MATHEWS Open Access Journals

ISSN: 2474-6797

Research Article Mathews Journal of Cancer Science

Distinguish Between Benign and Malignant Prostate Using the Trace Element Content Ratios in Prostatic Tissue as Tumor Markers

Vladimir Zaichick[1], Sofia Zaichick[2]

[1]Department of Radionuclide Diagnostics, Medical Radiological Research Centre, Russia.

[2]Department of Medicine, University of Illinois College of Medicine, USA.

Corresponding Author: Vladimir Zaichick, Department of Radionuclide Diagnostics, Medical Radiological Research Centre, Koroleva Str- 4, Obninsk 249036, Kaluga Region, Russia, **Tel:** +7 (48439) 60289; **Email:** vezai@obninsk.com

Received Date: 28 Jul 2016

Accepted Date: 18 Aug 2016

Published Date: 24 Aug 2016

Citation: Zaichick V and Zaichick S. (2016). Distinguish Between Benign and Malignant Prostate Using the Trace Element Content Ratios in Prostatic Tissue as Tumor Markers. M J Canc. 1(1): 006.

ABSTRACT

The aim of the study was the development of new highly precise testing methods for early diagnosis of prostate cancer. For this purpose the values of the zinc/silver (Zn/Ag), zinc/cobalt (Zn/Co), zinc/chromium (Zn/Cr), zinc/iron (Zn/Fe), zinc/mercury (Zn/Hg), zinc/rubidium (Zn/Rb), zinc/antimony (Zn/Sb), zinc/scandium (Zn/Sc), and zinc/selenium (Zn/Se) mass fraction ratios in normal (n = 37), benign hypertrophic (n = 43) and cancerous tissues (n = 60) of the human prostate gland were calculated using data obtained by instrumental neutron activation analysis. Mean values ± standard error of mean (M ± SEM) for trace element mass fraction ratios in normal tissue were: Zn/Ag – 32270 ± 5360, Zn/Co – 27010 ± 3720, Zn/Cr – 2650 ± 360, Zn/Fe – 10.9 ± 1.4, Zn/Hg – 27010 ± 3720, Zn/Rb – 73.6 ± 6.6, Zn/Sb – 34330 ± 6160, Zn/Sc – 46790 ± 7870, and Zn/Se – 1550 ± 170, respectively. It was observed that in benign hypertrophic tissues the Zn/Cr, Zn/Hg, Zn/Sb, and Zn/Se mass fraction ratios were lower than in normal tissues. In cancerous tissue all investigated mass fraction ratios were significantly ($p < 0.001$) lower than in normal and benign hypertrophic tissues of the prostate. Finally, we propose to use the estimation of Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Sensitivity, specificity, and accuracy of these tests were in ranges 96-100%, 89-100%, and 94-100%, respectively. Application of the Zn/trace element mass fraction ratios instead the Zn mass fraction is more suitable for PCa diagnosis, because accuracy of Zn/trace element mass fraction ratios does not depend on sample mass and changes in moisture content.

KEYWORDS: Trace Element; Trace Element Mass Fraction Ratios; Prostate; Benign Prostatic Hypertrophy; Prostatic Carcinoma; Neutron Activation Analysis.

INTRODUCTION

The prostate gland may be a source of many health problems in men past middle age, the most common being benign prostatic hyperplasia (BPH), and prostatic carcinoma (PCa). BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra and can significantly impair quality of life [1]. The prevalence of histological BPH is found in approximately 50-60% of males age 40-50, in over 70% at 60 years old and in greater than 90% of men over 70 [2, 3]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of non-cutaneous malignancy in males and, except for lung cancer, is the leading cause of death from cancer [4-9]. Although the etiology of BPH and PCa is unknown, many trace elements have been highlighted in the literature in relation to the development of these prostate diseases [10-29].

Trace elements have essential physiological functions such as maintenance and regulation of cell function and signalling, gene regulation, activation or inhibition of enzymatic reactions, neurotransmission, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively [30]. Excessive accumulation, deficiency or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death and malignant transformation [31].

