Tumor marker α -fetoprotein receptor does not discriminate between benign prostatic disease and prostate cancer

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Conflict of interest

RECAF concentration in patients' blood samples was measured free of charge by BioCurex, Inc., Suite 215, 7080 River Road, V6X 1X5 Richmond, BC, Canada. BioCurex Inc. had no role in the design of the study, the collection and analysis of the data or the decision to publish. No other conflicts of interest are declared.

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Abstract

Background. The α -fetoprotein receptor (RECAF) is a proposed novel tumor marker for detecting several different types of tumors, including prostate cancer (PCa).

Objectives. The aim of the study was to evaluate RECAF in discriminating benign prostatic conditions from PCa and to compare it with prostate-specific antigen (PSA).

Material and methods. A total of 64 patients with elevated serum PSA levels and/or abnormal digital rectal examination of the prostate referred to a tertiary center for transrectal ultrasound (TRUS) biopsy of the prostate were prospectively enrolled in the study from January 2009 to April 2010. Serum RECAF, total PSA (tPSA) and free PSA (fPSA) concentrations were measured. The results were correlated with histopathologic findings using the Mann-Whitney U test and Kruskal-Wallis χ^2 test.

Results. The median RECAF concentration was 5.34 U/L in the benign pathology group of patients and 4.72 U/L in the malignant pathology group. The difference was not statistically significant. RECAF density, tPSA and fPSA concentrations and tPSA density were significantly different between the benign and malignant pathology groups ($p = 0.033$, $p = 0.000$, $p = 0.002$ and $p = 0.000$, respectively). RECAF concentration and RECAF density did not differ significantly in the subgroups of PCa patients stratified according to Gleason score, predominant primary Gleason grade or maximum primary Gleason grade, but in predominant secondary Gleason grade and maximum secondary Gleason grade, significant differences were found ($p = 0.007$ and $p = 0.004$, respectively).

Conclusions. The results of the study did not confirm the RECAF tumor marker as an alternative way to discriminate between groups of patients with benign prostatic conditions and PCa, and its concentration and density do not differ among PCa histopathologic groups.

Key words: prostate cancer, histopathology, prostate-specific antigen, tumor marker, α -fetoprotein receptor

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In the last decade, prostate cancer (PCa) has become the most frequently diagnosed cancer among all solid tumors in men in developed countries.¹ Its increased incidence is the result of widespread screening programs and improved awareness of the disease among the general population. The discovery of the serum tumor marker prostate-specific antigen (PSA) and its adoption in clinical practice has lowered the stage of newly discovered PCa.² PSA is organ specific but not disease specific; it can be elevated in benign prostatic hyperplasia (BPH), prostatitis, as well as in PCa.³ PSA values in BPH and prostatitis often resemble PSA levels in the early stages of PCa, which is a curable disease.

A diagnosis of PCa is confirmed by a transrectal ultrasound (TRUS)-guided biopsy of the prostate indicated by increased PSA levels, a suspect digital rectal examination (DRE), or suspicious areas detected on a TRUS; however, up to 60% of prostate biopsy procedures still prove to be negative using these criteria.^{4–6} Several modifications of serum PSA value have been proposed to improve its specificity in the early detection of PCa, for example the ratio of free PSA (fPSA) to total PSA (%fPSA), PSA density, PSA velocity and doubling time and detection of PSA isoforms. However, their use in clinical practice is limited.³ Other molecules that may be useful as PCa markers are under investigation. Of these, prostate cancer antigen 3 (PCA3) is the most extensively studied and has already been proposed for clinical use due to its slightly better performance compared with tPSA.^{7,8}

The α -fetoprotein receptor (RECAF) is an oncofetal antigen present in high concentrations during fetal growth and development; however, its concentration drops to very low levels after birth and normally remains low even in adult life.^{9,10} It has been suggested that RECAF concentrations increase in some malignant diseases (e.g., teratocarcinoma, hepatocellular, breast, lung, prostate, ovary and gastric carcinoma), whereas in benign tumors, RECAF levels do not appear elevated.^{11–13}

Moro et al. studied the clinical usefulness of RECAF in the diagnostics of BPH and PCa, and they reported a sensitivity of 99% and a specificity of 95%.¹¹ However, no correlation to PSA concentration, histopathologic grading (Gleason grade), or the T stage of PCa was made.

