

Expression of soluble urokinase plasminogen activator receptor may be related to outcome in prostate cancer patients

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Abstract. The urokinase-type plasminogen activator receptor (uPAR) exists as a GPI anchored glycoprotein ($M_r = 50-60$ kDa) on the surface of various cell types. This receptor can be bound by or cleaved by urokinase. The cleaved receptor, soluble urokinase-type plasminogen activator receptor (suPAR), with a $M_r = 35$ kDa has no known physiological function and can be identified circulating in the blood of normal individuals. Although no function has been characterized, the soluble receptor has been reported to be of clinical significance. The objective of this study is to characterize novel serum markers that can be used for the early detection of prostate cancer and to predict patient prognosis. Thirty-nine patients at the University of Yaounde I, Yaounde, Cameroon, West Africa were examined for prostatic disorders. 46% of serum of patients with BPH or prostate cancer contains elevated levels of suPAR. To examine the patients were diagnosed with benign prostate hyperplasia (BPH), while 44% of the patients were diagnosed via biopsy with prostate cancer and graded accordingly. Here we show that the significance of suPAR as a diagnostic factor, we used a suPAR ELISA kit and compared these results with serum levels of prostate specific antigen (PSA), the current diagnostic marker for prostate cancer. PSA and serum suPAR levels in BPH and cancer patients were greatly elevated in the majority of patients, while others had undetectable levels of either. Serum levels of suPAR were high in cancer patients as well as, although to a lesser degree, in patients with BPH. Cancer patients who died during the follow up period were found to have consistently higher serum suPAR levels than correlating serum PSA levels. These preliminary findings are the first

evaluating serum suPAR levels as a possible diagnostic marker for the early detection of prostate cancer and for prediction patient prognosis.

Introduction

Proteolytic enzymes are crucial players in physiological processes such as tissue restructuring and the pathological processes of cancer cell invasion and metastasis (1). Urokinase-type plasminogen activator (uPA) has been established as a vital constituent in these processes through the conversion of inactive plasminogen into active plasmin (2). Plasmin, a serine protease, is involved in the degradation of several extracellular matrix components and thus contributes to the metastatic properties of cancer cells. uPA has also been shown to be associated with such non-proteolytic processes as cellular adhesion and chemotaxis (3). The high affinity receptor (uPAR) for uPA exists on the cell surface of various human leukocytes (4,5), several nonhematopoietic cells, as well as various epithelioid tumor cell types such as colon, breast, lung, and prostate carcinoma (6-11). uPAR is a three domain, highly glycosylated, 55-60 kDa protein that is anchored to the cell surface through the carboxy-terminal attachment of a glycosyl phosphatidyl-inositol moiety. The proteolytic activity of uPA has been shown to possess the ability to cleave the uPA receptor (12) leading to detectable levels of soluble urokinase plasminogen activator receptor (suPAR) in the blood. Elevated levels of suPAR have been associated with breast and colon cancer (13), non-small cell lung carcinoma (14), and ovarian cancer (15). Tumor extracts from breast (16), colon (17), and lung cancer patients (18) were found to contain high uPAR levels and correlated with poor prognosis. Further, ovarian cancer patients exhibited increased serum suPAR levels that related to poor survival prognosis (15). Recently it was shown that increased levels of plasma suPAR correlate to poor prognosis in colorectal cancer patients (19). These findings suggest that increased plasma/serum levels of suPAR may be useful for predicting patient prognosis.

Prostate specific antigen (PSA) is currently recognized as the tumor marker of choice for early detection of prostate cancer. PSA tests, when used in conjunction with a digital rectal exam have facilitated an increase in

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the detection of localized disease. Elevated serum PSA levels, however, only suggests the presence of prostate cancer and can be affected by the age of the patient, as PSA concentrations tend to increase with age. A prostate biopsy is currently required for the diagnosis of prostate cancer.

The ability to use serum samples to determine the presence of prostate cancer, its progression, and relate them to patient prognosis would prove to be highly beneficial by reducing the need for patient biopsies. Prostate tumor tissue analysis is useful in disease diagnosis, while peripheral blood samples can allow the monitoring of treatment and disease progression without the need for invasive procedures. We have begun a study to analyze the levels of serum suPAR in BPH and prostate cancer patients and a relation to prognosis. Although these results are preliminary, they could lead to better detection of disease when used alone or in conjunction with other prostate diagnostic factors and give reliable patient prognosis, both of which would be of great clinical significance.