In previous studies significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed [32-53]. Moreover, a significant informative value of Zn content as a tumor marker for PCa diagnostics was shown [54]. Hence it is possible that besides Zn, some other trace elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Current methods applied for measurement of trace element contents in samples of human tissue include a number of methods. Among these methods the instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) is a non-destructive and one of the most sensitive techniques. It allows measure the trace element contents in a few milligrams tissue without any treatment of sample. Analytical studies of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal, BPH and PCa tissue were done by us using INAA-LLR [16, 21, 29, 48, 51, 55]. Non-destructive method of analysis avoids the possibility of changing the content of chemical elements in the studied samples, which allowed for the first time to obtain reliable results [56-59]. In particular, it was shown that the average mass fraction of Zn in PCa tissues is 7 times lower than in healthy or BPH tissue [55]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which was to determine the content of zinc in the material of trans-rectal needle biopsy of prostate indurated site. For the first time it was proposed to use INAA-LLR to determine zinc and other trace element contents in needle-biopsy cores [55]. Moreover, it was shown in the study that in a normal prostate tissue mass fraction of some trace elements tend to be correlated with Zn, while in BPH and PCa tissues these relationships are partially broken or changed [55]. These findings open the additional possibilities for developing new methods of PCa diagnostics using Zn content/trace element content ratio.

Thus, this work had three aims. The first was to calculate individual Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios using data about the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, Zn contents in intact prostate of healthy men aged over 40 years obtained by INAA. The second aim was to compare the levels of mass fraction ratios in the prostate gland of age-matched patients, who had either BPH or PCa, and the third was to evaluate mass fraction ratios to aid diagnosis of prostate cancer. All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

MATERIAL AND METHODS

Samples

All patients studied (n = 103) were hospitalized in the Urological Department of the Medical Radiological Research Centre. Trans-rectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases the diagnosis BPH (n = 43) and PCa (n = 60) has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials.

Intact (normal) prostates were removed at necropsy from 37 men who had died suddenly. The majority of deaths were due to trauma. Tissue samples were collected from the peripheral zone of dorsal and lateral lobes of their prostates, within 2 days of death and then the samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. A histological examination was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer [16, 21, 29].

Sample preparation, instrumentation, methods and certified reference materials

Details of sample preparation, the relevant nuclear reactions, radionuclides, gamma energies, methods of analysis and the results of quality control were presented in our earlier publications concerning the chemical elements of human prostate tissue investigated by INAA-LLR [16, 21, 29, 48, 51, 60].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [61]. All prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. Using the Microsoft Office Excel software, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for trace element mass fraction ratios in normal, benign hyperplastic and cancerous prostate tissue. The difference in the results between BPH and Norm, PCa and Norm, and PCA and BPH was evaluated by Student's t-test and nonparametric Wilcoxon-Mann-Whitney U-test. We applied a significance level of 0.05. For the construction of "individual data sets for Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/

Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate tissue" diagrams the Microsoft Office Excel software was also used.

RESULTS

The age of 43 patients with BPH ranged from 38 to 83 years, the mean being 66 ± 8 years (M ± SD). The 60 patients aged 40-79 suffered from PCa. Their mean age was 65 ± 10 years. Mean age of men with intact prostates (Normal group) was 55 ± 11 years (range 41-79).

Table 1 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate tissue.

Table 1: Some statistical parameters of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate.

