Research Article

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Journal of Cancer Epidemiology and Prevention

2020
Vol. 5 No. 1:1

A Nobel Multivariate Index for Cancer Risk Detection Based On the Serum Trace Elements: Metallo-Balance Method

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Received date: December 26, 2019; **Accepted date:** January 16, 2020; **Published date:** January 23, 2020

Citation: Okamoto N, Mikami H, Nakamura Y, Kusakabe M, Yamamoto N, et al. (2020) A Nobel Multivariate Index for Cancer Risk Detection Based On the Serum Trace Elements: Metallo-Balance Method Cancer Epidemiology & Prevention Vol.5 No.1 DOI: 10.36648/cancer.5.1.4

Abstract

Objective: There are increasing reports that many trace elements are playing important roles for biological forms. We used ICP-MS and developed a novel method for precise measurement of serum trace elements called “Metallo-Balance (MB) test” for the screening of cancer risk.

Methods: 463 patients with colorectal cancer (men:278; women 185), 277 patients with prostate cancer (men) and 429 patients with breast cancer (women) were collected in Kanagawa cancer center and Chiba cancer center, Japan, while 850 controls (men: 471; women: 379) w/o cancers were obtained from general population. Under IRB approved protocol serum samples were collected and subjected to ICP-MS analysis to measure 17 trace elements (Na, Mg, P, S, K, Ca, Fe, Zn, Cu, Se, Rb, Sr, As, Mo, Cs, Co, Ag). Cancer cases and controls were statistically measured the sensitivity, specificity and area under ROC curve (AUC).

Results: High sensitivity, specificity and AUC were confirmed by binominal logistic regression analysis. AUC for colorectal cancer (men) was 0.943 (95% CI, 0.920-0.966), 0.842 (0.801-0.882) for prostate cancer, 0.898 (0.861-0.935) for colorectal cancer (women), and 0.824 (0.783-0.865) for breast cancer. In particular, sensitivity of 80% or more in stages I and II can be considered effective as a cancer risk screening. Elements that became significant in connection with cancer were Mg, Co, Cu and Mo. Conversely, S, Zn and Rb were less relevant to cancer.

Conclusion: These results are considered to indicate that "the balance of trace elements in the blood is largely disrupted from the early stage and onset of cancer". MB test is a promising method for cancer detection and maybe risk assessment.

Keywords: Trace elements; Serum; Cancer risk; Screening; Multi-variate analysis

Introduction

Development of cancer control programs has been an urgent task in many countries in the world, with many programs focusing on the introduction of screening methods aimed at early detection and treatment in specific regions. Various screening methods, such as palpation, imaging (X-ray, CT scan, MRI scan, PET scan, etc.), endoscopy, and xenodiagnoses (blood test, cytology, biopsy, etc.) have been introduced in many regions. However, accuracy and efficacy of these have not necessarily been sufficiently established [1]. For example, palpation is ocular inspection for which use is limited to external organs, breasts, colon, rectum, and stomach, while imaging requires expensive equipment and technicians and carries risks such as poor reading and radiation exposure. On the other hand, testing of body fluids, etc. has fewer burdens on the examinees and is considered to be easier to use during mass examination.

In Japan, screening using body fluids to identify blood tumor markers for unspecified sites (CA125, CA19-9, CEA, SCC etc.), PSA for prostate cancer, serum pepsinogen for stomach cancer, urine NMPP22 and CK8-18 for bladder cancer are implemented.

Furthermore, measurement of MicroRNA [2], nucleosomes [3], and apoAII [4] in the blood or saliva has recently been developed for cancer screening.

Trace elements are essential in a living body, but an excess or defficiency may inhibit normal vital reactions, leading to malfunction or disease. Many cases, for example anemia due to iron(Fe) deficiency [5], osteoporosis due to calcium(Ca) deficiency [6], Minamata disease due to excess mercury(Hg) [7], Keshan disease due to selenium(Se) deficiency [8], schizophrenia due to low zinc(Zn) [9], heart failure due to low magnesium(Mg), high phosphorus(P), and Ca [10], have been reported. It has also been reported that serum zinc is lower and calcium, copper, iron and magnesium are higher in breast cancer patients compared to the general population [11]. In addition, the Cu/Zn ratio has been reported to be higher in breast cancer patients [12] and ovarian cancer patients [13]. Similarly, several element concentrations in renal cell cancer [14], lung cancer [15], pancreatic cancer [16], colorectal cancer [17,18], oral cancer [19], prostate cancer [20] etc. have been reported to differ from healthy individuals. In this study, "cancer patients with abnormal concentrations of elements in the blood", as indicated in the above reports, were considered. A case-control study to compare blood samples from patients with colorectal cancer, breast cancer or prostate cancer, with those of and healthy normal persons was carried out in order to develop and implement a new index for use in screening for cancer risk.

