The role of Fetuin-A and Leucine-rich α-2-glycoprotein in the diagnosis of prostate cancer – a pilot study

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Background. Prostate cancer (PC) is one of the most frequently diagnosed non-skin solid cancers and is a leading cause of cancer-related death and the incidence increasing. Early diagnosis of the disease improves the outcomes. There is an urgent need for new biomarkers with greater discriminative precision for diagnosis, risk-stratification and treatment. The aim of our study was to evaluate the diagnostic and prognostic potential of Fetuin-A and LRG1 in patients with PC.
Methods. Serum levels of Fetuin-A and LRG1 were compared in patients with PC (n=46), a control group 1 including young, healthy subjects (n=26) and control group 2 including patients with negative prostate biopsy (n=46). In PC patients, the levels of both biomarkers were compared in subgroups with different tumour characteristics.
Results. We demonstrated a statistically significant higher concentrations of Fetuin-A in PC patients compared to control group 2 (439 mg/L vs. 372 mg/L), $P<0.001$. No statistically significant difference was found between PC patients and control group 1, nor for LRG1 levels between the three groups. In PC patients, higher serum levels of LRG1 were found in M1 patients compared to M0 (98 mg/L vs. 42 mg/L), $P=0.059$.
Conclusion. Fetuin-A levels are significantly higher in patients with prostate cancer than in patients without malignancy but LRG1 levels do not differ between patients with PC and controls.

Key words: biomarker, Fetuin-A, LRG1, prostate cancer, serum

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INTRODUCTION

Prostate cancer (PC) is the second most diagnosed cancer in adult men and the second leading cause of cancer death in men worldwide[1]. Each year, 1.6 million men are diagnosed with prostate cancer and 366 000 men die from prostate cancer[2]. The incidence increases with age, with nearly 60% of men over 65 years of age having histologically proven prostate cancer[3]. However, prostate cancer is very heterogeneous, with a significant proportion being clinically nonsignificant cancers, i.e. those that do not threaten the patient's life[4]. Despite its shortcomings, PSA remains the mainstay of laboratory diagnosis, risk stratification and monitoring of treatment of PC (ref.[5]). The search for additional diagnostic and prognostic biomarkers is the subject of intensive research.

The ideal biomarker must have high sensitivity and specificity, should be easy to use in clinical practice with low economic cost, and must provide clear results for the clinician[6]. Fetuin-A, also known as Alpha 2–Heremans Schmid Glycoprotein, is a 59kDa glycoprotein consisting of two amino-terminal cystatin domains and a smaller carboxyl-terminal domain. Fetuin-A is synthesized in the liver and subsequently secreted into the bloodstream. The concentration of Fetuin-A in adult male's ranges from 0.5–1.5 g/L (ref.[7]). Although it is described as a multifunctional protein, it plays the most important role in inhibiting ectopic calcification, where it interferes with calcium salt. As a major non-collagenous protein, it is deposited in bone and teeth. It is also able to modulate several signalling pathways and plays a role in diseases such as diabetes mellitus and renal failure[8]. Elevated levels of Fetuin-A have been associated with metabolic syndrome, whereas its reduced levels are associated with vascular calcifications and inflammation[9]. In addition, it interferes with several important pathways for tumour growth and metastasis[10]. Several studies have shown that it can also be ectopically synthesized by tumour cells and tissues[11].

Leucine-rich α-2 glycoprotein 1 (LRG1) is a serum glycoprotein that was originally associated with inflammatory responses in the body[12]. Since 1977, when it was first isolated from human serum, studies have shown that it regulates cell adhesion, apoptosis, and migration and is associated with various biological processes, including malignancy[13]. LRG1 has been reported to be a key regulator of not only inflammation but also angiogenesis[14].

The aim of our study was to evaluate the potential of these biomarkers in the diagnosis and monitoring of patients with PC by comparing serum levels of Fetuin-A and LRG1.

