

## Research Article

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## Ratios of Co/Trace Elements As Prostate Cancer Markers

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### Abstract

The aim of the study was to evaluate whether significant changes in the prostatic tissue levels of ratios Co/trace element contents exist in the malignantly transformed prostate. Contents of Co and other 42 trace elements (Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr) in normal (N, n=37, age range 41-87 years), benign hypertrophic (BPH, n=32, age range 56-78), and cancerous human prostate (PCa, n=60, age range 40-70) were investigated. Measurements of trace element contents were performed using a combination of instrumental neutron activation analysis and inductively coupled plasma mass spectrometry. Then the levels of ratios Co/trace element contents in every sample were calculated. It was observed that the ratio to Co of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Dy, Er, Gd, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, U, Y, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. It was proposed to use the Co/Ag, Co/Al, Co/B, Co/Bi, Co/Li, Co/Mn, Co/Pr, Co/Th, Co/Tl, and Co/Zr mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

**Keywords:** trace elements; trace element content ratios; prostate; benign prostatic hypertrophy; prostatic carcinoma; neutron activation analysis; inductively coupled plasma mass spectrometry

### Introduction

The prostate gland may be a source of many health problems in men past middle age, the most common 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. The prevalence of histological BPH is found in approximately 50-60% of males age 40-50 and greater than 90% of men over 70 years old [1, 2]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of noncutaneous malignancy in males. Except for lung cancer, PCa is the leading cause of death from cancer [3-8]. Although the etiology of BPH and PCa is unknown, some trace elements have been highlighted in the literature in relation to the development of these prostate diseases [9-29].

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 [30].

In earlier reported studies [31-69] significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed. In particular, it was shown that the average mass fraction of some trace elements in BPH were higher than normal levels, while those in adenocarcinoma were lower than in healthy prostatic parenchyma [60,61,66, 67,68]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which was to determine the ratios of chemical element contents changed in opposite directions during malignant transformation of prostate. For example, a significant informative

value of Zn/Co content as a tumor marker for PCa diagnostics was shown by us [70]. Hence it is possible that besides Zn, the ratio of Co to other trace elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Currently number of methods was applied for the measurement of chemical elements contents in samples of human tissue. 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 chemical element contents in few milligrams tissue without any treatment of sample. Nondestructive method of analysis avoids the possibility of changing the content of trace elements in the studied samples [71-74], which allowed for the first time to obtain reliable results. However, INAA-LLR allows only determine the mean mass fractions of 10-11 trace elements in the samples of normal and cancerous prostate glands [15,28]. The inductively coupled plasma mass spectrometry (ICP-MS) is more power analytical tool than INAA-LLR [18], but sample digestion is a critical step in elemental analysis by this method. In the present study two these analytical methods were used and the results, obtained for some trace elements by ICP-MS, were under the control of INAA-LLR data.

The present study had three aims. The main objective was to obtain reliable results about the 43 trace elements: Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr contents in intact prostate of healthy men aged over 40 years and also in the prostate gland of age-matched patients, who had either BPH or PCa, combining in consecutive order non-destructive INAA-LLR with destructive ICP-MS method. The second aim was to calculate Co/trace element content ratios for every samples and compare the levels of these ratios in normal, hyperplastic, and cancerous prostate. The third and final aim was to evaluate the ratios of Co/trace element contents for diagnosis of prostate cancer.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

## Material and Methods

### Samples

The patients studied (n=92) were hospitalized in the Urological Department of the Medical Radiological Research Centre (Obninsk, Russia). All of them were European-Caucasian, citizens of Moscow and Obninsk (a small city in a non-industrial region 105 km south-west of Moscow). Transrectal 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 has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. The age of 32 patients with BPH ranged from 56 to 78 years, the mean being 66±6 (M±SD) years. The 60 patients aged 40-79 suffered from PCa (stage T1-T4). Their mean

age was 65±10 (M±SD) years.

Intact prostates (N) were removed at necropsy from 37 men aged 41-87 who had died suddenly. All deceased were European-Caucasian, citizens of Moscow. Their mean age was 55±11 (M±SD) years. 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 [14,15,20,28].

