# Significance of Trace Element Quantities in Benign and Malignant Giant Cell Tumors of Bone

Zaichick Vladimir[1,*] and Zaichick Sofia[2]

[1]*Radionuclide Diagnostics Department, Medical Radiological Research Centre, Koroleyv St 4, Obninsk 249036, Russia*

[2]*Laboratory of Dr Gabriela Caraveo Piso, Feinberg School of Medicine, Northwestern University, 303 East Chicago Avenue, Ward 10-144, Chicago, IL 60611-4296, USA*

**Abstract:** To clarify the role of trace elements in the etiology and the pathogenesis of benign and malignant giant cell tumor (GCT) of bone, a nondestructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides were performed. The silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) mass fraction were measured in three groups of samples: normal bone samples from 27 patients with intact bone (12 females and 15 males), who had died from various non bone related causes, mainly unexpectedly from trauma, and also in samples, obtained from open biopsies or after operation of 10 patients with benign GCT (4 females and 6 males) and 10 patients with malignant GCT (4 females and 6 males). The difference in the results between trace element contents in the three groups was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test. In the bone affected by benign GCT the mean mass fractions of Ag, Co, Fe, Se, and Zn were significantly higher while the mean mass fraction of Rb was lower than in normal bone tissues. In malignant GCT tissue the mean mass fractions of Co, Fe, Sb, and Se were higher while the mean mass fraction of Rb was lower than in normal bone tissue. In malignant GCT tissue only the mean mass fractions of Fe and Rb were higher and the mean mass fractions of Ag and Zn were lower than in benign GCT tissue. Moreover, many correlations between trace elements found in the control group were no longer evident in the neoplastic bone. Thus, considerable changes in trace element content and their relationships were found in benign and malignant GCT and possible causes and effects of these alterations are discussed.

**Keywords:** Trace elements, human bone, benign and malignant giant cell tumor of bone, neutron activation analysis.

## 1. INTRODUCTION

Bone tumors are a heterogeneous group of tumors that all arise from bone tissue, which consists of cartilaginous, osteoid, osseous mineralized and fibrous tissue, and bone marrow elements. Each tissue can be subject to inflammation, and/or benign or malignant tumors. Bone neoplasms are often difficult to detect in their early stages because the associated signs and symptoms can be nonspecific, insidious in onset, and mimic more common disorders [1]. One of the most important differential diagnoses is between a benign and a malignant neoplasm with similar histology, for example, such as giant cell tumor (GCT) of the bone [2].

GCT is a relatively uncommon tumor. It is a heterogeneous tumor composed of three different cell populations and characterized by the presence of multinucleated giant cells (osteoclast-like cells). GCT is normally benign with unpredictable behavior including malignant transformation [3]. The World Health Organization classifies GCT as "an aggressive, potentially malignant lesion" [4]. Identification of malignant GCT is a great challenge [2]. GCT of bone was first described in 1818 and historically the lesion has been referred to by numerous terms, including *myeloid sarcoma*, *tumor of myeloplaxus*, *osteoclastoma*, and *osteoblastoclastoma* [5-9]. GCT is a tumor, accounting for 4%–9.5% of all primary osseous neoplasm and 18%–23% of benign bone neoplasm [9, 10]. GCTs predominately arise in long tubular bones (75-95%) with most cases (50%–65%) occurring near the knee. The next most common site is the distal radius (~10%). The epicenter of giant cell tumors is in the epiphysis [11, 12]. GCT is typically seen in early adulthood, with 80% of cases reported between the ages of 20 and 50, with a peak incidence between 20 and 30 years [9]. GCT may have aggressive features, including cortical expansion or destruction with a soft-tissue component [13]. The prevalence of malignant GCT is controversial, although a figure of 5%–10% of all GCT appears to be the most frequent consensus [9].

All imaging methods such as conventional roentgenography, functional nuclear medicine including scintigraphy and positron emission tomography, computed tomography, and magnetic resonance imaging are very important for the assessment of tumor

*Address correspondence to this author at the Medical Radiological Research Centre, Korolyev St.4, Obninsk 249036, Kaluga Region, Russia;
Tel: (48439) 60289; Fax: (495) 956 1440;
E-mail: vezai@obninsk.com; vzaichick@gmail.com

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location, shape, size, and infiltration of the adjacent tissue. However, clinical imaging and histopathologic evaluation of biopsy samples is not useful or practical as a routine examination which can be easily used to diagnose and to predict the malignant transformation of GCT [3]. Thus, the goals of many investigations are to assist the clinician in making an appropriate diagnosis by providing a rational method of selecting non-traumatic diagnostic tests that maximize specificity and minimize costs.