Tissue Mass fraction ratio	Mean	SD	SEM	Min	Max	Median	Per.	Per.
							0.025	0.975
Normal (n = 37)								
Zn/Ag	32271	27330	5360	3036	113139	23563	4263	90351
Zn/Co	27011	20354	3716	4236	82272	21419	4798	69552
Zn/Cr	2654	1780	356	248	7073	2404	330	6369
Zn/Fe	10.9	8.0	1.4	1.68	30.5	8.66	2.06	29.9
Zn/Hg	27011	18957	3717	2533	69231	24331	4110	64394
Zn/Rb	73.6	37.1	6.6	18.4	181	72.1	19.2	149
Zn/Sb	34333	33149	6156	2935	122891	18500	4970	118907
Zn/Sc	46794	34288	7866	14274	121364	35309	15874	116182
Zn/Se	1548	908	166	421	3652	1335	442	3439
BPH (n=43)								
Zn/Ag	39748	19357	4328	13499	83073	39623	14448	76749
Zn/Co	20798	14641	3359	6285	58902	15637	7496	54869
Zn/Cr	1161	678	156	282	2720	902	359	2619
Zn/Fe	10.4	6.82	1.15	1.88	30.2	9.30	2.22	28.8
Zn/Hg	6490	3302	688	1575	14404	6588	1829	13492
Zn/Rb	79.4	40.2	7.0	24.8	182	73.6	26.1	175
Zn/Sb	10115	9947	2344	1765	39016	7255	1903	36005
Zn/Sc	39678	12156	3372	18545	60576	40462	21415	58160
Zn/Se	886	413	90	236	1562	825	283	1540
PCa (n=60)								
Zn/Ag	723	680	133	64.5	2968	492	72.3	2474
Zn/Co	4293	2933	554	926	12136	3186	1021	11264
Zn/Cr	78.1	68.2	13.4	11.4	256	58.2	12.4	247
Zn/Fe	1.08	0.79	0.12	0.054	2.97	0.84	0.114	2.83
Zn/Hg	1216	618	115	184	2301	1106	207	2172
Zn/Rb	15.7	8.0	1.2	1.30	32.4	15.4	1.69	31.1
Zn/Sb	334	253	44	37.1	857	256	40.8	843
Zn/Sc	13157	8593	1624	2222	33750	10693	2703	30178
Zn/Se	270	155	28	69.2	706	234	90.0	644

M - arithmetic mean; SD – standard deviation; SEM – standard error of mean; Min – minimum value; Max – maximum value; Per. 0.025 – percentile with 0.025 level; Per. 0.975 – percentile with 0.975 level, n - number of samples.

The ratios of means and the difference between mean values of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in BPH and Norm, PCa and Norm, and PCA and BPH evaluated by Student's t-test and nonparametric Wilcoxon-Mann-Whitney U-test are presented in Table 2.

Table 2: Ratio of means and the difference between mean values of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate.

Mass fraction ratio	**BPH and Normal (N)**			**PCa and Normal (N)**			**PCa and BPH**		
	Ratio	p	p	Ratio	p	p	Ratio	p	p
	BPH/N	t-test	U-test	PCa/N	t-test	U-test	PCa/BPH	t-test	U-test
Zn/Ag	1.23	=0.28.	>0.05	0.022	**<0.001**	**≤0.01**	0.018	**<0.001**	**≤0.01**
Zn/Co	0.77	=0.22	>0.05	0.16	**<0.001**	**≤0.01**	0.21	**<0.001**	**≤0.01**
Zn/Cr	0.44	**<0.001**	**≤0.01**	0.029	**<0.001**	**≤0.01**	0.067	**<0.001**	**≤0.01**
Zn/Fe	0.95	=0.78	>0.05	0.099	**<0.001**	**≤0.01**	0.104	**<0.001**	**≤0.01**
Zn/Hg	0.24	**<0.001**	**≤0.01**	0.045	**<0.001**	**≤0.01**	0.19	**<0.001**	**≤0.01**
Zn/Rb	1.08	=0.55	>0.05	0.21	**<0.001**	**≤0.01**	0.20	**<0.001**	**≤0.01**
Zn/Sb	0.29	**<0.001**	**≤0.01**	0.0097	**<0.001**	**≤0.01**	0.033	**<0.001**	**≤0.01**
Zn/Sc	0.85	=0.41	>0.05	0.28	**<0.001**	**≤0.01**	0.33	**<0.001**	**≤0.01**
Zn/Se	0.57	**=0.001**	**≤0.01**	0.18	**<0.001**	**<0.001**	0.30	**<0.001**	**≤0.01**

t-test - Student's t-test, U-test - Wilcoxon-Mann-Whitney U-test, Bold significant differences.

Table 3 contains parameters of the importance (sensitivity, specificity and accuracy) of some trace element mass fraction ratios for the diagnosis of PCa calculated in this work.

Table 3: Parameters of the importance (sensitivity, specificity and accuracy) of some trace element mass fraction ratios for the diagnosis of PCa (an estimation is made for "PCa or intact and BPH prostate").

