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Trace element analysis of cancerous and non-cancerous breast tissues using inductively coupled plasma mass spectrometry

Abstract

In the most countries of the world, breast cancer ranks first in the structure of oncological morbidity in women. The etiology of this disease remains largely unclear, although it is known that disturbances of somatic elemental homeostasis play a certain role in oncogenesis. The aim of this study was to identify changes in the content of 35 trace elements during malignant transformation of breast tissue. For this purpose, we used the previously developed method of sample preparation, which allows determining the content of 35 trace elements in micro samples of breast tissue by using mass spectrometry with inductively coupled plasma. Using the developed technique, samples of cancerous and visually intact breast tissue adjacent to tumor were examined. The present study revealed a significant increase in the content of Al, As, Cd, Co, Cs, Cu, Mg, Mn, Mo, Nd, Rb, Se, Sn. Sr. Th, Ti, Tl, U, V, Zn and Zr in breast tissue during its malignant transformation. The detected multiple increase in the content of many trace elements in cancer tissue compared to adjacent intact breast tissue can be used to develop new methods for *in vitro* and *in vivo* cancer diagnostics, in which the ratios of trace element levels in these tissues will act as tumor markers. Further deeper study and understanding of the detected phenomenon will allow the development of new methods for the prevention and treatment of breast cancer.

Keywords: breast cancer, trace elements, inductively coupled plasma mass spectrometry; cancerous tissue; visually intact breast tissue adjacent to tumor

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Abbreviations: BCa, breast cancer; TEs, trace elements; ICP-MS, inductively coupled plasma mass spectrometry; Al, aluminum; As, arsenic; B, boron; Ba barium; Bi, bismuth; Cd, cadmium; Ce, cerium; Co, cobalt; Cr, chromium; Cs, cesium; Cu, copper; Ga, gallium; Ge, germanium; La, lanthanum; Li, lithium; Mg, magnesium; Mn, manganese; Mo, molybdenum; Nb, niobium; Nd, neodymium; Ni, nickel; Pb, lead; Rb, rubidium; Sb, antimony; Se, selenium; Sn, tin; Sr, strontium; Th, thorium; Ti, titanium, Tl, thallium; U, uranium; V, vanadium ; W, tungsten; Zn, zinc; Zr, and zirconium; CRM, certified reference materials;

Introduction

The widespread use of modern methods of early diagnostics of breast cancer (BCa) and progressive methods of treatment significantly reduce mortality from this disease.[1,2] However, despite the progress achieved, BCa remains a global health problem as it continues to occupy a leading position in cancer mortality worldwide.[3] BCa is not only a medical but also a social problem, since the incidence of this disease is steadily increasing and especially rapidly among young women.[1–4] BCa has a complex and still insufficiently studied etiology. It is obvious that the disclosure of the multifaceted nature of BCa, a deep understanding of its molecular basis and risk factors will contribute to the development of new highly effective methods of prevention, diagnosis and treatment of this disease.[5] Numerous epidemiological studies consider many factors that can contribute to the occurrence of breast cancer. These factors can be divided into four groups: 1) genetic predisposition, 2) hormonal status (long-term exposure to estrogens and reproductive history), 3) lifestyle (diet, obesity, physical activity, type of work, bad habits, etc.), and 4) environmental conditions (radiation and chemical exposure). The influence of some of these factors is confirmed by all studies, but there are still debates regarding many other factors and no consensus has been reached.[4–8]

In our previous studies, special attention was paid to the role of trace elements (TEs) of mammary gland tissue in the normal physiology of this organ.[9] In these studies, we proceeded from the idea that a violation of elemental somatic homeostasis (deficiency or excess) can provoke malignant transformation of mammary gland tissue.[10,11] To continue studying not only normal but also pathologically altered breast tissue we have developed the method of sample preparation, which allows us to determine the content of many TEs in small tissue samples by using inductively coupled plasma mass spectrometry (ICP-MS). This method made it possible. to use puncture biopsy material obtained in the clinic for research.[12]

Earlier, in our studies of malignant tumors of the bones, prostate and thyroid glands, significant differences were found in the content of many TEs, compared with the levels of these TEs characteristic of normal bone,[13–21] prostate,[22–29] and thyroid tissue,[30-40] respectively. By analogy, it can be assumed that the malignant transformation of breast tissue may also be associated with significant changes in the content of some TEs. Of particular interest in this case was the composition of intact breast tissue adjacent to a malignant tumor, since it can reflect the starting conditions from which the malignancy of glandular cells began.

To date, several articles have been published in which the content of TEs in malignant tumors, and visually intact breast tissue adjacent to the tumor was studied using various analytical methods.[41–74] However, due to the large scatter of published quantitative data, and sometimes their inconsistency, it was not possible to draw unambiguous conclusions about the changes in the content of TEs in malignant tumors and adjacent visually intact breast tissue. Also, no

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analytical reviews in the literature on this issue are found that could resolve the existing contradictions and draw adequate conclusions.

The present study is aimed at comparing the content of TEs in malignant breast tumors with the content of the same TEs in adjacent visually intact breast tissue. To achieve this goal, we used a previously developed technique.[12] To assess the reliability of our results, a systematic analysis of the published data on the content of TEs in malignant tumors and adjacent visually intact breast tissue is carried out. The analysis performed allows determine median values for data available in the literature and makes it possible comparison of the identified median values with our results.

Material and methods

Tissue samples

The study used a collection of malignant and non-malignant breast tissue samples obtained by percutaneous core needle biopsy. The collection was made by surgeons of the thoracic department of the Medical Radiological Research Center (Obninsk) in the 1990s. Tissue samples were obtained from 43 women with breast cancer (age 35 to 77 years, Caucasian race, Caucasian lifestyle). All patients were diagnosed with breast cancer for the first time and had not yet received any treatment. In all cases, the diagnosis was confirmed by clinical and morphological results obtained during the examination of biopsies and resected material. After biopsy, the obtained material was weighed, lyophilized,[75] and weighed again. Then, each dried tissue sample was sealed in polyethylene film pre-treated with rectified ethyl alcohol, and in sealed form placed in a numbered polyethylene capsule. Samples were stored in a fume hood at room temperature. As our studies have shown, lyophilized tissue samples in this form can be stored for decades without changing the levels of TEs in them.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards.