It is well known that the tissues of human body differ greatly in their proportions of chemical elements and that there is the homeostasis of both bulk and trace element (TE) contents [14]. Our detailed previous studies have confirmed this using a chemical composition analysis of bone tissue [15-41]. Thus, it can be expected that normal bone and bone tumors, possessing very different properties, have specific and different TE compositions. Moreover, as was shown by us in previous studies *in vivo* neutron activation analysis allows determination of some chemical element contents in intact bone, benign and malignant lesions of bone and has a potential to become a valuable diagnostic tool [17,18,20,42].

To our knowledge, no data are available for the TE contents of GCT, to permit distinction between benign and malignant tumor.

This work had three aims. The first was to obtain reliable data for silver (Ag), cobalt (Co), chromium (Cr), iron(Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) contents in three groups of bone tissue samples – intact bone, benign GCT and malignant GCT using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The second aim was to compare the TE contents in the different groups of samples and the third was to calculate inter-correlations between TE contents in each group of bone tissue samples.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

## 2. MATERIAL AND METHODS

### 2.1. Sample Preparation

Forty-seven children, adolescents and adults were included in this study. The subjects were divided into three groups: controls (1), benign GCT (2) and malignant GCT (3). The reference/control group consisted of 27 persons with intact bone (12 females and 15 males, aged from 16 to 49 years, mean±standard deviation, M±SD, 34±11 years) who had died from various non bone related causes, mainly unexpected from trauma. The intact bone samples mainly of femur and tibia were collected at the Department of Pathology, Obninsk City Hospital. Samples from 10 patients with benign GCT (4 females and 6 males aged from 15 to 47 years, M±SD 24±13 years) and 10 patients with malignant GCT (4 females and 6 males, aged from 14 to 56 years, M±SD 27±15 years) were obtained from open biopsies or after operation from resected specimens. All patients with bone diseases were hospitalized at the Medical Radiological Research Centre. In all cases the diagnosis was confirmed by clinical and histological data.

A titanium tool was used to cut and to scrape samples [43,44]. All bone and tumor tissue samples were freeze dried, until constant mass was obtained, and homogenized. Then samples weighing about 50-100 mg were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

### 2.2. Instrumentation and Method

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol–formaldehyde resins and aliquots of commercial, chemically pure compounds were used. Corrected certified values of BSS element contents were reported by us earlier [45,46]. Ten certified reference material (CRM) IAEA H-5 (Animal Bone) sub-samples and ten standard reference material (SRM) NIST 1486 (Bone Meal) sub-samples weighing about 100 mg were analyzed in the same conditions as bone and tumor samples to estimate the precision and accuracy of the results.

A vertical channel of the WWR-c research nuclear reactor was applied to determine the mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn by INAA-LLR. The quartz ampoule with bone samples, tumor samples, standards, CRM, and SRM was soldered, positioned in a transport aluminum container and exposed to a 100-hour neutron irradiation in a vertical channel with a thermal neutron flux about $10^{13} n \cdot cm^{-2} \cdot s^{-1}$. Two months after irradiation the samples were reweighed and repacked. The duration of each measurement was from 1 to 10 hours. To reduce the high intensity of $^{32}P$ $\beta$-particles ($T_{1/2}$=14.3 d) background, a berillium filter was used. A coaxial 98$cm^3$ Ge (Li) detector and a spectrometric unit (NUC

8100, Hungary), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9 keV resolution at the $^{60}$Co 1332 keV line. Information concerning the nuclear reactions, radionuclides and gamma-energies employed, together with other details of the analysis including the quality control of results were reported by us previously [34,36,37,46].

**Table 1: INAA Data of Trace Elements of CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal (mg/kg on dry mass basis)**

| Element | CRM IAEA H-5 | | This work results | SRM NIST 1486 | | This work results |
|---|---|---|---|---|---|---|
| | Mean | Type | Mean±SD | Mean | Type | Mean±SD |
| Ag | - | - | <0.002 DL | - | - | <0.002 DL |
| Co | 0.25 | N | 0.56±0.25 | - | - | 0.11±0.02 |
| Cr | 2.56 | N | <0.8 DL | - | - | ≤0.9 |
| Fe | 79±11 | C | 85±17 | 99±8 | C | 93±11 |
| Hg | 0.008 | N | ≤0.01 | - | - | ≤0.01 |
| Rb | 1.07 | N | ≤1.0 | - | - | ≤0.9 |
| Sb | 0.024 | N | ≤0.02 | - | - | ≤0.02 |
| Se | 0.054 | N | ≤0.05 | 0.13 | N | ≤0.05 |
| Zn | 89±15 | C | 86±7 | 147±16 | C | 153±29 |

M – arithmetic mean, SD – standard deviation, C – certified values, N – non-certified values.