Mass fraction ratio	Upper limit for PCa	Sensitivity	Specificity	Accuracy
	(M+2.5SD)	%	%	%
Zn/Ag	2420	96±4	100-2	99±1
Zn/Cr	250	96±4	98±2	97±2
Zn/Fe	3.0	100-2	89±4	94±2
Zn/Hg	2760	100-3	94±3	96±2
Zn/Sb	970	100-3	100-2	100-1

M - arithmetic mean, SD – standard deviation.

Figure 1 depicts individual data sets for Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in all investigated samples of normal, benign hypertrophic and cancerous prostate tissue.

DISCUSSION

As was shown by us the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate tissue can be seen as quite acceptable [16, 21, 29]. Good agreement of the trace element mass fractions analyzed by INAA-LLR with the certified data of CRM IAEA H-4 indicates an acceptable accuracy of the results obtained in the study of trace elements of the prostate presented in Tables 1-3 and Figure 1.

The mean values and all selected statistical parameters were calculated for 9 mass fraction ratios (Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se) (Table 1). The mass fraction ratio of these elements were calculated for all, or a major portion of normal, BPH and PCa samples. No published data referring to mass fraction ratios of trace elements in the human prostate was found.

From Tables 1 and 2, it is observed that in benign hypertrophic tissues the mass fraction ratios of Zn/Cr ($p < 0.001$), Zn/Hg ($p < 0.001$), Zn/Sb ($p < 0.001$), and Zn/Se ($p < 0.01$) are lower than in normal tissues. In cancerous tissue all investigated mass fraction ratios are lower ($p < 0.001$) than in normal and benign hypertrophic tissues of the prostate.

Analysis of trace element mass fraction ratios in prostate tissue could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to experience gained in a critical assessment of the limited capacity of the prostate specific antigen (PSA) serum test [62]. In addition to the PSA serum test and morphological study of needle-biopsy cores of the prostate, the development of other highly precise testing methods seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In all cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the