Sample preparation and ICP-MS measurements

To implement this study, we used the developed method of small mass samples preparation, which allows us to determine the content of 35 TEs in small tissue samples by using ICP-MS.[12] This method allows us to determine the content of the following elements: aluminum (Al), arsenic (As), boron (B), barium (Ba), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), copper (Cu), gallium (Ga), germanium (Ge), lanthanum (La), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), niobium (Nb), neodymium (Nd), nickel (Ni), lead (Pb), rubidium (Rb), antimony (Sb), selenium (Se), tin (Sn), strontium (Sr), thorium (Th), titanium (Ti), thallium (Tl), uranium (U), vanadium (V), tungsten (W), zinc (Zn), and zirconium (Zr) in milligram quantities of the sample under study. The ICP-MS method was chosen due to its high sensitivity, which made it possible to simultaneously determine the content of 35 TEs in a small tissue sample, which is usually obtained during a puncture biopsy.[12]

Deionized water was distilled without boiling in a PTFE Subboiler ECO IR Maassen "Water and acid purification system" (Germany) and nitric acid for analysis (65%, max 0.005 ppm Hg) from Merck (Germany) were used for sample preparation and element analysis. A solution of nitric acid (2%) was prepared by dilution of this Merck nitric acid with the deionized water and then used to prepare the solutions to be analyzed. A technique for microwave autoclave acid digestion of small mass (a few dozen mg) samples of the breast tissue samples had been developed earlier[76] and it was applied in the current study.

An X Series II inductively coupled plasma quadrupole mass spectrometer (ICP-MS) made by Thermo Scientific equipped with a concentric atomizer and a quartz cyclone atomization chamber cooled (up to 2°C) by a Peltier element was used. To calibrate the spectrometer for quantitative analysis the reference solutions of chemical elements obtained from High-Purity Standards (North Charleston, SC, USA) were used. Among them are CRM-TMDW (26 trace metals in drinking water), ICP-MS-68A (61 elements containing in solutions A and B) and single-element solutions (B, Mg, Al, Mn, Ni, Cu, Zn, Se, Rb, Sr, Cs, Ba). The parameters of the measurement procedure were as follows: generator output power 1400 W, plasma-forming gas (argon) consumption 13 L/min, auxiliary gas consumption 1.25 L/min, argon flow rate through the atomizer 0.88 L/min, plasma sampling depth 105 rel. units and sample flow rate 1 mL/min. Mass spectra were measured using two scanning modes: panoramic (Survey Scan) with 5 passes from 5 to 244 *m*/*z* and at points (Peak Jumping) with 1 channel per weight, the integration time of 20 ms, and with 25 passes. Subject to all the device settings, the level of oxide ions CeO^+/Ce^+ is no more than 2%, and the level of doubly charged ions (Ba^{2+}/Ba^+) is no more than 3%. To correct the possible registration efficiency, drift indium is used as an internal standard.

All measurements were performed using Plasma Screen software. The ICP–MS data were processed using the iPlasma Pro Quad software developed at our laboratory.[77] This program was designed to facilitate comprehensive processing of the information obtained from a mass spectrometer. The program involves all stages of processing beginning from calibration to calculation of element concentrations, estimation of measurement uncertainty, computation a set of various corrections, checking the quality of the results, etc. The program outputs multidimensional arrays of results, so allowing their comprehensive interpretation.

To verify the accuracy of the obtained results, Polish certified reference materials (CRM) MODAS-5 (Cod tissue) and MODAS-3 (Herring tissue) and the CRM prepared by the International Atomic Energy Agency IAEA-153 (Milk powder) were used. A more detailed description of the methodology developed and used by us was published earlier.[12,76,77]

Systematic mini review

A systematic search was performed using PubMed, Web of Science, Scopus, and Google Scholar to identify literature published up to February 2025 on the considered elements (Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr) in cancerous and adjacent visually intact breast tissue. The key terms used in the search strategy included "chemical elements" or "trace elements" in combination with "breast cancer", "breast tumor", "breast carcinoma", or "adjacent visually intact breast tissue". In addition, we searched for all results reported in previous reviews and relevant meta-analyses on the topic of interest.

The identified studies were included only if they met the following standards: (1) only studies involving human participants were included; (2) quantitative data on the TEs of interest were presented; (3) in patients with breast cancer, the diagnosis was confirmed morphologically. In some cases, review articles were included in our

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study if they were relevant to the topic and met the above requirements, but the main focus was on original works. There were no restrictions on the language of published papers.

Subsequently, the literature data were collected and classified for each TEs depending on the breast tissue (tumor or adjacent intact tissue). From the published data, the median of the mean values for tumor tissue and adjacent intact breast tissue was found for each specific TEs.

Statistics

The main statistical parameters such as arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975 for mass fractions of TEs (mg/kg dry weight) were calculated using MS Excel. The significance of differences in the results between the two groups (cancer and adjacent breast tissue) was assessed using the parametric Student's *t*-test and the nonparametric Wilcoxon-Mann-Whitney *U*-test. MS Excel was also used to determine the median values of the mean contents of Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr in tumor and adjacent breast tissue found in the published articles.

Results

The results of ICP-MS determination of mass fraction of Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr in three different international certified reference materials MODAS-5 (cod tissue), MODAS-3 (herring tissue) and IAEA-153 (milk powder) in solutions obtained using the developed sample preparation method are presented in Table 1.

Table 1 ICP-MS data (Mean±SD) of trace elements mass fraction (mg kg^{-1}, dry mass basis) in certified reference material MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue), and IAEA-153 (Powdered milk) compared to their certified values