**Table 2: Basic Statistical Parameters for Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn Mass Fractions (mg/kg, dry mass basis) in Tissue of Intact Bone (N), benign (bGCT) and Malignant Giant Cell Tumor (mGCT)**

| Element | M | SD | SEM | Min | Max | Med | P0.025 | P0.975 |
|---|---|---|---|---|---|---|---|---|
| **Intact bone (N), n=27** | | | | | | | | |
| Ag | 0.00274 | 0.00152 | 0.00051 | 0.000256 | 0.00468 | 0.00282 | 0.000320 | 0.00458 |
| Co | 0.0107 | 0.0070 | 0.0014 | 0.00370 | 0.0345 | 0.00785 | 0.00464 | 0.0288 |
| Cr | 0.274 | 0.182 | 0.057 | 0.110 | 0.669 | 0.202 | 0.117 | 0.629 |
| Fe | 51.2 | 46.3 | 9.3 | 9.20 | 173 | 30.2 | 9.68 | 155 |
| Hg | 0.0057 | 0.0044 | 0.0014 | 0.00100 | 0.0138 | 0.00525 | 0.00100 | 0.0133 |
| Rb | 3.68 | 1.58 | 0.48 | 0.970 | 6.57 | 3.30 | 1.40 | 6.41 |
| Sb | 0.0151 | 0.0102 | 0.0032 | 0.00600 | 0.0420 | 0.0139 | 0.00600 | 0.0364 |
| Se | 0.176 | 0.092 | 0.029 | 0.0550 | 0.358 | 0.169 | 0.0633 | 0.336 |
| Zn | 80.6 | 15.4 | 3.0 | 45.4 | 115 | 82.1 | 51.7 | 109 |
| **Benign giant cell tumor (bGCT), n=10** | | | | | | | | |
| Ag | 0.00449 | 0.00126 | 0.00045 | 0.00180 | 0.00590 | 0.00450 | 0.00219 | 0.00585 |
| Co | 0.0341 | 0.0190 | 0.0063 | 0.00300 | 0.0608 | 0.0308 | 0.00558 | 0.0599 |
| Cr | 0.320 | 0.223 | 0.074 | 0.0560 | 0.761 | 0.224 | 0.0758 | 0.719 |
| Fe | 353 | 128 | 43 | 155 | 606 | 353 | 172 | 577 |
| Hg | 0.00554 | 0.00284 | 0.00095 | 0.000800 | 0.00910 | 0.00610 | 0.000940 | 0.00904 |
| Rb | 1.17 | 2.14 | 0.71 | 0.17 | 6.55 | 0.17 | 0.17 | 5.71 |
| Sb | 0.031 | 0.034 | 0.011 | 0.00300 | 0.0973 | 0.0179 | 0.00426 | 0.0942 |
| Se | 1.51 | 0.96 | 0.32 | 0.373 | 3.21 | 1.51 | 0.410 | 3.02 |
| Zn | 115.4 | 23.5 | 7.8 | 64.5 | 140 | 119 | 71.5 | 139 |
| **Malignant giant cell tumor (mGCT), n=10** | | | | | | | | |
| Ag | 0.00178 | 0.00189 | 0.00067 | 0.000200 | 0.00500 | 0.000785 | 0.000219 | 0.00484 |
| Co | 0.0365 | 0.0184 | 0.0065 | 0.0200 | 0.0780 | 0.0328 | 0.0202 | 0.0714 |
| Cr | 0.420 | 0.283 | 0.100 | 0.114 | 0.914 | 0.387 | 0.120 | 0.864 |
| Fe | 640 | 410 | 145 | 118 | 1362 | 559 | 148 | 1291 |
| Hg | 0.0077 | 0.0046 | 0.0017 | 0.00180 | 0.0167 | 0.00745 | 0.00225 | 0.0155 |
| Rb | 2.17 | 1.52 | 0.54 | 0.170 | 4.06 | 2.06 | 0.170 | 4.04 |
| Sb | 0.0328 | 0.0184 | 0.0065 | 0.0160 | 0.0620 | 0.0259 | 0.0164 | 0.0617 |
| Se | 1.84 | 0.88 | 0.31 | 0.730 | 3.14 | 1.77 | 0.784 | 3.11 |
| Zn | 65.6 | 38.0 | 13.4 | 20.6 | 127 | 59.6 | 22.8 | 124 |

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.

### 2.3. Computer Programs and Statistic

A dedicated computer program of INAA mode optimization was used [47]. Using the Microsoft Office Excel software, the following quantities of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for the TE mass fractions. The differences in the results between intact bone, benign GCT and malignant GCT were evaluated using the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test. For the estimation of the Pearson correlation coefficient between different pairs of the TE mass fractions in each group of bone and tumor tissue samples the Microsoft Office Excel software was also used.

## 3. RESULTS

Table **1** depicts our data for nine TE mass fractions determined by INAA-LLR in ten sub-samples of CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal reference material and the certified values of this material.

Table **2** 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 Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in the samples of intact bone, benign GCT and malignant GCT.

Information concerning the effect of benign or malignant transformation on the TE mass fractions in bone is presented in Table **3**.