MATERIAL AND METHODS

Patient characteristics

Three groups of patients were enrolled in the study which was conducted at our institution between January 2023 and March 2024. The first group consisted of patients with histologically confirmed prostate cancer. Patients were enrolled in the study after diagnosis and then were prospectively followed up. All patients were initially evaluated by digital rectal examination (DRE), PSA, Gleason score and extent of disease, and were classified into risk groups according to the European Association of Urology (EAU). Exclusion criteria were the presence of another cancer within the last 10 years and acute infection at the time of sampling. Control group 1 consisted of young, healthy male patients (age up to 35 years). Control group 2 consisted of patients who underwent transrectal prostate biopsy with a finding of benign prostatic hyperplasia (no finding of malignancy, no finding of prostatitis). Exclusion criteria for controls were the same as for patients with PC. The study was approved by the Ethics Committee of the University Hospital Brno (04-110123/EK, 11 January 2023) and informed consent was obtained from all patients. All patients were treated in accordance with the current recommendations of the European Urological Association.

A total of 121 patients were enrolled in the study, of whom 46 (38%) were in the prostate cancer group, 26 (21.5%) were in control group 1, and 49 (40.5%) were in control group 2 (Fig. 1). The baseline characteristics

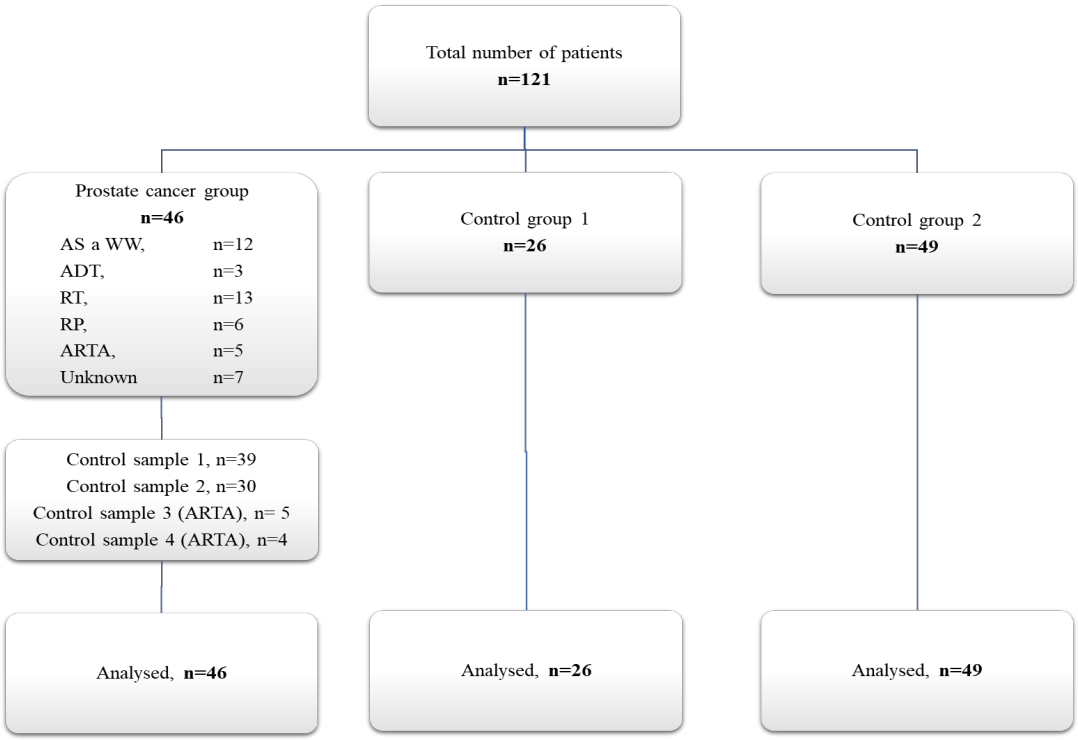

Fig. 1. CONSORT flowchart of the study.
AS, Actevi surveillance; VW, Watchfull waiting; ADT, Androgen deprivation therapy; RT, Radiotherapy; RP, Radical prostatectomy; ARTA, Androgen receptor targeted agents.