Element	MODAS-5		MODAS-3		IAEA-153	
	Certificate	Our result	Certificate	Our result	Certificate	Our result
Al	-	6±1	-	14±1	-	-
As	1.64±0.27	1.7±0.1	9.24±0.81	8.8±0.4	-	-
B	-	0.34±0.05	-	9.0±0.3	-	2.03±0.07
Ba	0.162±0.028	0.18±0.02	2.71±0.28	2.6±0.1	-	0.67±0.04
Bi	0.007	0.006±0.001	-	-	-	-
Cd	0.005	0.0046±0.0004	0.33±0.03	0.32±0.01	-	-
Ce	-	0.006±0.002	-	0.021±0.008	-	-
Co	0.014	0.012±0.001	0.08±0.01	0.110±0.003	-	0.016±0.001
Cr	0.201	0.3±0.1	0.90±0.11	0.9±0.2	-	-
Cs	0.059±0.005	0.059±0.002	0.085±0.008	0.086±0.005	-	-
Cu	1.38±0.09	1.5±0.1	3.19±0.22	3.2±0.1	0.6±0.2	0.42±0.03
Ga	-	0.012±0.001	-	0.036±0.002	-	-
Ge	-	0.006±0.001	-	0.018±0.002	-	-
La	-	0.007±0.002	-	0.017±0.005	-	-
Li	0.026	0.030±0.002	0.90±0.11	0.76±0.03	-	0.034±0.005
Mg	1200±200	1178±38	3000±200	2739±75	1060±75	1023±19
Mn	0.92±0.08	0.89±0.05	5.78±0.61	5.3±0.1	-	0.22±0.04
Mo	-	-	0.13±0.02	0.14±0.01	0.3± 0.3	0.228±0.004
Nb	-	-	-	0.006±0.002	-	-
Nd	-	-	-	0.006±0.003	-	-
Ni	0.136	0.14±0.02	0.32±0.05	0.5±0.1	-	0.13±0.02
Pb	0.045	0.05±0.01	0.104±0.013	0.13±0.01	-	-
Rb	4.54±0.33	4.5±0.1	2.33±0.20	2.24±0.07	14.0±1.9	14.9±0.4
Sb	-	-	0.016±0.004	0.017±0.002	-	-
Se	1.33±0.1	1.2±0.1	2.63±0.2	2.8±0.1	-	-
Sm	-	-	0.0018	0.0015±0.0003	-	-
Sn	-	0.14±0.01	-	0.23±0.02	-	0.05±0.02
Sr	4.07±0.36	3.5±0.4	192±15	180±6	4.1±0.6	3.76±0.07
Th	-	0.002±0.001		0.003±0.001	-	0.0009±0.0008
Ti	-	<0.9	-	<2.1		<0.2
Tl	-	0.0013±0.0002	-	0.0014±0.0005	-	-
U	-	-	0.075±0.008	0.063±0.002	-	-
V	-	-	0.78±0.11	0.62±0.01	-	-
W	-	0.024±0.008	-	-	-	-
Zn	20.1±1.1	21±1	111±6	114±3	39.5±1.8	33±1
Zr	-	0.10±0.02	-	0.09±0.03	-	0.014±0.008

Mean, arithmetical mean; SD, standard deviation

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Figure 1 demonstrates the mean mass fraction and the range of the standard error of the mean (M±SEM) for each of the 35 studied TEs in the compared pairs - malignant tumor and adjacent visually intact tissue of the mammary gland.

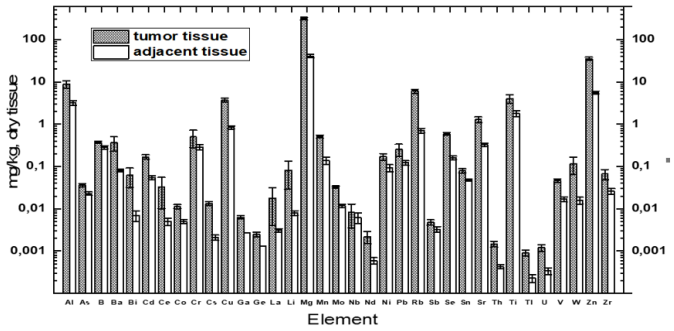

Figure 1 The mean mass fraction (M) and the range of the standard error of the mean (±SEM) for each of the 35 studied TE in cancerous and intact breast tissue adjacent to tumor.

In all or most of the tissue samples with malignant transformation it was possible to determine all TEs, which allowed us to calculate all the main statistical characteristics for these TEs. The mean values of the mass fraction and standard deviation (M±SD) of the TEs in malignant tumors, obtained using the ICP-MS micro method developed by us, were (mg/kg dry tissue): Al 8.9±11.9, As 0.036±0.018, B 0.373±0.149, Ba 0.366±0.949, Bi 0.062±0.196, Cd 0.169±0.133, Ce 0.033±0.152, Co 0.0113±0.0088, Cr 0.50±1.41, Cs 0.0134±0.0098, Cu 3.71±2.61, Ga 0.0063±0.0037, Ge 0.0025±0.0018, La 0.018±0.093, Li 0.082±0.351, Mg 317±154, Mn 0.509±0.269, Mo 0.0328±0.0150, Nb 0.0082±0.0307, Nd 0.0022±0.0043, Ni 0.170±0.210, Pb 0.254±0.537, Rb 6.07±4.27, Sb 0.0048±0.0047, Se 0.588±0.284, Sn 0.080±0.059, Sr 1.29±1.27, Th 0.00148±0.00147, Ti 4.04±6.17, Tl 0.00091±0.00090, U 0.00120±0.00137, V 0.0463±0.0247, W 0.117±0.331, Zn 35.4±19.1, and Zr 0.067±0.119. The Ga and Ge mass fractions in adjacent breast tissue were determined just in a few samples. The possible upper limit of the mean (M_{max}) for these TEs was calculated as the average mass fraction, using the value of the detection limit (DL) instead of the individual value when the latter was found to be below the DL:

$$M_{max} = (\sum_{1}^{n_i} C_i + DL \times n_j)/n \quad (1)$$

where C_i is the individual value of the TE mass fraction in sample i, n_i is the number of samples with mass fraction higher than the DL, n_j is number of samples with mass fraction lower than the DL, and $n = n_i + n_j$ is number of samples that were investigated. The content of the remaining TEs analyzed (Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr) was determined in all or most samples of adjacent breast tissue. This made it possible to calculate all the main statistical characteristics for the mass fractions of these TEs. In the visually intact breast tissue adjacent to the tumor the mean values of the mass fraction (M±SD) were (mg/kg dry tissue): Al 3.15±3.01, As 0.023±0.012, B 0.286±0.173, Ba 0.081±0.040, Bi 0.0074±0.0136, Cd 0.055±0.045, Ce 0.0048±0.0033, Co 0.0050±0.0034, Cr 0.29±0.25, Cs 0.0021±0.0022, Cu 0.84±0.45, Ga (0.0027), Ge (0.0013), La 0.0031±0.0023, Li 0.0082±0.0065, Mg 41.1±28.1, Mn 0.14±0.18, Mo 0.0117±0.0072, Nb 0.0062±0.0116, Nd 0.0006±0.0008, Ni 0.093±0.121, Pb 0.123±0.111, Rb 0.707±0.594, Sb 0.0033±0.0035, Se 0.161±0.102, Sn 0.048±0.017, Sr 0.33±0.21, Th 0.00043±0.00036, Ti 1.80±1.82, Tl 0.00023±0.00033, U 0.00034±0.00038, V 0.017±0.015, W 0.0158±0.0208, Zn 5.63±2.89, and Zr 0.026±0.028.