The aim of the present study was to evaluate the RECAF tumor marker in the diagnostics of benign prostatic diseases and PCa and to compare it with PSA, an established clinical marker. To the authors' knowledge, this is the first study to report on correlations between RECAF and PSA and the histopathologic grading of PCa.

Material and methods

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia, and informed consent was obtained from each participant. This was

a single-center study. The patients included in the study had been routinely scheduled for a TRUS-guided biopsy of the prostate by the referring urologist due to an elevated PSA concentration, suspect DRE or both. The exclusion criteria for the study were: a history of previous malignant disease, an indwelling urinary catheter or a previous positive biopsy. Any previous medical therapy for the treatment of lower urinary tract symptoms was recorded. The inclusion of the patients in the study and the TRUS-guided biopsies were performed by two certified urologists. Consecutive patients scheduled on a date on which 1 of the 2 participating urologists was performing biopsies were considered and included, if the inclusion criteria were met. While patients were scheduled for TRUS-guided biopsies up to 3 months in advance, the urologist performing the TRUS-guided biopsies was scheduled on day-to-day basis, so the patients included were a random sample. A total of 64 patients were prospectively enrolled in the study from January 2009 to April 2010. Other certified urologists and residents were performing TRUS-guided biopsies at the time of the study, but their patients were not included in the study.

Detection methods

Blood samples for analysis were obtained from each patient just before the TRUS-guided biopsies. Full blood count, serum electrolytes, alkaline phosphatase, tPSA, fPSA and RECAF markers were determined from the samples. If a patient had been taking a 5 α -reductase inhibitor for over 6 months, the tPSA and fPSA concentrations measured were doubled for the statistical analysis.

The concentration of serum RECAF was measured with a competitive chemiluminescence immunoassay (CLIA) by BioCurex Inc. (Richmond, BC, Canada). The test was a solid-phase competitive immunoassay using microwell plates in which a constant amount of RECAF-acridinium competed with the RECAF in the serum sample to bind to the monoclonal antihuman RECAF antibody immobilized in the solid phase. The amount of labeled RECAF bound to the solid phase was inversely proportional to the amount of RECAF in the sample.

PSA measurements were performed on a LIAISON CLIA analyzer (DiaSorin, Saluggia, Italy) with the LIAISON tPSA and LIAISON fPSA assays. The method for quantitative determination of tPSA and fPSA is a sandwich CLIA. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units and indicates the concentration of tPSA or fPSA present in the samples.

Before each biopsy, the clinical stage according to the DRE was noted, and the volume of the prostate was measured by TRUS examination. The TRUS-guided prostate biopsies were performed using the proto-

Table 1. Patient characteristics stratified according to histopathologic results

Patient characteristics	Benign group median (interquartile range)	Malignant group median (interquartile range)	Mann-Whitney U (sig.)
Number of patients	35	29	
Age (years)	63.0 (59.0–69.0)	71.0 (61.5–76.5)	333.5 (p = 0.019)
Prostate volume (mL)	46.0 (38.0–56.8)	30.0 (23.0–40.1)	223.5 (p = 0.000)
Blood leukocytes (10 ⁹ /L)	7.2 (5.9–8.2)	6.9 (6.2–7.6)	476.5 (p = 0.676)
Hemoglobin level (g/L)	150.0 (142.0–155.0)	152.0 (140.5–158.0)	475.0 (p = 0.661)
Blood thrombocytes (10 ⁹ /L)	210.0 (176.0–262.0)	230.0 (207.0–288.5)	392.0 (p = 0.119)
Serum alkaline phosphatase (μkat/L)	1.100 (0.940–1.300)	1.80 (0.995–1.260)	496.5 (p = 0.882)