Materials and Methods

Patients. Blood samples were obtained with informed consent from thirty-nine patients with Urological disorders at the University of Yaounde I in Yaounde, Cameroon, West Africa between January 1999 and June 1999. Histological examination results showed that 18 individuals suffered from benign prostate hyperplasia (BPH) and 16 were found to have varying degrees of prostate cancer. Seven prostate cancer patients died during the follow up period. Five patients refused histological examination or were unavailable for follow up visits and thus were dropped from the study. The age of the patients used in this study ranged from 51 to 81 years (median 67.5 years).

Blood collection. Peripheral venous blood was collected in dry tubes and clotted at room temperature. Serum was collected within 30 minutes after clotting blood. Samples were then frozen in liquid nitrogen (-180°C) for storage for a period of up to 6 months. Frozen samples were thawed at 37°C prior to use and diluted 1:8 with 1% w/v BSA in PBS/Triton X-100 buffer.

Measurement of Serum suPAR. The IMUBIND soluble uPAR ELISA kit for quantification of suPAR antigen in human blood samples was obtained from American Diagnostica Inc., Greenwich, CT. A 3 ng/ml uPAR standard was serially diluted to generate a standard curve. The assay was performed according to manufacturer specifications (20). Quantification of serum suPAR was completed using a spectrophotometer. Sample values were then obtained by association with the standard curve and multiplied by the dilution factor to obtain correlating suPAR concentrations.

Measurement of serum PSA. Serum PSA was measured using the AxSYM PSA assay, Abbot Laboratories, Abbott Park, IL. Assay was performed according to manufacturer specifications (21). The AxSYM PSA assay is based on Microparticle Enzyme Immunoassay (MEIA) technology, through which Anti-PSA coated microparticles are bound by sample PSA

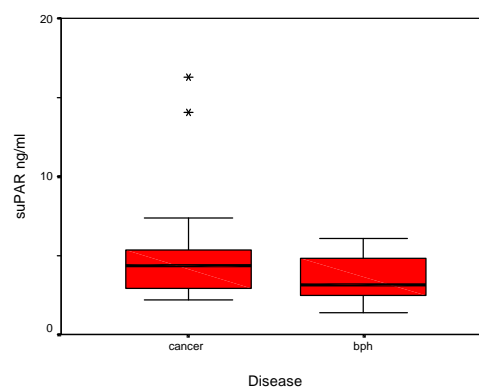


Figure 1. Boxplot representation of suPAR in patients with prostate cancer and those with BPH. Box bottom represents the first quartile (Q1), box top represents the third quartile (Q3), and the central line indicates the median. The top and bottom whiskers represent the highest and lowest values within the range $Q3 + 1.5 \text{ IQR}$ ($Q3 - Q1$) and $Q1 - 1.5 \text{ IQR}$ respectively. Outliers are shown as asterisks (*).

forming an antigen-antibody complex. This complex is then labeled by an Anti-PSA: Alkaline Phosphatase conjugate. The resulting complex will fluoresce upon the addition of substrate (4-methylumbelliferyl Phosphate). Fluorescence is measured using the AxSYM systems optical assembly. Sample values were multiplied by the dilution factor to determined coinciding serum PSA concentrations.

Statistics. Group means were compared using unpaired Student's t test with Levene's test for equality of variance. The results are given as mean \pm SE. Correlation coefficients were determined using Spearman's. Significance of all data was considered at the level $P \leq 0.05$.

Results

The distribution of serum suPAR levels in men with prostate cancer or BPH is shown in Figure 1. Serum suPAR in prostate cancer patients ($n = 16$) ranged between 2.18 and 16.29 ng/ml (median = 4.21 ng/ml, mean = 5.43 ng/ml \pm 1.02). BPH patients ($n = 18$) serum suPAR levels ranged between 1.36 and 6.07 ng/ml (median = 3.15 ng/ml, mean = 3.51 ng/ml \pm 0.32). There was no statistically significant difference in serum suPAR levels between prostate cancer patients and BPH patients when using a independent samples Student's t-test with equal variances not assumed ($P = 0.090$). The distribution of serum suPAR levels in surviving men and men that died during the follow-up period is shown in Figure 2. Serum suPAR in surviving patients ($n = 9$) ranged between 2.24 and 5.19 ng/ml (median = 3.11 ng/ml, mean = 3.64 ng/ml \pm 0.39). Deceased patients ($n = 7$) had serum suPAR ranging between 2.18 and 16.29 ng/ml (median = 5.48 ng/ml, mean = 7.73 \pm 2.03). Statistically there is no difference between deceased and surviving patients in serum suPAR levels among prostate cancer patients using a independent samples Student's t-test with equal variances not assumed ($P = 0.091$). There is a small ($r = 0.321$) but insignificant ($P = 0.064$) correlation between serum suPAR levels in all patients when the data is analyzed using Pearson's.