Materials and Methods

Cases and controls

463 patients with colorectal cancer (men: 278; women: 185), 277 patients with prostate cancer (men), 429 patients with breast cancer (women) who were treated at kanagawa cancer center and chiba cancer center in Japan from April 2005 to March 2015 were targeted in this study. After receiving an explanation from the primary physician, participants submitted a written informed consent form. After admission and diagnosis at the hospital and before the start of treatment, a 7 mL blood sample was drawn from the cubital vein in the morning fasting state. The sample was centrifuged at 3,000 rpm for 10 minutes with a preservative at room temperature and stored at -80°C until measurement of elements. The pathologic stage of each cancer after discharge was recorded and used.

The control group was formed from persons who received a standard examination in the region (mass examination) conducted by chiba cancer center each year. 850 people (men: 471; women: 379) who had not developed any cancer during 15-year follow-up period as part of the Japan Multi-Institutional Collaborative Cohort Study: J-MICC study [21] and who provided consent for the provision of a blood sample for cancer study were selected. Blood (7 mL) was drawn at the baseline for an independent study in the Chiba region and the same process was performed and samples were stored at -80°C until measurement. Absence of cancer was confirmed in this control group at the end of 2015 by record linkage with the Chiba Population based Cancer Registry.

Study design

The flow of this study is shown in **Figure 1**. Ages in the targeted cases and the control group were between 30 to 79 years and were matched as close as possible. Subjects were then randomly divided into two groups by sex and by cancer sites, with one group for training data and the other for validation data. Training and validation data consisted of colorectal cancer (men) 139 and 139, colorectal cancer (women) 93 and 92, breast cancer 214 and 215, prostate cancer 139 and 138. For the control group, 236 and 235 men, 190 and 189 women, respectively.

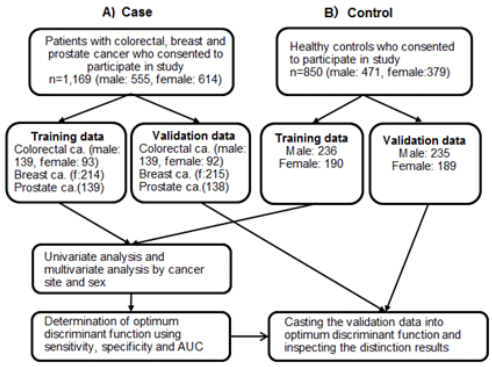

Figure 1: Flow-chart of the study design.

Binomial logistic regression analysis of the training data by sex and by site was performed. After determining the best indicator, indicative power of the validation data of the subject and control groups was examined. However, there were no persons in the control group who corresponded to prostate cancer patients under the age of 49 years, so 218 persons from the control group were analyzed.

Reagents and instruments

Reagents: 61% nitric acid solution: Kanto Chemical Co., Ltd.,

30% Hydrogen peroxide solution: Kanto Chemical Co., Ltd.,

XSTC-622B (Mixed standard solution for ICP-MS): SPEX,

Standard solution (Na, Mg, P, S, K, Ca, Be, Te) for atomic absorption analysis: Kanto Chemical Co., Ltd.,

ICP-MS metal standard solution (Y, Rh) for ICP-MS: AccuStandard

Equipment: All devices used for measurement of trace elements, such as sample storage bins, sampling bins and micropipette tips, were made of polypropylene. All containers were washed with ultra-pure water (18.00 M Ω•CM or more), then 500 µL of 61% nitric acid was added to the containers and heated overnight. The containers were then washed again with ultra-pure water, filled with 10% nitric acid, and heated for 2 nights. The containers were then washed with ultra-pure water. The measuring instrument used ICP-MS (Agilent 7800).