Table 2. A comparison of median RECAF concentration, median PSA concentration and derived parameters between benign and malignant groups of patients

Tumor marker	Benign group median (interquartile range)	Malignant group median (interquartile range)	Mann-Whitney U (sig.)
Number of patients	35	29 ¹	
RECAF concentration (U/L)	5.34 (3.87–9.70)	4.72 (3.86–9.41)	472.5 (p = 0.637)
RECAF density (U/L/cm ³)	0.1187 (0.0734–0.2167)	0.1995 (0.1344–0.3451)	349.0 (p = 0.033)
tPSA concentration (μg/L)	3.57 (2.40–5.10)	7.71 (4.58–13.50)	197.0 (p = 0.000)
fPSA concentration (μg/L) ¹	0.540 (0.380–0.960)	0.895 (0.605–1.390)	270.0 (p = 0.002)
tPSA density (μg/L/cm ³)	0.0795 (0.0529–0.1020)	0.2658 (0.1199–0.5195)	137.0 (p = 0.000)
Free to total PSA ratio (%) ¹	18.5 (10.2–24.2)	13.0 (8.0–18.9)	363.0 (p = 0.079)

¹ Due to missing data, the number of patients in this group in rows fPSA and free to total PSA ratio is 28; RECAF – α-fetoprotein receptor; tPSA – total PSA; fPSA – free PSA.

col established in the authors' institution, targeting the lateral parts of the peripheral zone of both prostatic lobes.

The biopsy cores were analyzed in the histopathology laboratory of the Institute for Pathology at the Medical Faculty of Ljubljana University (Slovenia). The results were divided into benign and malignant categories and further subclassified as BPH, inflammation (prostatitis), high-grade prostatic intraepithelial neoplasia (HGPIN), suspected PCa and confirmed PCa. In the PCa reports, the Gleason grades were noted.

Statistical analysis

The data were analyzed using SPSS software (v. 21.0, IBM Corp., Armonk, USA). The measured concentrations of RECAF and PSA and their derived values were compared using the Mann-Whitney U test for the 2 groups of patients. The Kruskal-Wallis χ^2 test was used to compare the values of more than 2 groups of patients. The Kendall τ -b test was used to calculate the statistical correlation between PSA and RECAF concentrations and RECAF density.

Results

Complete data was obtained from 63 out of the 64 patients; in 1 patient, the fPSA concentration was not measured. Table 1 shows a comparison of general patient data stratified into benign and malignant pathology groups, in which the benign group consisted of the patients whose pathology report diagnosed BPH, prostatitis or HGPIN, and the malignant group consisted of patients with either a suspicious report for PCa or confirmed PCa.

The median RECAF concentration was 5.34 U/L (3.87–9.70) in the benign pathology group and 4.72 U/L (3.86–9.41) in the malignant pathology group. The difference was not statistically significant (Mann-Whitney U test = 472.5; p = 0.637). However, when RECAF concentration was normalized to prostate volume (RECAF density), a significant difference was found between the 2 pathology groups (p = 0.033). The tPSA and fPSA concentrations and tPSA density were also significantly different between the benign and malignant pathology groups, whereas %fPSA was not (Table 2).

The group of patients diagnosed with PCa was further stratified according to the following histologic pa-

rameters: Gleason score (Gsum), predominant primary Gleason grade (Gprim), predominant secondary Gleason grade (Gsec), maximum primary Gleason grade (maxGprim), and maximum secondary Gleason grade (maxGsec), where predominant Gleason grade refers to the predominant Gleason grade reported by a pathologist after considering all the biopsy cores, and maximum grade refers to the highest Gleason grade found in any of the biopsy cores. The median RECAF concentrations, RECAF densities, tPSA concentrations, fPSA concentrations, PSA densities and %fPSA were statistically com-

pared among these subgroups. Out of 29 patients diagnosed with PCa, 28 had complete histopathologic data reported; in 1 patient the exact pathologic grading could not be determined due to the small amount of PCa in his biopsy cores (Table 3).