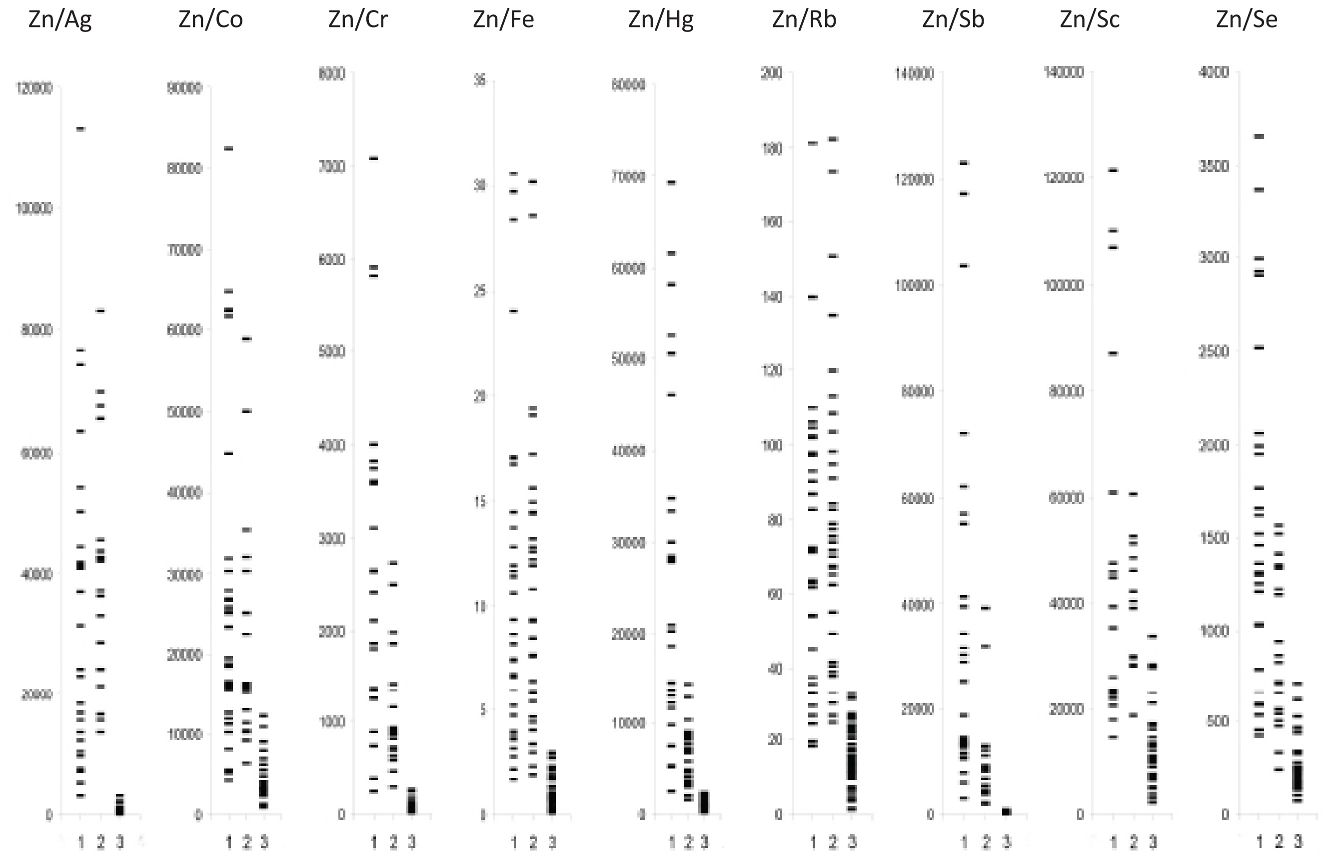

Figure 1: Individual data sets for Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.

indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of trace element ratios information for the diagnosis of PCa. As is evident from individual data sets (Figure 1), the Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb mass fraction ratios are the most informative for a differential diagnosis. For example, if 2420 (M ± 2.5SD) is the value of Zn/Ag mass fraction ratio assumed to be the upper limit for PCa (Figure 1) and an estimation is made for "PCa or intact and BPH tissue", the following values are obtained:

Sensitivity = {True Positives (TP)/[TP + False Negatives (FN)]} •100% = 96 ± 4%;

Specificity = {True Negatives (TN)/[TN + False Positives (FP)]} •100% = 100-2%;

Accuracy = [(TP+TN)/(TP+FP+TN+FN)] •100% = 99 ± 1%.

The number of people (samples) examined was taken into account for calculation of confidence intervals [63]. In other words, if Zn/Ag mass fraction ratio in a prostate biopsy sample do not exceed 2420, one could diagnose a malignant tumor with an accuracy of 99 ± 1%. Thus, using the (Zn/Ag)-test makes it possible to diagnose cancer in 96 ± 4% cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb mass fraction ratios for the diagnosis of PCa were calculated (Table 3).

Mass fraction ratios of Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb in the needle-biopsy cores could be used as a tool to diagnose PCa and are comparable with characteristics of the Zn mass fraction-test [55]. However, it is our opinion that application of the trace element mass fraction ratios is more suitable for PCa diagnosis. Trace element mass fraction depends on the sample mass, which decreases with loss of its moisture. The needle-biopsy core is a small piece of tissue with a relatively high "surface/volume" ratio. After sampling, it begins to lose mass very fast. Weight loss of samples depends on the humidity of operating and store rooms [56]. Thus, it is very difficult to determine the fresh mass of needle-biopsy cores and to calculate the precise mass fraction of trace elements. Sample freeze-dry, storage in air-tight vials until weighing, and then calculating mass fraction on dry weight basis is the only possible method that eliminates the variation in sample weight. Conversely, accuracy of trace element mass fraction ratios does not depend on sample mass and changes in moisture content. Therefore, this method does not require dry samples. Moreover, the use of the relations between mass fractions of chemical elements is particularly promising for the development of in vivo diagnostic methods, including the diagnosis of PCa.

CONCLUSION

In this work, mean values of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/

Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate tissue were calculated using data of INAA-LLR. It was observed that in benign hypertrophic tissues the Zn/Cr, Zn/Hg, Zn/Sb, and Zn/Se mass fraction ratios are lower than in normal tissues. In cancerous tissue all investigated mass fraction ratios are lower than in normal and benign hypertrophic tissues of the prostate. It was shown that the Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb mass fraction ratios are the most informative for a differential diagnosis among all investigated mass fraction ratios. Finally, we propose to use the estimation of Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer.

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