**Table 3: Differences between Mean Values (M±SEM) of Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn Mass Fractions (mg/kg, dry mass basis) in Tissue of Intact Bone (N), Benign (bGCT) and Malignant Giant Cell Tumor (mGCT)**

| Groups of samples | Element | Norm (N) | Benign GCT (bGCT) | *t*-test $p\leq$ | U-test *p* | Ratio bGCT/N |
|---|---|---|---|---|---|---|
| bGCT and N | Ag | 0.00274±.00051 | 0.00449±0.00045 | **0.010** | **≤0.01** | 1.64 |
| | Co | 0.0107±0.0014 | 0.0341±0.0063 | **0.006** | **≤0.01** | 3.19 |
| | Cr | 0.274±0.057 | 0.320±0.074 | 0.627 | >0.05 | 1.17 |
| | Fe | 51.2±9.3 | 353±43 | **0.00008** | **≤0.01** | 6.89 |
| | Hg | 0.0057±0.0014 | 0.00554±0.00095 | 0.914. | >0.05 | 0.97 |
| | Rb | 3.68±0.48 | 1.17±0.71 | **0.011** | **≤0.01** | 0.32 |
| | Sb | 0.0151±0.0032 | 0.031±0.011 | 0.224 | >0.05 | 2.05 |
| | Se | 0.176±0.029 | 1.51±0.32 | **0.003** | **≤0.01** | 8.58 |
| | Zn | 80.6±3.0 | 115.4±7.8 | **0.002** | **≤0.01** | 1.43 |
| Groups of samples | Element | Norm (N) | Malignant GCT (mGCT) | *t*-test $p\leq$ | U-test *p* | Ratio mGCT/N |
| mGCT and N | Ag | 0.00274±.00051 | 0.00178±0.00067 | 0.254 | >0.05 | 0.65 |
| | Co | 0.0107±0.0014 | 0.0365±0.0065 | **0.005** | **≤0.01** | 3.41 |
| | Cr | 0.274±0.057 | 0.420±0.100 | 0.229. | >0.05 | 1.53 |
| | Fe | 51.2±9.3 | 640±145 | **0.005** | **≤0.01** | 12.5 |
| | Hg | 0.0057±0.0014 | 0.0077±0.0017 | 0.352 | >0.05 | 1.35 |
| | Rb | 3.68±0.48 | 2.17±0.54 | 0.052 | **≤0.01** | 0.59 |
| | Sb | 0.0151±0.0032 | 0.0328±0.0065 | **0.034** | **≤0.01** | 2.17 |
| | Se | 0.176±0.029 | 1.84±0.31 | **0.001** | **≤0.01** | 10.5 |
| | Zn | 80.6±3.0 | 65.6±13.4 | 0.309 | >0.05 | 0.81 |
| Groups of samples | Element | Benign GCT (bGCT) | Malignant GCT (mGCT) | *t*-test $p\leq$ | U-test *p* | Ratio mGCT/bGCT |
| mGCT and bGCT | Ag | 0.00449±0.00045 | 0.00178±0.00067 | **0.005** | **≤0.01** | 0.40 |
| | Co | 0.0341±0.0063 | 0.0365±0.0065 | 0.728 | >0.05 | 1.07 |
| | Cr | 0.320±0.074 | 0.420±0.100 | 0.437 | >0.05 | 1.31 |
| | Fe | 353±43 | 640±145 | 0.093 | **≤0.05** | 1.81 |
| | Hg | 0.00554±0.00095 | 0.0077±0.0017 | 0.248 | >0.05 | 1.39 |
| | Rb | 1.17±0.71 | 2.17±0.54 | 0.283 | **≤0.05** | 1.85 |
| | Sb | 0.031±0.011 | 0.0328±0.0065 | 0.863 | >0.05 | 1.06 |
| | Se | 1.51±0.32 | 1.84±0.31 | 0.463 | >0.05 | 1.22 |
| | Zn | 115.4±7.8 | 65.6±13.4 | **0.008** | **≤0.01** | 0.57 |

M – arithmetic mean, SEM – standard error of mean, *t*-test - parametric Student's *t*-test, U-test - non-parametric Wilcoxon-Mann-Whitney test, Statistically significant values are in bold.

The data for inter-correlation calculations (values of *r*–coefficient of correlation) including all pairs of the TE identified by us in the samples of intact bone, benign GCT and malignant GCT are shown in Table **4**.