### Sample preparation

All tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized. The sample weighing about 10 mg (for biopsy materials) and 50-100 mg (for resected materials) was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with double rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

After INAA-LLR investigation, the prostate samples were taken out and used for ICP-MS method. The samples were decomposed in autoclaves; 1.5 mL of concentrated HNO<sub>3</sub> (nitric acid at 65 %, maximum (max) of 0.0000005 % Hg; GR, ISO, Merck) and 0.3 mL of H<sub>2</sub>O<sub>2</sub> (pure for analysis) were added to prostate tissue samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) and then heated for 3 h at 160–200 °C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to the plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO<sub>3</sub>+H<sub>2</sub>O<sub>2</sub>+ deionized water), and the resultant solutions were used as control samples.

### Instrumentation and methods

A vertical channel of a nuclear reactor was applied to determine the trace element mass fractions by INAA-LLR. The quartz ampoule with prostate samples and certified reference materials was soldered, positioned in a transport aluminum container, and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of  $1.3 \times 10^{13} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ . Ten days after irradiation samples were reweighed and repacked. The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer used for INAA-LLR included the 100 cm<sup>3</sup> Ge(Li) detector and on-line computer-based multichannel analyzer. The spectrometer provided a resolution of 1.9 keV on the <sup>60</sup>Co 1332 keV line. Other details of the INAA-LLR analysis were presented in our previous publication [15].

An ICP-MS Thermo-Fisher “X-7” Spectrometer (Thermo Electron,

USA) was used to determine the content of trace elements by ICP-MS. The element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements. Information detailing with the ICP-MS method used was presented in our previous publication [18].

### Certified reference materials

For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal muscle and IAEA HH-1 Human hair from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated prostate tissue samples. All samples of CRMs were treated in the same way as the prostate samples. Detailed results of this quality assurance program were presented in earlier publications [15,18].

### Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [76]. All prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. For elements investigated by both INAA-LLR and ICP-MS methods the mean of all results was used. Using the Microsoft Office Excel software Co/trace element contents for each trace element in every sample were calculated. Then arithmetic mean  $\pm$  standard error of mean was calculated for trace element mass fraction and for ratios of Co/trace element mass fraction in normal, benign hyperplastic and cancerous prostate. The difference in the results between BPH and N, PCa and N, as well as PCa and BPH was evaluated by parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test. Values of  $p < 0.05$  were considered to be statistically significant. For the construction of "individual data sets for Co/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate" diagrams the Microsoft Office Excel software was also used.

### Results

Table 1 represents mean values  $\pm$  standard error of mean ( $M \pm SEM$ ) of the Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr mass fraction in normal, benign hypertrophic and cancerous prostate.

Table 2 depicts mean values  $\pm$  standard error of mean ( $M \pm SEM$ ) of the ratio to Co of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction in normal, benign hypertrophic and cancerous prostate.

The ratios of means and the difference between mean values of the Co/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate are presented in Table 3.

Individual data sets for Co/Ag, Co/Al, Co/B, Co/Bi, Co/Li, Co/Mn, Co/Pr, Co/Th, Co/Tl, and Co/Zr mass fraction ratios in all investigated samples of normal, benign hypertrophic and cancerous prostate, respectively, are shown in Figure 1.

Table 4 contains parameters of the importance (sensitivity, specificity and accuracy of Co/Ag, Co/Al, Co/B, Co/Bi, Co/Li, Co/Mn, Co/Pr, Co/Th, Co/Tl, and Co/Zr mass fraction ratios for the diagnosis of PCa calculated in this work.

### Discussion

As was shown by us [14,15,17,18], the use of CRM IAEA H-4 Animal muscle, IAEA HH-1 Human hair, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs as certified reference materials for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the trace element contents in these CRMs, measured by us using INAA-LLR and ICP-MS methods, with the certified data [14,15,17,18] indicates an acceptable accuracy of the results obtained in the present study.