Experimental operations

Sample pretreatment: 50 µL of the blood sample was weighed

in a pluggable polypropylene container, 125 µL of 61% nitric acid solution was added and 25 µL of 30% hydrogen peroxide water was mixed and heated at 70 °C for 16 hours.

Preparation of calibration curve: XSTC-622B was appropriately diluted with a 3% nitric acid solution, and calibration curves of iron (Fe), copper (Cu), zinc (Zn), arsenic (As), strontium (Sr), cobalt (Co), rubidium (Rb), selenium (Se), molybdenum (Mo), cesium (Cs), and silver (Ag) were prepared. In addition, single element standard solutions were mixed and appropriately diluted with a 3% nitric acid solution to prepare calibration curves of sodium (Na), magnesium (Mg), phosphorus (P), sulfur (S), potassium (K) and calcium (Ca). The correlation coefficient was 0.9998 or more for any of the 17 elements.

Analysis conditions of ICP-MS: Apparatus conditions were high frequency output 1550 W, plasma gas flow rate 15 L/min, nebulizer gas flow rate 1.05 L/min, and auxiliary gas flow rate L/min. Samples were introduced into the apparatus by suction using a peristaltic pump. As internal standard elements, beryllium (Be), yttrium (Y), Rhodium (Rh) and tellurium (Te) were added to 50, 5, 1 and 50 µg/L, respectively. Under the above conditions, 17 elements including Na (ppm), Mg (ppm), P (ppm), S (ppm), K (ppm), Ca (ppm), Fe (ppb), Co (ppb), Cu (ppb), Zn (ppb), As (ppb), Se (ppb), Rb (ppb), Sr (ppb), Mo (ppb), Ag (ppb) and Cs (ppb) were measured by ICP-MS.

Statistical processing

Data items used for analysis included 18 items according to age-class (integer value obtained by dividing age by 10) and 17 logarithmically converted elements. For each of the 18 measurement items, Student's t-test was used to determine any difference between target cases and the control group. Furthermore, binomial logistic regression analysis was performed using multivariate analysis according to all 18 items. Sensitivity, specificity, area under the ROC (Receiver Operating Characteristic) Curve (AUC) [22,23] were calculated. Excel Analysis (BellCurve, Japan) and SPSS Ver.24 (IBM, USA) were used for all processing.

Results

For the training data and validation data according to the number of cases by age-class, the number of patients with cancer by stage and affected site is shown in **Table 1**. No differences in age and stage distribution were observed.

Mean and Standard Deviation (SD) of the measurements of training data of the 17 elements and age-class divided by cancer site, between the target cases and control group are shown in **Table2a and 2b** (gray area is less than 5% with significant difference). For colorectal cancer (men), Na, Mg, K, Ca, Fe, Co, Cu and Zn were significantly higher and S, Fe, Rb, Mo and Cs were lower compared to the control group. For prostate cancer, Na, Ca, Fe, Co, Cu, As and Se were significantly higher and Rb and Cs were significantly lower. For colorectal cancer (women), Na, Mg, Ca, Co, Cu, Zn and Ag were significantly higher and Rb and Cs were lower element. For breast cancer, Na, Mg, P, S, Ca, Fe, Co, Cu, Zn, Se and Sr were significantly higher and Rb and Cs

Table 1: Age distribution of subjects and stage of cancer patients by cancer site and data type.

Cancer site	Item	Class	Training data			Validation data		
			Control	Case	Total	Control	Case	Total
Colorectal cancer (male)	Age	30-39	5	3	8	5	3	8
		40-49	13	12	25	12	11	23
		50-59	45	31	76	45	31	76
		60-69	98	57	155	98	58	156
		70-79	75	36	111	75	36	111
	Stage	0	-	4	-	-	3	-
		I	-	33	-	-	28	-
		II	-	39	-	-	36	-
		III	-	44	-	-	52	-
		IV	-	18	-	-	20	-
	Total		236	139	375	235	139	374
Prostate cancer	Age	50-59	45	14	59	45	15	60
		60-69	98	59	157	98	57	155
		70-79	75	66	141	75	66	141
	Stage	0	-	0	-	-	2	-
		I	-	23	-	-	27	-
		II	-	71	-	-	52	-
		III	-	34	-	-	40	-
		IV	-	10	-	-	16	-
	Total		218	139	357	218	138	356