## 4. DISCUSSION

The non-destructive INAA-LLR was used in this research study because this method has many definite advantages over other analytical methods, particularly, in the clinical chemistry. For example, after non-destructive INAA-LLR there is a possibility to check the results for some TE and to receive additional information about other TE contents by destructive analytical methods such as atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry, inductively coupled plasma mass spectrometry and so on, using the same bone samples. Moreover, if a deep-cooled channel of nuclear reactor is available, the non-destructive INAA-LLR allows determining TE contents in the fresh bone/tumor samples and combining TE study with histological investigation. It is also necessary to keep in mind that the non-destructive methods are the current gold-standard solution to control destructive analytical techniques [14]. The destructive analytical methods are based on measurements of processed tissue. In such studies tissue samples are ashed and/or acid digested before analysis. There is evidence that certain quantities of TE are lost as a result of such treatment

**Table 4: Intercorrelations of Pairs of the Trace Element Mass Fractions in Tissue of Intact Bone, Benign and Malignant Giant Cell Tumor**

| Tissue | Element | Co | Cr | Fe | Hg | Rb | Sb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|
| Intact | Ag | -0.23 | 0.51 | -0.80[b] | -0.02 | 0.62[a] | 0.31 | -0.45 | 0.38 |
| bone | Co | 1.00 | 0.16 | 0.55[a] | 0.79[b] | -0.10 | 0.08 | 0.52 | 0.17 |
| n=27 | Cr | 0.16 | 1.00 | -0.48 | 0.51 | 0.56[a] | -0.31 | -0.08 | 0.46 |
| | Fe | 0.55[a] | -0.48 | 1.00 | 0.09 | -0.54 | -0.25 | 0.60[a] | -0.17 |
| | Hg | 0.79[b] | 0.51 | 0.09 | 1.00 | 0.18 | -0.13 | 0.35 | -0.14 |
| | Rb | -0.10 | 0.56[a] | -0.54 | 0.18 | 1.00 | -0.05 | -0.06 | 0.34 |
| | Sb | 0.08 | -0.31 | -0.25 | -0.13 | -0.05 | 1.00 | 0.04 | 0.22 |
| | Se | 0.52 | -0.08 | 0.60[a] | 0.35 | -0.06 | 0.04 | 1.00 | 0.24 |
| | Zn | 0.17 | 0.46 | -0.17 | -0.14 | 0.34 | 0.22 | 0.24 | 1.00 |
| bGCT | Ag | -0.35 | -0.82[b] | 0.23 | 0.27 | -0.80[b] | -0.55[a] | 0.18 | -0.23 |
| n=10 | Co | 1.00 | 0.18 | -0.30 | 0.12 | -0.01 | 0.67[a] | 0.19 | -0.47 |
| | Cr | 0.18 | 1.00 | -0.60[a] | -0.47 | 0.85[b] | 0.01 | 0.02 | 0.14 |
| | Fe | -0.30 | -0.60[a] | 1.00 | 0.47 | -0.53 | 0.10 | 0.26 | 0.39 |
| | Hg | 0.12 | -0.47 | 0.47 | 1.00 | -0.55 | 0.31 | 0.50 | -0.11 |
| | Rb | -0.01 | 0.85[b] | -0.53 | -0.55 | 1.00 | -0.12 | 0.09 | 0.12 |
| | Sb | 0.67[a] | 0.01 | 0.10 | 0.31 | -0.12 | 1.00 | -0.08 | -0.39 |
| | Se | 0.19 | 0.02 | 0.26 | 0.50 | 0.09 | -0.08 | 1.00 | 0.35 |
| | Zn | -0.47 | 0.14 | 0.39 | -0.11 | 0.12 | -0.39 | 0.35 | 1.00 |
| mGCT | Ag | -0.13 | -0.08 | 0.13 | -0.18 | -0.01 | -0.08 | -0.57[a] | -0.30 |
| n=10 | Co | 1.00 | 0.30 | 0.75[a] | 0.73[a] | 0.02 | 0.35 | 0.56[a] | 0.67[a] |
| | Cr | 0.30 | 1.00 | 0.31 | -0.16 | -0.07 | 0.24 | 0.52 | -0.03 |
| | Fe | 0.75[a] | 0.31 | 1.00 | 0.52 | -0.34 | 0.04 | 0.46 | 0.10 |
| | Hg | 0.73[a] | -0.16 | 0.52 | 1.00 | 0.33 | -0.23 | 0.54 | 0.57[a] |
| | Rb | 0.02 | -0.07 | -0.34 | 0.33 | 1.00 | 0.24 | 0.29 | 0.37 |
| | Sb | 0.35 | 0.24 | 0.04 | -0.23 | 0.24 | 1.00 | 0.10 | 0.74[a] |
| | Se | 0.56[a] | 0.52 | 0.46 | 0.54 | 0.29 | 0.10 | 1.00 | 0.27 |
| | Zn | 0.67[a] | -0.03 | 0.10 | 0.57[a] | 0.37 | 0.74[a] | 0.27 | 1.00 |

bGCT – benign giant cell tumor of bone, mGCT – malignant giant cell tumor of bone, statistically significant difference: [a] - $p \leq 0.05$, [b] - $p \leq 0.01$, [c] - $p \leq 0.001$.