No statistically significant correlations were found between tPSA and fPSA concentrations on one hand, and RECAF concentration and density on the other hand (Table 4).

Positive statistically significant correlations were found between the age of the patient and tPSA and fPSA con-

Table 3. A comparison of median RECAF concentration, median total and free PSA concentration and derived parameters between histopathologic subgroups in the patients with prostate cancer

Gleason category	Gleason value	RECAF concentration (U/L)	RECAF density (U/L/cm ³)	tPSA concentration (µg/L)	fPSA concentration (µg/L)	tPSA density (µg/L/cm ³)	Free to total PSA ratio (%)
no. of patients		28	28	28	271	28	271
Gsum	6	9.42	0.28	4.42	0.62	0.152	18.6
	7	5.105	0.215	8.005	0.895	0.414	11.4
	8	8.025	0.324	10.94	0.90	0.361	9.0
	9	4.42	0.123	15.45	1.78	0.558	13.4
	Kruskal Wallis χ^2 (sig.)	6.539 (p = 0.088)	6.850 (p = 0.077)	12.057 (p = 0.007)	9.859 (p = 0.020)	9.389 (p = 0.025)	3.496 (p = 0.321)
Gprim	3	6.245	0.206	5.395	0.765	0.199	14.7
	4	4.42	0.168	13.50	1.60	0.558	11.0
	Mann Whitney U (sig.)	68.000 (p = 0.194)	83.000 (p = 0.546)	34.000 (p = 0.004)	35.000 (p = 0.009)	29.000 (p = 0.002)	69.000 (p = 0.348)
Gsec	3	9.29	0.336	5.57	0.68	0.214	18.0
	4	6.245	0.176	6.445	0.815	0.255	11.5
	5	4.32	0.103	15.40	1.57	0.454	11.9
	Kruskal Wallis χ^2 (sig.)	4.555 (p = 0.103)	10.063 (p = 0.007)	9.199 (p = 0.01)	8.057 (p = 0.018)	4.356 (p = 0.113)	2.216 (p = 0.330)
maxGprim	3	0.6245	0.206	5.395	0.765	0.199	14.7
	4	4.245	0.168	13.50	1.60	0.558	11.0
	5	4.52	0.143	10.81	1.40	0.415	23.4
	Kruskal Wallis χ^2 (sig.)	1.694 (p = 0.429)	0.908 (p = 0.635)	9.234 (p = 0.010)	6.894 (p = 0.032)	10.990 (p = 0.004)	0.995 (p = 0.608)
maxGsec	3	0.929	0.336	5.57	0.68	0.214	18.0
	4	0.595	0.205	6.47	0.87	0.281	11.0
	5	4.145	0.099	14.55	1.78	0.447	13.4
	Kruskal Wallis χ^2 (sig.)	4.802 (p = 0.091)	11.102 (p = 0.004)	7.784 (p = 0.020)	8.219 (p = 0.016)	3.136 (p = 0.078)	2.136 (p = 0.344)

¹ Due to missing data, the number of patients in this group in columns fPSA and free to total PSA ratio is 27; Gsum – Gleason score; Gprim – predominant primary Gleason grade; Gsec – predominant secondary Gleason grade; maxGprim – maximum primary Gleason grade; maxGsec – maximal secondary Gleason grade; RECAF – α -fetoprotein receptor; tPSA – total PSA; fPSA – free PSA.

Table 4. Statistical correlation between PSA and RECAF

Kendall τ -b (sig.)	RECAF concentration	RECAF density
Total PSA	-0.049 (0.570)	0.074 (0.388)
Free PSA	0.017 (0.840)	-0.034 (0.691)

RECAF – α -fetoprotein receptor; tPSA – total PSA; fPSA – free PSA.

centrations and PSA density, but no statistically significant correlations were found between the age of the patient and RECAF concentration and density (Table 5).