Colorectal Cancer(female)	Age	30-39	7	2	9	8	2	10
		40-49	28	5	33	28	4	32
		50-59	53	22	75	51	22	73
		60-69	58	37	95	59	37	96
		70-79	44	27	71	43	27	70
	Stage	0	-	4	-	-	5	-
		I	-	27	-	-	24	-
		II	-	20	-	-	24	-
		III	-	37	-	-	28	-
		IV	-	5	-	-	11	-
Breast cancer	Total		190	93	283	189	92	281
	Age	30-39	7	29	36	8	29	37
		40-49	28	63	91	28	62	90
		50-59	53	16	69	51	18	69
		60-69	58	72	130	59	71	130
		70-79	44	34	78	43	35	78
	Stage	0	-	12	-	-	14	-
		I	-	21	-	-	23	-
		II	-	30	-	-	25	-
		III	-	2	-	-	2	-
		IV	-	0	-	-	0	-
	Total		190	214	404	189	215	404

Table 2a: Results to Student's t-test between case and control of training data by cancer site (Male).

Item	Colorectal cancer					Prostate cancer				
	Control (n=236)		Case (n=139)		p value	Control (n=218)		Case (n=138)		p value
	mean	SD	mean	SD		mean	SD	mean	SD	
Age-class	5.953	0.96	5.799	0.994	0.1374	6.138	0.731	6.377	0.664	0.002
ln(Na)	8.048	0.035	8.072	0.024	<0.001	8.05	0.034	8.058	0.027	0.0209
ln(Mg)	2.948	0.079	2.997	0.077	<0.001	2.949	0.078	2.949	0.079	0.9448
ln(P)	4.7	0.118	4.691	0.132	0.5176	4.695	0.119	4.699	0.124	0.7864
ln(S)	6.986	0.078	6.956	0.083	<0.001	6.985	0.078	6.99	0.074	0.6246
ln(K)	5.088	0.088	5.123	0.086	<0.001	5.09	0.088	5.107	0.078	0.0727
ln(Ca)	4.478	0,074	4.501	0.053	0.0016	4.481	0.073	4.516	0.051	<0.001
ln(Fe)	6.807	0.372	6.698	0.667	0.0433	6.809	0.373	6.985	0.345	<0.001
ln(Co)	-2.407	0.374	-1.876	0.635	<0.001	-2.402	0.38	-2.274	0.282	<0.001
ln(Cu)	6.663	0.173	6.819	0.2	<0.001	6.665	0.169	6.753	0.201	<0.001
ln(Zn)	6.586	0.181	6.667	0.197	<0.001	6.58	0.181	6.582	0.148	0.8908
ln(As)	0.95	0.594	0.998	0.708	0.4836	0.989	0.573	1.129	0.573	0.0256
ln(Se)	4.918	0.143	4.909	0.139	0.5337	4.915	0.143	4.972	0.17	<0.001
ln(Rb)	5.109	0.186	5.013	0.191	<0.001	5.099	0.185	4.989	0.204	<0.001
ln(Sr)	3.417	0.314	3.412	0.296	0.8776	3.421	0.319	3.478	0.3	0.0957
ln(Mo)	0.324	0.551	0.13	0.47	<0.001	0.349	0.551	0.474	0.464	0.0281
ln(Ag)	-1.422	0.865	-1.255	0.801	0.0645	-1.437	0.879	-1.456	0.993	0.857
ln(Cs)	-0.349	0.28	-0.434	0.302	0.0059	-0.356	0.279	-0.468	0.297	<0.001

Table 2b: Results to Student's t-test between case and control of training data by cancer site (Female).