Table 1. Basic characteristics of study participants.

Characteristic of patients	n (%)	Average (SD)	Median (Min–Max)
Age	121 (100 %)	56.9 (17.0)	62.0 (20.0–84.0)
Patients (all)	46 (38.0 %)	69.6 (7.3)	69.0 (52.0–84.0)
Risk groups			
Low	14 (11.6 %)	67.8 (5.6)	67.5 (59.0–77.0)
Medium	15 (12.4 %)	69.0 (8.5)	70.0 (52.0–79.0)
High	17 (14.0 %)	71.6 (7.4)	72.0 (60.0–84.0)
Control group 1*	26 (21.5 %)	28.2 (4.5)	29.0 (20.0–36.0)
Control group 2*	49 (40.5 %)	60.3 (7.0)	62.0 (44.0–78.0)
PSA	72 (100 %)	23.7 (146.5)	2.8 (0.2–1246.0)
Patients	46 (63.9 %)	36.7 (182.8)	6.7 (0.4–1246.0)
Control group 1	26 (36.1 %)	0.7 (0.3)	0.6 (0.2–1.2)
PHI	71** (100 %)	56.0 (56.3)	37.5 (10.8–295.5)
Patients	45 (63.4 %)	75.5 (62.9)	54.4 (11.7–295.5)
Control group 1	26 (36.6 %)	22.3 (6.9)	19.8 (10.8–36.3)

Table 2. Summary characteristics of patients with prostate cancer (n=46).

Characteristic of patients	Categories	n	%
DRE (Digital Rectal Examination)	cT1c	28	60.9
	cT2a	5	10.9
	cT2b	4	8.7
	cT2c	9	19.6
	cT3	0	–
Gleason score	6 (3+3)	15	32.6
	7 (3+4, 4+3)	17	37.0
	8 (4+4)	9	19.6
	9 (4+5, 5+4)	5	10.9
	10 (5+5)	0	–
Risk	Low	14	30.4
	Medium	15	32.6
	High	17	37.0
Extent of disease	N0M0	40	87.0
	N1	0	–
	M1	6	13.0
Characteristic	n (%)	Average (SD)	Median (Min–Max)
iPSA (μg/L)	46 (100 %)	34.1 (130.0)	6.9 (0.3–870.0)
iPSA according to risk			
low	14 (30.4 %)	5.1 (2.7)	4.9 (0.3–9.0)
medium	15 (32.6 %)	5.7 (3.1)	4.5 (2.5–14.0)
high	17 (37.0 %)	82.9 (208.6)	16.2 (3.9–870.0)

of the groups are shown in Table 1. Characteristics of patients with PC according to palpation findings, Gleason score, risk groups and extent of disease are shown in Table 2.

Laboratory analysis

Laboratory processing of the samples was performed at the Department of Laboratory Methods, University Hospital Brno. All handling of the collected samples was performed blindly and in accordance with institutional procedures for handling human biological material. In all groups of patients, blood samples were collected for standard biochemical tests including PSA and PHI (prostate health index), differential blood count, and Fetuin-A and LRG1 levels. The analyses of Fetuin-A and LRG1 serum levels were performed using the Human Fetuin-A ELISA kit, KAPEPKT800, DIAsource ImmunoAssays S.A. (Belgium) and the Human LRG1 ELISA kit, E-ELK-H6067, Elabscience Biotechnology Inc. (Germany). 10 μL of human serum was used for Fetuin-A determination. The blood sample was allowed to clot for at least 30 min at room temperature before serum separation by centrifugation (850–1500 x g for 10 min). The serum was then separated into clean tubes and frozen at −20 °C until measurement. Serum samples were left at room temperature for 30 min before dilution (1:10000). Subsequently, the samples were processed using an ELISA kit. The samples were analysed once, and the results were interpreted in mg/l. The analytical sensitivity of the ELISA for Fetuin-A is 0.005 mg/L (ref.[15]).