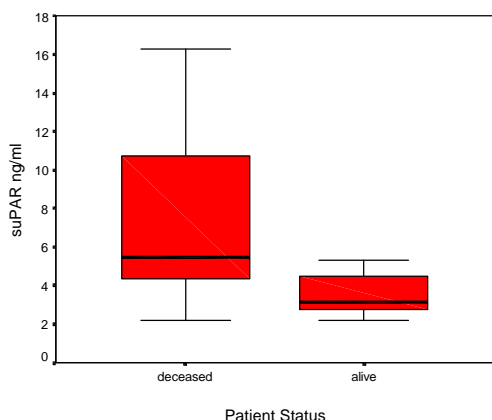


Figure 2. Boxplot representation of suPAR in prostate cancer patients that died during the follow up period and those that remain alive

Discussion

Studies have shown that the receptor for urokinase can be found on the surface of colon, lung, breast, and prostate epithelioid tumor cells (6-11). This receptor can be cleaved and solubilized in the blood. Levels of this soluble receptor have been shown to be elevated in several cancers including breast, lung, ovarian, and colon (13-15). Ovarian and colorectal cancer patients with elevated levels of suPAR have exhibited poor survival prognoses (15,19). Soluble urokinase-type plasminogen activator seems to be a cancer indicator with broad specificity. Thus, it can be speculated that elevated blood suPAR levels can be indicative of carcinogenesis and poor survival prognosis.

In the present study, we have shown that prostate cancer patients have elevated levels of serum suPAR. Others have shown that healthy men have serum suPAR present in low quantities (median = 1.1 ng/ml) (13). The prostate cancer patients in the current study exhibit elevated levels of serum suPAR (median = 4.21 ng/ml) when compared to the healthy men in the aforementioned study. Additionally, prostate cancer patients that died had a median serum suPAR value of 5.48 ng/ml. Also, we were not able to establish if patients died of cancer or unrelated causes. Our data indicates that there is no statistical difference ($P = 0.090$) between men with BPH and those with cancer of the prostate. The difference may become evident in future studies when a larger sample size is used. Serum PSA, the currently accepted diagnostic marker for prostate cancer, and serum suPAR levels were also examined and compared in this study. Our data shows that there is a poor correlation ($r = 0.321$, $P = 0.064$) between the indicators. This could be the result of several factors. The small sample size of this study can lead to misleading results, as well as differences in the techniques used for assay. The concentration of suPAR and PSA in a given sample, when quantitated by assays from independent manufacturers can vary due to differences in reagent specificity and assay methods. Also, it is known that men can exhibit increased PSA levels as they age, whereas; suPAR levels have been shown to remain relatively constant in healthy individuals regardless of age (13). Further, medical treatments received by several of the cancer patients in this study have given rise to undetectable PSA levels, making a correlation between serum suPAR and PSA difficult to

evaluate. Results and a relation to patient ethnicity and environment are undeterminable and were not included in the scope of the present paper but may be included in the future.

Early detection of prostate carcinoma is hindered by the asymptomatic nature of men with localized tumors. Currently, questionable PSA test results (between 4.0 and 10.0 ng/ml) along with a positive DRE are indicative of possible prostate cancer. To verify if a patient indeed has a localized tumor, a biopsy is necessary. The need for simple, safe, and inexpensive early detection tests for carcinomas of the prostate are evident. Novel highly sensitive assays for the early detection of prostate cancer could prove beneficial by eliminating the need for patient biopsies among men with questionable PSA test results. Some new diagnostic markers under investigation for their ability to detect prostate cancer include human glandular kallikrein-2 (hk2), percentage of free PSA, complexed PSA, prostate-specific membrane antigen (PMSA), and N-telopeptide (NTx).

In summary, we have shown that serum suPAR as a diagnostic marker for prostate cancer and for patient survival prognosis deserves further investigation. With this preliminary and future data, we believe that suPAR will join the list of novel markers for prostate cancer and show that suPAR tests can be valuable for early detection of localized and metastatic disease while also enabling persistent disease status monitoring after medical or surgical treatment.

Acknowledgments

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