Item	Colorectal cancer					Breast cancer				
	Control (n=190)		Case (n=93)		p value	Control (n=190)		Case (n=214)		p value
	mean	SD	mean	SD		mean	SD	mean	SD	
Age-class	5.547	1.11	5.887	0.965	0.0137	5.547	1.11	5.222	1.314	0.0091
ln(Na)	8.052	0.036	8.072	0.027	<0.001	8.052	0.036	8.069	0.022	<0.001
ln(Mg)	2.971	0.076	3.009	0.083	<0.001	2.971	0.076	2.987	0.073	0.0419
ln(P)	4.787	0.11	4.77	0.127	0.2535	4.787	0.11	4.812	0.112	0.0248
ln(S)	6.981	0.068	6.945	0.086	<0.001	6.981	0.068	7.021	0.065	<0.001
ln(K)	5.055	0.099	5.068	0.085	0.2581	5.055	0.099	5.069	0.118	0.1967
ln(Ca)	4.475	0.081	4.498	0.054	0.013	4.475	0.081	4.53	0.043	<0.001
ln(Fe)	6.61	0.427	6.63	0.541	0.7441	6.61	0.427	6.769	0.465	<0.001
ln(Co)	-2.105	0.671	-1.788	0.63	<0.001	-2.105	0.671	-1.896	0.662	0.0023
ln(Cu)	6.748	0.163	6.907	0.252	<0.001	6.748	0.163	6.801	0.174	0.0016
ln(Zn)	6.583	0.19	6.658	0.165	0.0013	6.583	0.19	6.635	0.149	0.0033
ln(As)	0.712	0.661	0.839	0.732	0.1424	0.712	0.661	0.765	0.692	0.44
ln(Se)	4.89	0.139	4.904	0.163	0.4435	4.89	0.139	4.946	0.138	<0.001
ln(Rb)	5.051	0.18	4.917	0.305	<0.001	5.051	0.18	4.972	0.192	<0.001
ln(Sr)	3.339	0.269	3.387	0.28	0.17	3.339	0.269	3.401	0.31	0.0381
ln(Mo)	0.216	0.478	0.186	0.471	0.6081	0.216	0.478	0.177	0.406	0.3853
ln(Ag)	-1.412	0.85	-1.145	0.893	0.0154	-1.412	0.85	-1.422	0.838	0.8461
ln(Cs)	-0.384	0.307	-0.589	0.319	<0.001	-0.384	0.307	-0.5	0.285	<0.001

Table 3: Partial regression coefficient and significance by binominal logistic regression analysis.

Item	Colorectal Ca. (Male)		Prostate Ca.		Colorectal Ca. (Female)		Breast Ca.	
	Partial regression coefficient	p value	Partial regression coefficient	p value	Partial regression coefficient	p value	Partial regression coefficient	p value
Age-class	-0.6347	0.0039	0.2877	0.1702	-0.0555	0.7885	-0.4455	<0.001
ln(Na)	30.4559	<0.001	-6.7256	0.2242	22.2788	0.0095	5.5959	0.3471
ln(Mg)	6.3226	0.0208	2.6142	0.1928	4.5762	0.1001	1.0818	0.5768
ln(P)	-1.4564	0.3927	-1.9335	0.114	1.3537	0.4873	-0.9784	0.4676
ln(S)	-20.8789	<0.001	-13.2833	<0.001	-22.3568	<0.001	-3.7657	0.2022
ln(K)	5.5054	0.0658	3.6056	0.122	3.5334	0.2214	1.0895	0.4306
ln(Ca)	14.5858	0.0137	19.1967	<0.001	9.2026	0.083	19.0774	<0.001
ln(Fe)	0.2338	0.6396	1.595	<0.001	0.4622	0.308	0.8731	0.0072
ln(Co)	2.8334	<0.001	1.5398	<0.001	0.8677	0.0052	0.8257	<0.001
ln(Cu)	3.608	0.0013	2.444	0.0028	3.2998	0.0028	0.7888	0.3574
ln(Zn)	3.2695	0.0073	-0.6342	0.469	2.6192	0.0101	0.6533	0.4236
ln(As)	0.4994	0.1163	0.0449	0.8578	-0.0079	0.9785	0.0624	0.7761
ln(Se)	-1.2302	0.5075	2.3001	0.0682	1.5885	0.2781	0.9217	0.3999
ln(Rb)	-6.6253	<0.001	-4.3976	<0.001	-4.8747	0.002	-4.006	<0.001
ln(Sr)	-0.9881	0.108	-0.5453	0.2595	-0.2144	0.7561	-0.505	0.2847
ln(Mo)	-1.6054	<0.001	0.0364	0.9029	-0.1996	0.6124	-3.382	0.2233
ln(Ag)	0.0187	0.937	0.0722	0.6478	0.3136	0.1562	-0.2353	0.1313
ln(Cs)	0.2258	0.7716	-0.5048	0.4229	-0.8379	0.2934	-0.4571	0.438

were significantly lower. Six elements were significantly different for all affected sites-Na, Ca, Co, Cu, Rb and Cs, suggesting that these elements are strongly related to the development and progress of cancer. In the univariate analysis, some elements showed significant difference between the target cases and the control group. However, identification of the affected cancer site by only one element was not possible. Therefore, age-class measurements based on training data were added to the 17 elements as the analysis data, and multivariate analysis using binomial logistic regression analysis was performed.