Table 2 depicts the differences between the mean values of the mass fractions of the studied TEs in the malignant tumor and the intact breast tissue adjacent to the tumor, assessed using the parametric Student *t*-test and the nonparametric Wilcoxon-Mann-Whitney *U*-test.

Table 2 Comparison of mean values (M±SEM) of trace elements mass fraction (mg/kg dry tissue) in cancerous (BCa) breast tissue and intact breast tissue adjacent to tumor (IA)

Element	Mass fraction of chemical elements in female breast tissue				Ratio
	BCa	IA	t-test	U-test	BCa/IA
	M±SEM	M(Mmax) ±SEM	p	p	
Al	8.9±1.8	3.2±0.4	0.0043*	<0.01*	2.78*
As	0.036±0.003	0.023±0.002	0.00012*	<0.01*	1.57*
B	0.373±0.023	0.286±0.025	0.59	>0.05	1.3
Ba	0.366±0.140	0.081±0.006	0.11	>0.05	4.52
Bi	0.062±0.030	0.007±0.002	0.42	>0.05	8.86
Cd	0.169±0.020	0.055±0.006	<0.000001*	<0.01*	3.07*
Ce	0.033±0.023	0.005±0.001	0.23	>0.05	6.6
Co	0.0113±0.0013	0.0050±0.0005	0.000049*	<0.01*	2.26*
Cr	0.50±0.22	0.29±0.04	0.34	>0.05	1.72
Cs	0.0134±0.0015	0.0021±0.0003	<0.00000001*	<0.01*	6.38*
Cu	3.71±0.40	0.84±0.07	<0.00000002*	<0.01*	4.42*
Ga	0.0063±0.0006	-0.0027	-	-	>2.33
Ge	0.0025±0.0003	-0.0013	-	-	>1.92
La	0.0180±0.0140	0.0031±0.0003	0.31	>0.05	5.81
Li	0.082±0.053	0.008±0.001	0.2	>0.05	10
Mg	317±23	41±4	<0.00000001*	<0.01*	7.73*
Mn	0.509±0.041	0.137±0.027	<0.00000001*	<0.01*	3.72*
Mo	0.0328±0.0023	0.0117±0.0010	<0.00000001*	<0.01*	2.80*
Nb	0.0082±0.0047	0.0062±0.0017	0.27	>0.05	1.32
Nd	0.0022±0.0007	0.0006±0.0001	0.043*	<0.05*	3.67*

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Table 2 Continued...

Ni	0.170±0.032	0.093±0.018	0.11	>0.05	1.83
Pb	0.254±0.082	0.123±0.016	0.12	>0.05	2.07
Rb	6.07±0.65	0.71±0.09	<0.00000001*	<0.01*	8.55*
Sb	0.0048±0.0007	0.0033±0.0005	0.16	>0.05	1.45
Se	0.588±0.043	0.161±0.015	<0.00000001*	<0.01*	3.65*
Sn	0.080±0.009	0.048±0.003	0.0023*	<0.01*	1.67*
Sr	1.29±0.20	0.33±0.03	0.00018*	<0.01*	3.91*
Th	0.00148±0.00022	0.00043±0.00005	0.000028*	<0.01*	3.44*
Ti**	4.04±0.94	1.80±0.27	0.047*	<0.05*	2.24*
Tl	0.00091±0.00014	0.00023±0.00005	0.000017*	<0.01*	3.96*
U	0.00120±0.00021	0.00034±0.00006	0.00022*	<0.01*	3.53*
V	0.0463±0.0038	0.0169±0.0022	<0.00000001*	<0.01*	2.74*
W	0.117±0.051	0.016±0.003	0.34	>0.05	7.31
Zn	35.4±2.9	5.6±0.4	<0.00000001*	<0.01*	6.32*
Zr	0.067±0.018	0.026±0.004	0.032*	<0.01*	2.58*

M, arithmetic mean; SEM, standard error of mean; *t*-test, Student's *t*-test; U-test, Wilcoxon-mann-whitney *U*-test,* Significant values.

Comparison of our results with literature data for the mass fractions of Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr in cancerous and visually intact breast tissue adjacent to the tumor is shown in Table 3 & 4, respectively. Column 3 of these tables presents the median of the published mean values for each TEs, and in parentheses the number of studies that contain quantitative data on the content of this TEs in cancerous or adjacent to tumor intact breast tissue is indicated. Columns 4 and 5 indicate, respectively, the minimum and maximum values (arithmetic mean ± standard deviation or median) of the mass fraction of each TEs found from the reported data; the number of samples studied is indicated in parentheses and the corresponding link is given in square brackets.

Table 3 Median, minimum and maximum value of means of trace element mass fractions (mg/kg dry tissue) in cancerous breast tissue of females according to data from the literature in comparison with this work results