[14,44,48]. There is no doubt that every method available for the measurement of TE contents in bone and tumor samples can be used. However, when using destructive analytical methods it is necessary to control for the losses of TE, for complete acid digestion of the sample, and for the contaminations by TE during sample decomposition, which needs adding some chemicals.

The results of mean values for Fe and Zn - two representative TE of CRM IAEA H-5 (Animal Bone) and SRM NIST1486 (Bone Meal) were in the range of 95% confidence interval (M±2SD) of the certificates' values (Table **1**). Good agreement with the certified data of CRM and SRM for Fe and Zn mass fractions determined by INAA-LLR indicate an acceptable accuracy and for other TE mass fractions obtained in the study of intact bone and tumor tissue samples presented in Tables **2-4**.

The mean values and all selected statistical parameters were calculated for nine TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) mass fractions (Table **2**). The mass fraction of these TE mass fraction were measured in all, or a major portion of normal bone, benign GCT and malignant GCT samples.

From Table **3** it is observed that in the benign GCT tissue the mean mass fractions of Ag, Co, Fe, Se, and Zn are respectively 1.6, 3.2, 6.9, 8,6, and 1.4 times higher and the mean mass fraction of Rb is almost 3 times lower than in normal bone tissues. In malignant GCT tissue the mean mass fractions of Co, Fe, Sb, and Se are respectively 3.4, 12.5, 2.2, and 10.5 times higher and the mean mass fraction of Rb is 41% lower than in normal bone tissues. In malignant GCT tissue only the mean mass fractions of Fe and Rb are significantly higher (1.8 and 1.9 times, respectively) and the mean mass fractions of Ag and Zn some lower (60% and 43%, respectively) than in benign GCT tissue.

In the control group a statistically significant direct correlation was found between the Ag and Rb ($r$ = 0.62, $p$ ≤0.05), Co and Fe ($r$ = 0.55, $p$≤0.01), Co and Hg ($r$ = 0.79, $p$ ≤0.01), Cr and Rb ($r$ = 0.56, $p$ ≤0.05), and between Fe and Se ($r$ = 0.60, $p$ ≤0.05) mass fractions (Table **4**). In the same group a pronounced inverse correlation was observed between the Fe and Ag ($r$ = -0.80, $p$ ≤0.05). If some positive correlations between the TE were predictable (e.g., Fe–Co), the interpretation of other observed relationships requires further study for a more complete understanding.

In the benign GCT tissue many significant correlations between TE found in the control group are no longer evident, for example, direct correlation between Fe and Co or between Fe and Se, etc. (Table **4**). However, direct correlations between Co and Sb ($r$ = 0.67, $p$ ≤0.05) and Cr and Rb ($r$ = 0.85, $p$ ≤0.01), as well as inverse correlation between Ag and Cr ($r$ = -0.82, $p$ ≤0.01), Ag and Rb ($r$ = -0.80, $p$ ≤0.01), Ag and Sb ($r$ = -0.55, $p$ ≤0.05), and also Cr and Fe ($r$ = -0.60, $p$ ≤0.05) were observed (Table **4**).

Similarly, in the malignant GCT tissue many significant correlations between TE found in the control group are also no longer evident, for example, direct correlation between Fe and Se, etc. (Table **4**). However, direct correlations between Co and Fe ($r$ = 0.75, $p$ ≤0.05), Co and Hg ($r$ = 0.73, $p$ ≤0.05), Co and Se ($r$ = 0.56, $p$ ≤0.05), Co and Zn ($r$ = 0.67, $p$ ≤0.05), Zn and Hg ($r$ = 0.57, $p$ ≤0.05), and also Zn and Sb ($r$ = 0.74, $p$ ≤0.05), as well as inverse correlation between Ag and Se ($r$ = -0.57, $p$ ≤0.05) were observed (Table **4**).

Thus, if we accept the levels and relationships of TE mass fraction in the intact bone samples of control group as a norm, we have to conclude that with a tumor transformation the levels and relationships of TE in bone significantly change. No published data referring to contents of TE or correlations between TE mass fractions in the benign and malignant GCT of bone were found.

Characteristically, elevated or reduced levels of TE observed in cancerous tissues are discussed in terms of their potential role in the initiation and promotion of cancer. In other words, using the low or high levels of the TE in cancerous tissues researchers try to determine the carcinogenic role of the deficiency or excess of each TE in investigated organ. In our opinion, abnormal levels of many TE in tumor could be and cause, and also effect of malignant transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in TE level in pathologically altered tissue is the reason for alterations or vice versa.