The mean values and standard error of mean ( $\pm SEM$ ) were calculated for 43 trace element contents including Co (Table 1), as well as for 42 ratios of Co/trace element mass fractions (Table 2). The mass fraction of Co and other 42 trace elements were measured in all, or a major portion of normal prostate samples. The masses of BPH and PCa samples varied very strong from a few milligrams (sample from needle biopsy material) to 100 mg (sample from resected material). Therefore, in BPH and PCa prostates mass fraction ratios of Co/Ag, Co/Cr, Co/Fe, Co/Hg, Co/Rb, Co/Sb, Co/Sc, Co/Se, and Co/Zn were measured in all, or a major portion of samples, while ratios to Co of other trace element content were determined in 21 samples (11 BPH and 10 PCa samples, respectively).

From Table 3, it is observed that in benign hypertrophic tissues the Co/Ag, Co/Au, Co/B, Co/Be, Co/Br, Co/Cd, Co/Fe, Co/La, Co/Li, Co/Nb, Co/Ni, Co/Pb, Co/Sc, Co/Se, Co/Th, Co/Ti, Co/Tl, Co/Yb, Co/Zn, and Co/Zr mass fraction ratios not differ from normal levels, but the mass fraction ratios of Co/Al, Co/Ce, Co/Cs, Co/Dy, Co/Er, Co/Gd, Co/Ho, Co/Mn, Co/Mo, Co/Nd, Co/Pr, Co/Rb, Co/Sm, Co/Sn, Co/Tb, Co/Tm, Co/U, and Co/Y are higher, while the mass fraction ratios of Co/Bi, Co/Cr, Co/Hg, and Co/Sb are significantly lower. In cancerous tissue the all Co/trace element mass fraction ratios investigated in the study are significantly lower, than in BPH and normal prostate, with the exception of Co/Cd, Co/Cs, Co/Fe, Co/Hg, Co/Mo, Co/Nb, Co/Rb, Co/Sc, Co/Se, Co/Yb, and Co/Zn ratios.

Analysis of the mass fraction ratios for trace element 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 [77,78]. 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 BPH and PCa cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of Co/trace element mass fraction ratios for the diagnosis of PCa. As is evident from Table 3 and, particularly, from individual data sets of ratios (Fig. 1), the Co/Ag, Co/Al, Co/B, Co/Bi, Co/Li, Co/Mn, Co/Pr, Co/Th, Co/Tl, and Co/Zr mass fraction ratios are potentially the most informative test for a differential diagnosis. For example, if 0.5 is the value of Co/Ag mass fraction ratio assumed to be the upper limit for PCa (Fig. 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% = 90±6%;

Specificity = {True Negatives (TN)/[TN + False Positives (FP)]} • 100% = 89±5%.

Accuracy = [(TP+TN)/(TP+FP+TN+FN)] • 100% = 93±3%.

The number of people (samples) examined was taken into account for calculation of confidence intervals [79]. In other words, if Co/Ag mass fraction ratio in a prostate biopsy sample is lower 0.5, one could diagnose a malignant tumor with an accuracy 93±3%. Thus, using the Co/Ag mass fraction ratio-test makes it possible to diagnose cancer in 90±6% cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of for Co/Al, Co/B, Co/Bi, Co/Li, Co/Mn, Co/Pr, Co/Th, Co/Tl, and Co/Zr mass fraction ratios for the diagnosis of PCa were also calculated (Table 4).

## Conclusion

The combination of nondestructive INAA-LLR and destructive ICP-MS methods is satisfactory analytical tool for the precise determination of 43 trace element mass fractions in the tissue samples of normal, BPH and carcinomatous prostate glands. The sequential application of these methods allowed precise quantitative determinations of mean mass fraction of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr. It was observed that the ratio to Co of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Dy, Er, Gd, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, U, Y, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Co/Ag, Co/Al, Co/B, Co/Bi, Co/Li, Co/Mn, Co/Pr, Co/Th, Co/Tl, and Co/Zr mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

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Competing interests

All other authors declare no competing interests.

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