Results of the ROC curve and AUC by binominal logistic regression analysis are shown in **Figure 2**. In colorectal cancer (men), AUC and 95% coincident interval (CI) were 0.943 and 0.920-0.966, 0.842 and 0.801-0.882 in prostate cancer, 0.898 and 0.861-0.935 in colorectal cancer (women), and 0.824 and 0.783-0.865 in breast cancer, respectively. From these results, differentiation between target cases and the control group by binomial logistic regression analysis was possible using the 17 elements plus age-class, and four indicator functions could be calculated from 4 cancer sites as indicators for each tumor to ensure validity by validation analysis using the validation data.

Partial regression coefficient and statistical significance of four indicators obtained by the binomial logistic regression analysis are shown in **Table 3**. In colorectal cancer (men), Na, Mg, Ca, Co, Cu, and Zn were strongly associated with cancer, and S, Rb, and Mo were strongly associated with non-cancer. In prostate cancer, Ca, Fe, Co, and Cu were highly associated with cancer, and S and Rb were strongly associated with non-cancer. In colorectal cancer (women), Na, Co, Cu, and Zn were highly associated with cancer, and S and Rb were strongly associated with non-cancer. In breast cancer, Ca, Fe, Co, and others were highly associated with cancer, and Rb was strongly associated with non-cancer.

In order to verify the four indicators, analysis using the validation data was performed. For cancer, sensitivity was calculated by stage, and for the control group, specificity was calculated. Results are shown in **Figure 3**. For colorectal cancer (men), specificity was 83.4% (196/235), sensitivities for stage 0, stage I, stage II, stage III, and IV were 100.0% (3/3), 75% (21/28) 77.8% (28/36), 80.8% (42/52), and 100.0 (20/20) respectively. For prostate cancer, specificity was 67, 4% (147/218), sensitivities were 100.0% (2/2), 81.5% (22/27), 69.2% (36/52), 72.5% (29/40), 87.5% (14/16), respectively. For colorectal cancer (women), specificity was 89.4% (169/189), sensitivities were 60.0% (3/5), 54.2% (13/24), 83.3% (20/24), 82.1% (23/28), 81.8% (9/11) respectively. In breast cancer, specificity was 67.7% (128/189), sensitivities were 81.8% (18/22), 82.7% (67/81), 75.6% (59/78), and 64.3 (9/14) respectively. There were no stage IV breast cancer patients.

Discussion

Minerals, which contain trace elements, along with proteins, carbohydrates, fats, vitamins and water, compose the six basic nutrients essential for human and animal life. When an element is not sufficiently supplied a deficiency occurs, and when in excess, poisoning symptoms occur. In addition, some elements which are not classified as essential elements of the body are still considered necessary for enzyme activity, especially for substance metabolism and intercellular communication. When trace elements are deficient or in excess, metabolic balance of the body, etc. is lost, and symptoms specific to each element appear. In addition, the composition of trace elements is said to change due to the onset of a disease, poor physical condition or an immune system disorder, and homeostasis (homeostasis) breaks down. Various reports have suggested a link between the biological significance of each element and disease, such as reports on pollution-related illness, anemia [5], type II diabetes [24], cardiovascular disease [25], age-related macular degeneration [26], schizophrenia [9], Alzheimer's disease [27], and a wide range of other health conditions. Many reports have suggested a correlation between trace elements and malignant neoplasms [28]. From this, the possibility of estimating the risk of cancer incidence was examined in this study using a case-control method to compare trace element concentrations in the blood of cancer patients and general healthy persons. In addition to serum and plasma, hair [29,30], saliva [31], nails [32,33], urine [34] have been used as samples for trace element measurement, but blood, which is easy to collect and has standardized handling methods, was used in this study.