Bone is a mineralized connective tissue. It is formed by osteoblasts, that deposit collagen and release Ca, Mg, and phosphate ions that combine chemically within the collagenous matrix into a crystalline mineral, known as bone hydroxyapatite. On average, bone tissue contains about 10-25% water, 25% protein fibers like collagen, and 50% hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. Many TE are bone-seeking elements and they are

closely associated with hydroxyapatite [36,37,40]. Benign and malignant GCT is classified as a bone tumor. Our previous findings showed that the means of the Ca and P mass fraction in the benign and malignant GCT of bone tissue are lower than in normal bone, but the mean of Ca/P ratio is similar [49]. It suggested that GCT continues to form bone hydroxyapatite but to a lesser degree than normal bone.

**Silver:** Ag is a TE with no recognized trace metal normal physiological function in the human body [50]. Ag in metalic form and inorganic Ag compounds ionize in the presence of water, body fluids or tissue exudates. The silver ion $Ag^+$ is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes [51]. Besides such interactions, chronic exposure to Ag causes a permanent bluish-gray discoloration of the skin (argyria) or of the sclera (argyrosis). Exposure to soluble Ag compounds may produce other toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory, and intestinal tract, and changes in blood cells [52]. More detailed knowledge of Ag toxicity can lead to a better understanding of its impact on human health, including bone conditions. Why there is Ag accumulation by benign GCT tissue is not completely understood and requires further studies. In any case an elevated level of Ag in benign GCT, in comparison with normal bone and malignant GCT, could possibly be explored to aid a differential diagnosis between benign and malignant GCT.

**Cobalt:** Health effects of high exposure to Co, whether resulting from occupational, environmental, dietary and medical contact are characterized by a complex clinical syndrome, including mainly neurological, cardiovascular and endocrine deficits [53,54]. Co is genotoxic and carcinogenic. This is mainly caused by oxidative DNA damage by reactive oxygen species (ROS), perhaps combined with inhibition of DNA repair [55]. Indeed, Co ions affect osteoblast proliferation, size, and shape. Co ions also promote secretion of cytokines from osteoblasts, which leads to inflammation and osteoclast differentiation, maturation, and stimulation [56]. Thus, a carcinogenic effect of elevated Co level in benign and malignant GCT tissue may be assumed. It was found in the present study, that there is a direct correlation between Fe and Co levels in normal bone and malignant GCT tissue (Table **4**). Therefore an increased level of Co in both benign and malignant GCT is closely connected to a very high Fe content in tumor tissue (Table **3**). Anyway, the accumulation of Co in benign and malignant GCT tissue could possibly be explored as a diagnostic marker for GCT.

**Iron:** Our findings show that the mean of the Fe mass fractions in the benign and malignant GCT tissue samples were respectively 6.9 and 12.5 times greater than in normal bone tissues (Table **3**). It is well known that the Fe mass fraction in a tissue sample depends mainly on the blood volume in that tissue. Thus, one can speculate that benign and malignant GCT are characterized by an increase of the mean values of the Fe mass fractions because its levels of tumor vascularization are higher than that of normal bone. Moreover, one can deduce that the level of malignant GCT vascularization is almost 2 times higher than that of benign GCT. Thus, this difference could possibly be explored to aid the diagnosis of GCT malignancy.

**Rubidium:** There is very little information about the effects of Rb in organisms. No negative environmental effects have been reported. Rb is only slightly toxic on an acute toxicological basis and would pose an acute health hazard only when ingested in large quantities [57]. Rb has some function in immune responce [58], probably by supporting cell differentiation [59]. The reason for a lower level of Rb in benign and malignant GCT tissue than that in normal bone is not completely understood and requires further studies.

**Selenium:** In the benign and malignant GCT tissue the mean Se mass fractions were respectively 8.6 and 10.5 times 11.0 times higher than in normal bone (Table **3**). A high Se level was reported in malignant tumors of the ovary [60], lung [61], prostate [62-70], breast [71,72], gastro intestinal tract [73], and also in cancers of the stomach [73] and thyroid [75]. Moreover, in our previous study elevated levels of Se were found in such malignant tumors of bone as osteogenic sarcoma [76], chondrosarcoma [77], and Ewing's sarcoma [78]. The role played by Se in those tumors remains unknown, but in general it is accepted that certain proteins containing Se can mediate the protective effects against oxidative stress. A literature-based analysis found the association of malignant tissue transformation with local oxidative stress. Studies have shown that oxidative stress conditions play an important role in both the initiation and the progression of cancer by regulating molecules such as DNA, enhancers, transcription factors, and cell cycle regulators [79]. However the cause of increased Se in cancerous tissue and particularly in benign and

malignant GCT of bone is not completely understood and requires further studies. However the great accumulation of Se in benign and malignant GCT tissue could possibly be explored to aid the diagnosis of GCT.

**Antimony:** Animal carcinogenicity data were concluded sufficient for Sb [80]. Possible mechanisms of Sb's action include its potential to produce active ROS and to interfere with the DNA repair system [80]. The cause of Sb accumulation by malignant GCT tissue is not completely understood and requires further studies. An elevated level of Sb in malignant GCT in comparison with normal bone and benign GCT could possibly be explored for aid in making the differential diagnosis between benign and malignant GCT.