Discussion

This study focused on a comparison of novel tumor marker RECAF vs PSA, a tumor marker that has been established for PCa for 2 decades.² A previous study reported a sensitivity of 99% and specificity of 95% for RECAF.¹¹ However, the present study found no statistically significant difference in RECAF concentration between the benign and malignant pathology groups. Consequently, the sensitivity and specificity of the RECAF tumor marker were not determined. The median RECAF concentration was lower in the patients with PCa than in the benign pathology group. Since it has been suggested that RECAF, as an oncofetal protein, is expressed in large quantities in physiologic and pathologic states of high cell division and turnover rates, such as fetal development and some cancers, higher concentrations of RECAF in the PCa patients than in the benign pathology group were expected.^{10–13} On the other hand, the median RECAF density was almost 1.7 times higher in the patients with PCa than in the group with benign conditions, which was a statistically significant difference. It should be noted that the median prostatic volume was almost 1.6 times lower in the malignancy group than in the benign group, possibly introducing a bias. A much larger difference in RECAF density was expected, because RECAF concentrations in benign conditions have been reported to be nearly the same as in healthy men, suggesting a very low concentration in benign prostatic conditions.¹¹ In contrast, the PSA measurements in the present study showed significantly higher tPSA and fPSA concentrations and tPSA density in the group of patients with PCa when compared with the benign group.

The age of the patient is a proven risk factor for PCa. The present study has shown a positive statistical correlation between age and tPSA, fPSA and PSA density, which were higher in the group of PCa patients, who were significantly older than those in the benign group.

No such correlation was found for RECAF concentration or RECAF density. The positive correlation between PSA density and age underscores the fact that in older men, PSA produced per volume of prostatic tissue increases due to PCa and not due to benign prostatic enlargement.

Generally, tPSA concentration is higher in PCa patients with a higher Gsum.^{14,15} The results of the present study show significantly higher median tPSA and fPSA concentrations in the subgroups of patients with increasing Gsum, Gprim, Gsec and maxGsec. Similarly, tPSA density also significantly increases in patients with increasing Gprim, implying higher prostatic cell division and turnover rates in patients with higher grade cancer. In contrast, RECAF concentration and RECAF density do not differ significantly in the subgroups of patients stratified according to Gsum, Gprim, and maxGprim; it was only in the subgroups with increasing Gsec and maxGsec that significant differences in RECAF density were found. Median RECAF concentrations decrease with increasing Gprim, Gsec, and maxGsec; median RECAF density decreases with increasing Gprim, Gsec, maxGprim, and maxGsec, even though one would expect that a higher rate of cell division and turnover in higher grade cancers would be reflected in higher RECAF concentration and density.

The results of this study did not confirm that higher tPSA and fPSA concentrations imply higher RECAF concentration and RECAF density, because no statistically significant correlations were found in the entire study group. As this study is the first to report on the RECAF marker in relation to the histopathologic grading of PCa and correlations to PSA, there are no other studies to compare the results with.

In conclusion, the results of the present study did not show RECAF to be an alternative tumor marker for discrimination between groups of patients with benign prostatic conditions and PCa. Its concentration and density do not correlate with either tPSA or fPSA, which are established markers for PCa and which differ significantly between the benign and malignant pathology groups of patients in the study. In contrast to tPSA and fPSA, RECAF concentration does not significantly differ between histopathologic subgroups according to Gsum, Gprim, and Gsec grades or maxGprim and maxGsec grades. RECAF density is, paradoxically, lower in higher-end Gleason grade and maxGsec grade subgroups. To elucidate the issue of the RECAF marker in different histopathologic groups, it is necessary to conduct further studies including histopathologic staining of prostate biopsy cores using a tissue-section staining kit approved by the US Food and Drug Administration.¹¹

Table 5. Statistical correlation between PSA and RECAF with the age of the patient

Kendall τ -b (sig.)	tPSA	fPSA	PSA density	RECAF concentration	RECAF density
Age	0.245 (0.005)	0.306 (0.001)	0.213 (0.015)	-0.149 (0.088)	-0.015 (0.866)

RECAF – α -fetoprotein receptor; tPSA – total PSA; fPSA – free PSA.

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