For LRG1 determination, thawed serum samples were diluted (1:10000) and then left in solution for 30 min at room temperature. The levels of LRG1 in the serum samples were measured in duplicate using the above ELISA kit. The results were interpreted in mg/L. The analytical sensitivity of the ELISA for LRG1 is 0.00017 mg/L (ref.[16]).

Statistical analysis

Statistical data analysis was performed at the Institute of Health Information and Statistics of the Czech Republic in Brno. Standard descriptive statistics were used to summarize the data. Continuous data were evaluated with respect to the observed categories using the nonparametric Mann-Whitney test and the nonparametric Kruskal-Walli's test, respectively. The observation of data over time was evaluated using the Wilcox paired nonparametric test and the Spearman correlation coefficient was used to relate some selected variables. Statistical significance was assessed at the 5% significance level, *P*-values less than 0.05 were considered statistically significant.

RESULTS

First, Fetuin-A and LRG1 levels were compared between prostate cancer patients and control group 1, i.e. young and healthy men. The median LRG1 value was 46 mg/L in prostate cancer patients and 40 mg/L in control group 1, *P*=0.201. The median value of Fetuin-A in group 1 was 439 mg/L and in control group 1 was 398 mg/L, *P*=0.227. Thus, when comparing the values of the two biomarkers studied, there was no statistically significant difference between prostate cancer patients and young,

Table 3. Comparison of biomarker levels among the study groups.

Potential oncomarker	Patients with PC (n = 46)			Control group 1 (n = 26)			*P*
	n	Average; median	Min–Max	n	Average; median	Min–Max	
Fetuin (mg/L)	46	459; 439	227–879	26	423; 398	240–607	0.227
LRG 1 (mg/L)	46	70; 46	13–254	26	47; 40	15–106	0.201
				Control group 2 (n = 49)			
Fetuin (mg/L)	46	459; 439	227–879	49	370; 372	206–695	<0.001

Table 4. Evaluation of the relationship between tumour characteristics and Fetuin-A and LRG1 concentrations in prostate cancer patients.

Categorization		n	Fetuin (mg/L)			LRG 1 (mg/L)		
			average; median	Min–Max	*P*	average; median	Min–Max	*P*
Risk	low	14	474; 432	258–879		79; 66	17–236	
	medium	15	437; 435	300–550	0.756	52; 42	18–135	0.469
	high	17	465; 504	227–739		78; 48	13–254	
Gleason score	6	15	471; 430	258–879		75; 59	17–236	
	7	17	458; 439	300–584	0.884	64; 42	19–254	0.702
	8–10	14	446; 449	227–739		72; 54	13–166	
DRE	T1	28	450; 433	237–879	0.405	69; 54	17–236	0.702
	T2	18	473; 491	227–739		71; 43	13–254	
iPSA*	< 4	10	452; 437	367–578		68; 40	13–236	
	4–20	29	467; 439	237–879	0.999	66; 59	17–144	0.652
	> 20	7	432; 513	227–584		90; 48	17–254	
Disemination	negative	40	463; 439	237–879	0.714	63; 42	13–236	0.059
	M1	6	432; 422	227–666		117; 98	37–254	

healthy subjects (Table 3). We also compared the values of Fetuin-A and LRG1 with variables describing the severity of the disease – risk group, Gleason score, DRE, and extent of disease. Of the selected variables, only the association of LRG1 levels with metastatic prostate cancer was at the borderline of statistical significance, with patients with M1 disease having higher LRG1 levels (98 mg/L vs. 42 mg/L), *P*=0.059 (Table 4). Next, we compared patients with prostate cancer and control group 2, i.e., patients without histologically proven cancer. Here we found statistically significantly higher Fetuin-A levels in the blood of patients with prostate cancer (439 mg/L vs. 372 mg/L), *P*<0.001 (Table 3, Fig. 2). There was also a statistically significant difference in Fetuin-A levels between control group 1 and control group 2 (median 398 mg/L vs. 372 mg/L, *P*=0.046).