**Zinc:** Zn is active in more than 300 proteins and over 100 DNA-binding proteins, including the tumor suppressor protein p53, a Zn-binding transcription factor acting as a key regulator of cell growth and survival after various forms of cellular stress. p53 is mutated in half of human tumors and its activity is tightly regulated by metals and redox mechanisms. Zn ions are cofactors of the superoxide dismutase enzymes, which prevent the onset and progression of tumors through cell protection against substances that cause the formation of free radicals and ROS. The role of zinc is to act as a membrane stabilizer and to participate in antioxidative protection and oxidative stress inhibition.

A low level of Zn was reported in malignant tumors of liver [81,82,83], kidney [81], uterus [84], lung [83,85], prostate [63-70,83,86-89], stomach [90], testis [90], thyroid [75,83,89,90] and in esophageal squamous cell cancer [91]. On the one hand, these facts imply that reduced Zn content in tumors is probably one of the factors in the etiology of malignant transformation of different tissues, because Zn deficiency has been linked to severe deficiency in immune function and disruption in T-Cell function. Zn deficiency also causes inactivation of p53, a tumor suppressor protein, which has been associated with many cancers [90]. On the other hand, it is possible to interpret the low levels of Zn in malignant GCT tissue as follows. Zn is a bone-seeking TE and its content in bone tissue is closely associated with that of hydroxyapatite [40]. Our previous findings showed that hydroxyapatite content of malignant GCT tissue is significantly lower than in normal bone [49]. Thus, bone tissue may lose Zn during malignant transformation. A reduced level of Zn in malignant GCT in comparison with benign GCT could possibly be explored as a means of differential diagnosis between these benign and malignant tumors.

Trace element inter-correlations**:** Each of the TE is distinct in its primary mode of action. Moreover, there are several forms of synergistic action of the TE as a part of intracellular metabolism, during which several reactive intermediates and byproducts are created [92-94]. These reactive species are capable of potent and surprisingly selective activation of stress-signaling pathways, inhibition of DNA metabolism, repair, and formation of DNA crosslinks, which are known to contribute to the development of human cancers [93,95]. Thus, in addition to TE contents changes of TE relationships (inter-correlations) might be involved in etiology and pathophysiology of bone tumors.

**Limitations:** This study has several limitations. Firstly, analytical techniques employed in this study measure only nine TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) mass fractions. There are many other TE associated with different levels of oxidative stress and carcinogenesis. Thus, future studies should be directed toward using other analytical methods which will extend the list of TE investigated in normal bone as well as in the benign and malignant GCT of bone. Secondly, the sample size of benign GCT and malignant GCT groups was relatively small. Despite these limitations, this study provides evidence that the levels of Ag, Co, Fe, Rb, Sb, Se, and Zn mass fractions have altered in GCT tumors and shows the necessity to continue TE research of benign and malignant bone GCT.

## CONCLUSION

INAA-LLR is a most satisfactory analytical tool to determine non-destructively the elemental content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn in samples of human intact bone and also in samples of benign and malignant GCT. In the bone affected by benign GCT the mean mass fractions of Ag, Co, Fe, Se, and Zn were significantly higher while the mean mass fraction of Rb was lower than in normal bone tissues. In malignant GCT tissue the mean mass fractions of Co, Fe, Sb, and Se were higher while the mean mass fraction of Rb was lower than in normal bone tissues. In malignant GCT tissue only the mean mass fractions of Fe and Rb were higher and the mean mass fractions of Ag and Zn were lower than in benign GCT tissue. In addition, in the tumor transformed bone many inter-correlations between TE contents found in the control group were no longer evident in the GCT groups. Thus, if we accept the levels and relationships of TE mass

fraction in the intact bone as a norm, we have to conclude that in benign and malignant GCT tissues the TE homeostasis was significantly disturbed. The studies on the role of TE in the etiology and pathogenesis of benign and malignant GCT should be continued, because of the limitations of numbers of different TEs studied in this work and to determine relevant mechanisms which may explain the findings. This paper has only considered two specific bone neoplasms. However the value of this approach to the determination of the malignant or benign nature of a tumor using TE analysis has been confirmed. It is likely to have many other useful applications and deserves to be included in the diagnostician's armamentarium after appropriate experimental confirmation.

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## AUTHORS' CONTRIBUTION

Vladimir Zaichick and Sofia Zaichick contributed equally to this work.

## CONFLICT OF INTERESTS

Authors have declared that no competing interests exist.

## ABBREVIATIONS

TE= Trace elements,

INAA= Instrumental neutron activation analysis,

INAA-LLR= INAA with high resolution spectrometry of long-lived radionuclidesm,

BSS= Biological synthetic standards,

CRM= Certified reference material,

SRM= Standard reference material,

ROS = Reactive oxygen species.

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