Element	**This work results**	**Published data (Reference)**		
	M±SD	**Median**	**Minimum**	**Maximum**
	n=43	of means (n)*	of means M or M±SD, (n)**	of means M or M±SD, (n)**
Al	8.9±11.9	14.4 (21)	0.157 (100) [41]	564±41 (10) [42]
As	0.036±0.018	0.077 (9)	0.0048 (63) [43]	4.12±1.64d (18) [44]
B	0.373±0.149	-	-	-
Ba	0.366±0.949	10.2 (2)	0.13 (17) [45]	20.2±4.4 (-) [46]
Bi	0.062±0.196	-	-	-
Cd	0.169±0.133	0.375 (16)	0.0042 (63) [43]	363±935d (7) [47]
Ce	0.033±0.152	-	-	-
Co	0.0113±0.0088	0.21 (16)	0.0049±0.0019 (6) [48]	9.1±16.2 (53) [49]
Cr	0.50±1.41	1.24 (21)	0.066 (63) [43]	55±15 (-) [46]
Cs	0.0134±0.0098	0.018 (9)	0.014 (46) [50]	3.2±1.4d (6) [51]
Cu	3.71±2.61	6.12 (43)	0.00062±0.00006 (-) [52]	1231±154 (-) [53]
Ga	0.0063±0.0037	2.8 (1)	2.8 (1) [51]	2.8 (1) [51]
Ge	0.0025±0.0018	0.64 (1)	0.64±0.38d (26) [54]	0.64±0.38d (26) [54]
La	0.018±0.093	5 (1)	5 (6) [55]	5 (6) [55]
Li	0.082±0.351	2.35 (1)	2.35±3.96 (53) [49]	2.35±3.96 (53) [49]
Mg	317±154	305 (17)	3 (6) [55]	1400±400 (6) [51]
Mn	0.509±0.269	1.42 (20)	0.026 (17) [45]	17.3±4.9 (18) [44]
Mo	0.0328±0.0150	2.45 (4)	1.00 (19) [56]	6.16±5.42 (53) [49]
Nb	0.0082±0.0307	-	-	-
Nd	0.0022±0.0043	-	-	-
Ni	0.170±0.210	2.54 (17)	0.1 (12) [57]	8.57±2.24 (18) [44]
Pb	0.254±0.537	1.55 (17)	0.05 (63) [43]	31.7±50.8 (53) [49]
Rb	6.07±4.27	13 (19)	0.75±0,12 (26) [58]	28.5 (-) [59]
Sb	0.0048±0.0047	0.0068 (5)	0.00035±0.00019 (6) [48]	18.3±12.8 (53) [49]
Se	0.588±0.284	0.915 (31)	0.22 (63) [43]	10231±808 (80) [60]

Table 3 Continued...

Sn	0.080±0.059	0.044 (3)	0.022 (12) [57]	0.26 (17) [45]
Sr	1.29±1.27	2.24 (12)	0.07 (17) [45]	34.6±11.9 (16) [61]
Th	0.00148±0.00147	-	-	-
Ti	4.04±6.17	13.0 (4)	1.0 (17) [45]	20.4±13.1 (16) [61]
Tl	0.00091±0.00090	-	-	-
U	0.00120±0.00137	0.019 (1)	0.019 ± 0.002 (6) [62]	0.019 ± 0.002 (6) [62]
V	0.0463±0.0247	1.34 (3)	1.34±0.76 (25) [63]	2.0 (6) [55]
W	0.117±0.331	-	-	-
Zn	35.4±19.1	49.6 (53)	0.25 (63) [43]	1158±15 (-) [64]
Zr	0.067±0.119	10 (1)	10±5 (19) [42]	10±5 (19) [42]

M, arithmetic mean; SD, standard deviation,

(n)* – number of all references; (n)** - number of samples.

Table 4 Median, minimum and maximum value of means of trace element mass fractions (mg/kg dry tissue) in intact breast tissue adjacent to malignant breast tumor according to data from the literature in comparison with this work results

Element	**This work results M±SD**	**Published data (Reference)**		
		Median of means	**Minimum of means**	**Maximum of means**
	n=43	(n)*	M or M±SD, (n)**	M or M±SD, (n)**
Al	3.15±3.01	3.5 (16)	0.51 (17) [45]	442±30 (10) [42]
As	0.023±0.012	0.19 (7)	0.0090±0.0030 (6) [51]	2.57±1.48 (18) [44]
B	0.286±0.173	-	-	-
Ba	0.081±0.040	0.93 (2)	0.06 (17) [45]	19.8±19.3 (9) [65]
Bi	0.0074±0.0136	-	-	-
Cd	0.055±0.045	0.38 (9)	0.04 (57) [66]	40.8±35.0 (43 [67]
Ce	0.0048±0.0033	-	-	-
Co	0.0050±0.0034	0.052 (13)	0.0031±0.0011 (6) [51]	1.19 (15) [59]
Cr	0.29±0.25	0.84 (12)	0.19(17) [45]	32.0±7.5 (17) [44]
Cs	0.0021±0.0022	0.008 (9)	0.007(46) [50]	3.7±0.6 (6) [51]
Cu	0.84±0.45	2.0 (30)	0.54 (37) [68]	42.0±6.9 (18) [44]
Ga	-0.0027	1.9 (1)	1.9±0.4 (6) [51]	1.9±0.4 (6) [51]
Ge	-0.0013	-	-	-
La	0.0031±0.0023	-	-	-
Li	0.0082±0.0065	-	-	-
Mg	41.1±28.1	79.6 (15)	11.9 (80) [69]	1100±700 [51]
Mn	0.14±0.18	0.88 (14)	0.06 (17) [45]	18.1±2.2 (18) [44]
Mo	0.0117±0.0072	0.24 (3)	0.20 (8) [56]	2.70±0.95 (26) [70]
Nb	0.0062±0.0116	-	-	-
Nd	0.0006±0.0008	-	-	-
Ni	0.093±0.121	1.84 (8)	0.9 (5) [71]	7.44±1.70 (18) [44]
Pb	0.123±0.111	1.33 (8)	0.14(8) [56]	9.6±2.6 (16) [61]
Rb	0.707±0.594	5.65 (16)	0,108±0,052 (5) [65]	12.6 (46) [50]
Sb	0.0033±0.0035	0.0046 (4)	0.00033±0.00013 (6) [48]	0.0120±0.0074 (5) [65]
Se	0.161±0.102	0.60 (22)	0.059±0.018 (17) [72]	1360±220 (80) [73]
Sn	0.048±0.017	0.13 (1)	0.13 (17) [45]	0.13 (17) [45]
Sr	0.33±0.21	0.95 (10)	0.03 (17 [45]	8.6±3.0 (16) [61]
Th	0.00043±0.00036	-	-	-
Ti	1.80±1.82	10 (3)	0.37 (17) [45]	14.4±2.8 (18) [44]
Tl	0.00023±0.00033	-	-	-
U	0.00034±0.00038	-	-	-
V	0.017±0.015	0.78 (2)	0.78±0.46 (25) [63]	0.78±0.46 (26) [70]
W	0.0158±0.0208	-	-	-
Zn	5.63±2.89	16.1 (35)	3.38 (37) [68]	99±32 (25) [74]
Zr	0.026±0.028	4 (1)	4±1 (10) [42]	4±1 (10) [42]

M, arithmetic mean; SD, standard deviation

(n)*,number of all references; (n)**, number of samples.

