja H 1998 Rapid exponential elimination of free prostate-specific antigen contrasts the slow, capacity-limited elimination of PSA complexed to alpha$_1$-antichymotrypsin from serum. Urology 51:57-62

Björk's article covers an experiment performed to determine the elimination rates of different forms of PSA. Patients undergoing radical retropubic prostatectomies were used in this study, and immuno assays were used to detect PSA levels at various times after surgery. This gives information for several of the parameters used in the mathematical model described in the methods section.

2. Lilja H 2003 Biology of prostate-specific antigen. Urology 62:27-33

Lilja provides a summary of many other journal articles covering the biochemistry of PSA. The article includes general information on the human kallikren gene family (on chromosome 19) of which PSA is a member. Specific information on PSA is also covered, including regulatory mechanisms, complexation, and factors affecting PSA expression.

3. Scripps Laboraties, Inc. 1997 The prostate-specific antigen-α2-macroglobulin complex: a significant form of total serum PSA. Scripps News 11:1-3

The presence and importance of the PSA-A2M complex is detailed in the Scripps article. This report describes an experiment in which PSA was introduced into female blood serum and the complexation and dissociation rates of PSA-ACT and PSA-A2M were studied. It provides values for several of the parameters used in the mathematical model that will be used for this project.

4. Stenman UH, Leinonen J, Zhang WM, Finne P 1999 Prostate-specific antigen. Sem in Cancer Bio 9:83-93

This article details the biochemistry and function of PSA, as well as gives an overview of its medical uses. It describes the production, modification, and metabolism of the enzyme. Stenman also gives the historical uses of PSA as a tumor marker and the improvements made in its use as a diagnostic technique.

5. Swanson KR, True LD, Lin DW, Buhler KR, Vessella R, Murray JD 2001 A quantitative model for the dynamics of serum prostate-specific antigen as a marker for cancerous growth: an explanation for a medical anomaly. American Journal of Pathology 158:2195-2199

The details of the development and use of xenografts are described in this article. A mathematical model for serum PSA levels is presented, which aided in the development of the model to be used for my thesis project.

6. True LD, Buhler K, Quinn J, Williams E, Nelson PS, Clegg N, Macoska JA, Norwood T, Liu A, Ellis W, Lange P, Vessella R 2002 Animal model: a neuroendocrine/small cell prostate carcinoma xenografts – LuCaP 49. American Journal of Pathology 161:705-715

This paper contains information on the androgen dependence of PSA production. It also describes the genetics of prostatic cancer xenografts in mice. For our purposes, this article contains information on the clearance rates of certain forms of PSA and provides useful information on the androgen dependence of cancer growth.