DISCUSSION

In our study, we evaluated the diagnostic potential of two biomarkers, Fetuin-A and LRG1. A few studies in the past have suggested that Fetuin-A might be important in the diagnosis of PC (ref.[17,18]). In our cohort, we observed significantly higher Fetuin-A levels in a group of patients with prostate cancer compared with a group of patients of similar age with benign prostatic hyperplasia, i.e., control group 2. When comparing patients with prostate cancer to young, healthy men, Fetuin-A levels were higher in patients with PC, but the difference was not statistically significant. This observation may be explained by naturally higher Fetuin-A levels at younger ages, with values gradually decreasing with increasing age[7,18]. Thus, this result has promising implications for clinical practice, where increased Fetuin-A levels at older ages could serve as an indicator of prostate cancer. Further studies on a larger number of patients are needed to confirm this important finding.

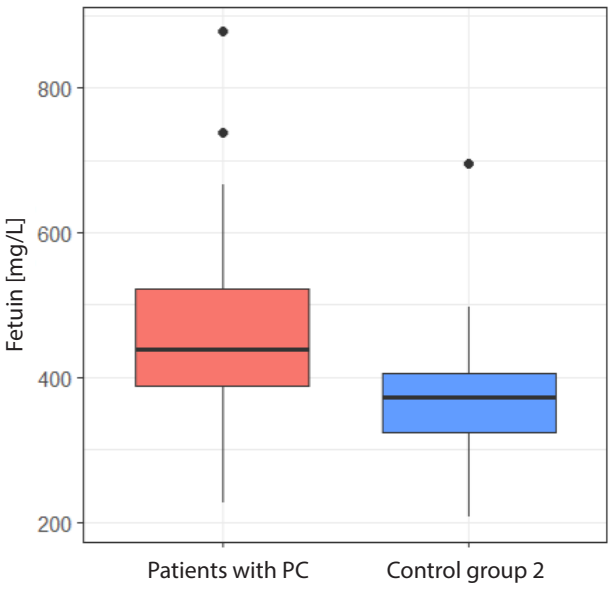

Fig. 2. Box plots of blood concentrations of Fetuin-A in prostate cancer patients and control group 2.

LRG1 is highly expressed in ovarian cancer, but some studies consider it a promising predictive marker for prostate cancer[14]. In our study, we did not demonstrate different levels between the study groups, thus not confirming the potential of LRG1 for the diagnosis of PC. Only in patients with metastatic disease were the levels higher than in non-metastatic PC (at the borderline of statistical significance). This is in correlation with the study by Guldvik et al.[14], who showed that elevated LRG1 blood levels are an adverse factor in patients with metastatic prostate cancer. Thus, a higher LRG1 level could be of prognostic significance.

The main limiting factor of our study is the small number of patients and the short recruitment period (15 months). The strength of the study is the prospective design of the study and the existence of a control group with negative prostate biopsy.

CONCLUSION

Fetuin-A is a potential new biomarker useful in the diagnosis of prostate cancer, its levels are significantly higher than in patients without malignancy. LRG1 levels do not differ between PC patients and controls. The association among elevated LRG1 levels and metastatic prostate cancer needs to be confirmed in a larger number of patients.

Author contributions: ASK: methodology, validation, investigation**,** writing-original draft; GV: resources, visualization; MB: resources; LK: formal analysis; MF: conceptualization, investigation, writing-review and editing. All authors read and approved the final manuscript.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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