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A comparison of the ratio of the mass fraction of TEs in cancerous and intact breast tissue adjacent to tumor obtained in this work with the corresponding ratios calculated from published results are presented in Table 5. To obtain the corresponding ratios according to the literature, we used the median values of the mass fractions of TEs in cancerous and intact breast tissue.

Table 5 Ratio of median of 35 trace element mean mass fractions (mg/kg dry tissue) in cancerous breast tissue (Med_C) and intact breast tissue adjacent to tumor (Med_A) according to literature data in comparison with this work results

Element	Medians of the reported means (n)*			This work result
	Cancerous tissue MedC(n)	Adjacent tissue MedA(n)	Ratio MedC/ MedA	Ratio MC/ MA
Al	14.4 (21)	3.5 (16)	4.11	2.78*
As	0.077 (9)	0.19 (7)	0.41	1.57*
B	-	-	-	1.3
Ba	10.2 (2)	0.93 (2)	11.9	4.52
Bi	-	-	-	8.86
Cd	0.375 (16)	0.38 (9)	0.99	3.07*
Ce	-	-		6.6
Co	0.21 (16)	0.052 (13)	4.04	2.26*
Cr	1.24 (21)	0.84 (12)	1.48	1.72
Cs	0.018 (9)	0.008 (9)	2.25	6.38*
Cu	6.12 (43)	2.0 (30)	3.06	4.42*
Ga	2.8 (1)	1.9 (1)	1.47	>2.33
Ge	0.64 (1)	-	-	>1.92
La	5 (1)	-	-	5.81
Li	2.35 (1)	-	-	10
Mg	305 (17)	79.6 (15)	3.83	7.73*
Mn	1.42 (20)	0.88 (14)	1.61	3.72*
Mo	2.45 (4)	0.24 (3)	10.2	2.80*
Nb	-	-	-	1.32
Nd	-	-	-	3.67*
Ni	2.54 (17)	1.84 (8)	1.38	1.83
Pb	1.55 (17)	1.33 (8)	1.17	2.07
Rb	13 (19)	5.65 (16)	2.3	8.55*
Sb	0.0068 (5)	0.0046 (4)	1.42	1.45
Se	0.915 (31)	0.60 (22)	1.53	3.65*
Sn	0.044 (3)	0.13 (1)	0.34	1.67*
Sr	2.24 (12)	0.95 (10)	2.36	3.91*
Th	-	-	-	3.44*
Ti	13.0 (4)	10 (3)	1.3	2.24*
Tl	-	-	-	3.96*
U	0.019 (1)	-	-	3.53*
V	1.34 (3)	0.78 (2)	1.72	2.74*
W	-	-	-	7.31
Zn	49.6 (53)	16.1 (35)	3.08	6.32*
Zr	10 (1)	4 (1)	2.5	2.58*

(n)*, the number of all found articles for each chemical element; M_C, mean mass fraction of trace element in malignant tumor; M_A, mean mass fraction of trace element in intact breast tissue adjacent to tumor

Good agreement between the multiple excess of many TEs in malignant tissue compared to the intact tissue adjacent to the tumor, found in the present study, and the data we obtained from the analysis of published data indicated not only the reliability of the identified phenomenon, but also the prospects for its use for diagnostic purposes. Obviously, in terms of developing new diagnostic methods, the individual ratio of TEs content in cancerous (Q_C) and intact breast tissue adjacent to the tumor (Q_A) is of particular importance. Such paired Q_C/Q_A ratios were calculated for each patient, and the main statistical characteristics of these ratios, such as the arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975 obtained for the entire group subjects examined are presented in Table 6.

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Table 6 Main statistical parameters of individual ratios of the mass fraction of 35 trace elements in cancerous breast tissue (Q_C) to those in intact breast tissue adjacent to the tumor (Q_A)

Element	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Al	2,74	3.13	0.61	0.13	13	1.53	0.2	10.5
As	1.78	1.09	0.2	0.5	5.5	1.5	0.5	4.45
B	1,66	1.29	0.24	0.42	7.55	1.61	0.52	4.36
Ba	2.43	1.72	0.34	0.33	7.6	2,13	0.42	5.98
Bi	2.38	2.29	0.45	0.14	10	1.63	0.18	7.5
Cd	3.05	2.6	0.49	1.06	12.4	2.02	1.08	9.2
Ce	2.17	2.01	0.39	0.15	8	1.4	0.16	7.35
Co	2.27	1.76	0.34	0.7	8	1.86	0.77	7.13
Cr	1.24	0.66	0.13	0.13	2.8	1.2	0.24	2.41
Cs	7.32	6.15	1.16	1.5	27	5.33	1.84	22.3
Cu	4.19	2.69	0.52	0.95	11.1	3.4	1.27	10.4
Ga	3.87	3.75	0.7	0.29	14	2.5	0.37	12.6
Ge	2.33	2.74	0.51	0.17	15	1.5	0.23	8
La	1.48	1.63	0.33	0.14	6	0.67	0.18	5.4
Li	0.89	1.21	0.23	0.03	5.5	0.5	0.07	4.2
Mg	9.64	8.23	1.55	2.84	39.2	7.11	3.25	32.9
Mn	5.25	4.14	0.78	1.67	20.3	3.96	1.67	16.2
Mo	3.9	3.61	0.69	0.5	16.7	3	0.7	12.3
Nb	1.3	1.57	0.3	0.05	8	1	0.08	4.75
Nd	2.75	3.38	0.68	0.03	10	1	0.04	10
Ni	2.85	2.29	0.55	0.14	10.5	2.06	0.18	8.49
Pb	2.47	3.77	0.71	0.04	14	0.8	0.07	12.7
Rb	9.44	5.72	1.1	2.82	26.9	7.76	2.98	21.3
Sb	2	1.8	0.34	0.14	7	1.42	0.17	5.65
Se	4.42	2.81	0.53	1.5	11	3.25	1.5	9.65
Sn	2	2.35	0.44	0.33	12.3	1.17	0.45	8.06
Sr	4.34	3.55	0.68	0.25	11.8	2.67	0.44	11
Th	4.28	4.9	0.96	0.2	15	1.83	0.25	15
Ti	2.22	1.97	0.39	0.19	7.08	1.55	0.25	6.73
Tl	5.83	6.11	1.14	0.5	20	3.33	0.5	20
U	2.86	3.3	0.65	0.2	10	1	0.2	10
V	7.01	6.55	1.26	0.83	30	4.5	1.1	22.6
W	3.13	3.53	0.72	0.08	10.8	1.67	0.09	10.3
Zn	7.37	5.44	1.03	1.53	25	5.93	2.14	22.9
Zr	2.48	2.68	0.52	0.21	12.5	1.6	0.38	9.85

M, arithmetic mean; SD, standard deviation; SEM, standard error of mean; Min, minimum value; Max, maximum value; Med, median; P0.025, percentile with 0.025 level; P0.975, percentile with 0.975 level

Discussion

Acceptable agreement of the values of the content of TEs in the international certified reference materials MODAS-5 (Cod tissue), MODAS-3 (Herring tissue), IAEA-153 (Milk powder) obtained in this study with the data of the corresponding certificates (Table 1) indicates sufficient accuracy of the developed ICP-MS micro method[12,76,77] and reliability of the mass fractions of Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr in samples of malignant and intact breast tissue, presented in Tables 2-6.

Mass fractions of the TEs presented in this study (Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr) were determined in all or most of the samples of both malignant tissue and intact adjacent breast tissue. This allowed us to calculate the main statistical characteristics for the mass fractions of these elements, such as the arithmetic mean (M), standard deviation of the mean (SD) and standard error of the mean (SEM) (Tables 2-5). The only exceptions were Ga and Ge, the mass fractions of which in the intact tissue adjacent to the tumor were higher than the DL in only a few samples. Therefore, the possible upper limit of the mean (M_{max}) for these TEs was calculated.

The M, SD and SEM values are valid only if the results of determination of the content of TEs in the samples studied are distributed normally. Only after making sure that the distribution of the results within each of the two studied groups of samples (malignant tumor and adjacent intact breast tissue) is normal, it is possible to use M, SD and SEM for comparison using parametric criteria, for example, Student's *t*-test. However, reliable detection of normal distribution of results with a relatively small number of samples in the presented study (n=43) is impossible, since the existing criteria for detection of the type of distribution of results require a large sample size, usually

several hundred samples. Since in our study it was not possible to prove or disprove the "normality" of the distribution of the obtained results due to the small sample size, in addition to the parametric Student's *t*-test, the nonparametric Wilcoxon-Mann-Whitney U-test was also used, which is applicable to any type of distribution of the results of the content of TEs in breast tissue.

To assess the effect of malignant transformation of breast tissue on the content of TEs in it, a comparison of the elemental composition of cancerous and intact breast tissue adjacent to the tumor was performed (Table 2). In the malignant tumor, the mass fractions of all the TEs studied were higher than the levels characteristic of the unaffected breast tissue adjacent to the tumor. To compare the two groups of samples (malignant tumor and adjacent to the tumor intact breast tissue), both the parametric Student's t-test and the nonparametric Wilcoxon-Mann-Whitney U-test were used, and both criteria confirmed the reliability of the difference in the mass fractions of all TEs except B, Ba, Bi, Ce, Cr, Ga, Ge, La, Li, Nb, Ni, Pb, Sb, and W.

In the study of TEs in the mammary gland in norm and pathology, both tissue samples obtained from healthy women and samples of visually intact tissue adjacent to the tumor are used as the "norm". However, mixing these two groups of samples is incorrect. For example, we have previously shown that in terms of the TEs content, intact tissue adjacent to thyroid tumors is not identical to normal thyroid tissue in practically healthy individuals.[78,79] Therefore, in our review of the literature data (Table 4), only the results obtained in the study of samples of visually intact breast tissue adjacent to a malignant breast tumor were used. Some values of the mass fractions of TEs were not expressed by the authors of the cited works in terms of dry tissue. However, we calculated these values using literature data on the water content of 50%[80] and ash content of 1% (in dry tissue)[45] in the mammary gland of adult women.

When considering the published data in Table 3 & 4, attention is drawn to the huge difference between the minimum (column 4) and maximum (column 5) values, which for almost all TEs amount to two, three or more orders of magnitude. Such a wide range of published data, in our opinion, is mainly due to the insufficient attention of many authors to proper quality control of their results including sampling. The lack of proper control allows for random errors both in the direction of underestimation and in the direction of overestimation of the analysis result. Since errors are random in nature, as the number of observations increases, the median of accumulated data on the content of one or another TE in breast tissue should approach the true value. This interpretation of the existing spread of accumulated data allows us to compare our results (column 2) with the medians of published mean mass fractions (column 3) for each TE.

The values of the mean mass fractions of Al, As, Cd, Cr, Cs, Cu, Mg. Mn. Rb. Sb, Se, Sn, Sr, Ti, and Zn in malignant breast tumors that we obtained were in good agreement with the median values of published data (Table 3). Our results for Ba, Co, Ni, and Pb were within the range of published mean values for these TEs. Our results for Ga, Ge, La, Li, Mo, U, V, and Zr were two to three mathematical orders of magnitude lower than the published mean values for these TEs, however, it should be noted that the number of publications on these elements was very small - 1-4 papers. No data on the mass fractions of B, Bi, Ce, Nb, Nd, Th, Tl, and W in breast cancer tissue were found in the literature available to us.

As follows from the data in Table 4, the results obtained for Al, Cr, Cs, Cu, Mg, Mn, Sb, Se, Sn, Sr, and Zn were in fairly good agreement with the medians of previously published mean values of TEs content in intact breast tissue adjacent to malignant breast tumor or fit within their min-max range (As, Ba, Cd, Co, Mn, Pb, Rb, and Ti). Our results for Mo, Ni, V, and Zr were one to two mathematical orders of magnitude lower than the published mean values for these TEs, but the discrepancy can be explained by the small number of studies. No data on the mass fractions of B, Bi, Ce, Ge, La, Li, Nb, Nd, Th, Tl, U, and W in intact breast tissue adjacent to the tumor were found in the literature available to us.

If to use the ratios of median values of mean mass fractions of TEs in cancerous tissue and intact breast tissue adjacent to the tumor, found from the analysis of literature data (Tables 3 & 4, respectively), they can be compared with the corresponding ratios of mean values of TEs content in these tissues obtained in the present study and presented in Table 2.

Calculation of these ratios showed that the increase in the content of all studied TEs during malignant transformation of breast tissue, except for data for As, Cd, and Sn, found in this work, is consistent with the results we obtained in analyzing published studies (Table 5). Thus, both from the data obtained in the present study and from our calculations made based on literature data, it clearly followed that the content of such elements as Al, Ba, Co, Cr, Cs, Cu, Mg, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ti, V, Zn, and Zr in malignant tissue is higher than in intact breast tissue adjacent to the tumor (Table 5).This indicated the potential of using the ratio of TEs content in malignantly transformed tissue (Q_C) and in intact tissue adjacent to the tumor (Q_A) - Q_C/Q_A for diagnostic purposes. To assess the diagnostic significance of the Q_C/Q_A ratio for each examined patient, individual Q_C/Q_A values were calculated and the main statistical characteristics obtained for the entire group of patients are presented in Table 6.

The "min" values in this table (column 5) showed that in all patients the Q_C/Q_A ratio for Cd, Cs, Mg, Mn, Rb, Se, and Zn was higher than 1.0. It followed that if we take $Q_C/Q_A > 1.0$ as the threshold value, then determining the content of any of these TEs in the tumor and adjacent tissue allows us to detect breast cancer with an accuracy of 100-2%.[81] If we focus not only on the "minimum" values, but also on the P0.025 values (table 6, column 8), then Cu and V will be added to the above-mentioned TEs that are potentially significant for diagnosing breast cancer. For these elements, only one patient had a Q_C/Q_A ratio of less than 1.0 (0.95 for Cu and 0.83 for V). For these TEs, the accuracy of detecting breast cancer was estimated at 97±3%.[81] The use of TEs levels in transformed breast tissue as tumor markers seems to be very promising, since the capabilities of modern analytical chemistry are rapidly increasing. For example, the distribution of such diagnostically promising TEs as Cu and Zn in the mammary gland can be determined non-invasively using neutron stimulated emission computed tomography (NSECT).[82]

It is known that healthy breast tissue consists of a glandular component and stroma (adipose tissue and ligaments surrounding ducts and lobules, blood and lymphatic vessels).[83] On average, the masses of the glandular component and adipose tissue together with the stroma are related approximately as 1:1.[84] It is also known that the content of many TEs in adipose tissue is significantly lower than in glandular tissue.[9] Although tumor tissue consists mainly of malignantly transformed glandular cells, even the complete absence of adipose tissue in the tumor cannot increase the mass fractions of TEs by more than two times. Thus, this factor cannot explain the more than threefold increase in cancer tissue of such elements as Cd, Cs, Cu, Mg, Mn, Rb, Se, Sr. Th, Tl, and Zn (Table 6). It is known that Mg ions, along with Ca, K, and Na ions, are the main electrolytes. Ions of all these metals regulate intracellular metabolism. Thus, the obtained results indicate colossal changes in intracellular metabolism during malignant transformation of mammary gland cells. Cu, Mn, Se, and Zn are part of many biologically active substances and are

extremely important TEs for the vital activity of the body. Their content inside the cells is under strict homeostatic control, since they form the epigenetic intracellular environment. A multiple increase in the intracellular concentration of these TEs can cause malignancy of cells.

One of the possible explanations for the observed phenomenon of multiple increase in the content of TEs in tumor tissue may be associated with disturbances in the metabolism of TEs and the mechanisms of their intracellular transport that occur during malignancy of glandular cells. As a result of such disturbances, changes in the permeability of cell membranes and subsequent excessive accumulation of TEs in cells may occur.

Another possible explanation may be associated with excessive intake of TEs into the body with food, water and air due to uncontrolled changes in the TEs content in the environment. Even a slight increase in the intracellular concentration of such TEs as Cd, Cs, Cu, Mg, Mn, Rb, Se, Sr. Th, Tl, and Zn may trigger the malignancy process. In this case, an increase in the content of these TEs should be detected not only in cancerous tissue, but also in visually intact tissue adjacent to the tumor. To confirm or refute the possibility of such a variant of breast tumor development, we plan to compare the TEs content in samples of visually intact tissue adjacent to the tumor with the TEs content levels characteristic of breast tissue in healthy women.

A limitation of this study is the relatively small sample size of the studied samples of malignant (n=43) and adjacent intact breast tissue (n=43). This did not allow us to determine the content of TEs considering the stage of the disease, the histological structure of the malignant tumor and molecular taxonomy, which is of particular interest for diagnostics, prognosis and choice of treatment tactics. Therefore, it is planned to continue collecting samples of malignant and adjacent intact breast tissue and analyzing the obtained material.

The detected multiple increase in the content of TEs in malignant tumors of the mammary gland opens great prospects for the development of new *in vitro* and *in vivo* methods for differential diagnostics of breast tumors, in which the levels of TEs will act as tumor markers. For this purpose, it is necessary to carry out further studies of the content of TEs in the tissue of the lesion in benign diseases of the mammary gland and compare the obtained results with the data of this work. We plan to conduct such a study in the future.

Conclusion

The developed method of sample preparation allows us to obtain reliable data of the content of Al, As, B, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, La, Li, Mg, Mn, Mo, Nb, Nd, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, W, Zn, and Zr in samples of cancerous and adjacent intact breast tissue with the help of ICP-MS. An important feature of the developed method is the ability to determine the content of TEs in samples weighing only a few milligrams, which is highly suitable for analyzing puncture biopsy materials. The present study revealed a significant increase in the content of Al, As, Cd, Co, Cs, Cu, Mg, Mn, Mo, Nd, Rb, Se, Sn. Sr. Th, Ti, Tl, U, V, Zn and Zr in breast tissue during its malignant transformation. All identified differences were statistically significant and generally consistent with the results of our analytical review of the literature. The results obtained in this work provide a solid basis for the development of new methods for diagnosing BCa based on the use of the ratio of TEs in the tissue of the lesion and adjacent intact tissue of the mammary gland as a tumor marker. Further detailed studies are needed to clarify the role of accumulation of many TEs in malignant tissue in the etiology and pathogenesis of BCa. Our further research will focus on increasing the sample of malignant breast tumors, as well as on studying the TE content in benign breast tumors.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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