Detection methods include ICP-OES (Inductively-Coupled Plasma Optical Emission Spectroscopy), atomic absorption spectrometry (AAS), X-Ray Fluorescence analysis (X-Ray Fluorescence analysis) (XRF) etc., but the ICP-MS (Inductively coupled plasma mass spectrometry) method was used due to its simplicity and because quantitative reliability of the measurement results could be ensured. In order to measure trace elements of a biological sample by ICP-MS, pretreatment in order to dissolve the mixture of proteins, fats, etc. into a homogeneous solution is needed. In general, nitric acid is used and the sample is dissolved by microwaves, and then the solution is used as a reference [35]. However, when microwaves were used, elution (contamination) of trace elements from the container due to high temperature decomposition was confirmed, so a new pretreatment method which did not require a high temperature was developed. (See Methods)

According to analysis of each of the 17 measured elements plus age-class, a significant difference in element concentrations in cancer patients at all cancer sites targeted (**Tables 2a and 2b**) was observed, suggesting a change in the balance of trace elements in the blood, and indicating carcinogenesis.

Na, Mg, Ca, Co, Cu, Zn were observed in high concentrations and were common to three or more cancer sites. Conversely, S, Fe, Rb and Cs were observed in low concentrations in cancer (**Tables 2a and 2b**). Na, Mg, Cu, and Zn are thought to be involved in division, differentiation, and metastasis of cancer cells through actions related to ion channels, ion pumps, and Na-K-ATPase [36]. Trace elements in the blood of cancer patients differ from those in healthy persons. For example, a decrease in Sulphur is thought to occur as large amounts are used as a component in amino acids and proteins in cancer cells and as a catalyst in the metabolism of sugars and fats. According to Al-Awadi, et al., when human cancer cells were transplanted into nude mice, serum cystine and homocysteine decreased [37]. Szabo, et al. reported that H2S derived from colon cancer cells by cystathionine-β-synthase

(CBS) stimulated cell activity (bioenergetics), cell proliferation and angiogenesis [38]. Similarly, other elements are suspected to be involved in cancer cell proliferation by activating energy metabolism and sugar/lipid metabolism through catalysis. In particular, it is reported that many trace elements are involved in angiogenesis [39-41], which is essential for the growth of cancer cells. Analysis of these mechanisms is thought to lead to the development of research in a new field named “Metallomics”.

From the univariate analysis results, it is hypothesized that each trace element is independently involved in cancer development. However, mutual influence may also occur since trace elements may exhibit antagonizing or opposing action. Therefore, analysis using multivariate analysis was carried out to further clarify the distinction between cancer patients and the general public.

The ROC curve and AUC by multivariate discriminant analysis using 18 items of 17 elements plus age class are shown in **Figure 2**. AUC for colorectal cancer (men) was 0.943 (95% CI, 0.920-0.966), 0.842 (0.801-0.882) for prostate cancer, 0.898 (0.861-0.935) for colorectal cancer (women), and 0.824 (0.783-0.865) for breast cancer. With regarding to current FOBT screening methods for colorectal cancer, Dodou et al. [42] calculated AUC=0.802 by 23 meta-analysis reports, and for digital mammography screening methods for breast cancer [43]. According to data analysis, AUC=0.79, and for prostate cancer screening with PSA, Louie et al. [44] reported that AUC=0.71 from six reliable reported meta-analyses. AUC in this report was determined to be high at all cancer sites, and the results of discriminant analysis using trace elements in blood were significant as a new screening method. Four binomial logistic regression equations derived from multivariate analysis using training data were found to be useful in distinguishing between early stage and advanced stage cancer by inputting validation data (**Figure 3**). In particular, sensitivity of 80% or more in stages I and II can be considered effective as a cancer risk screening. These results are considered to indicate that "the balance of trace elements in the blood is largely disrupted from the early stage and onset of cancer”.

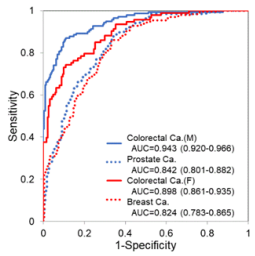

Figure 2: ROC curve and AUC (95% CI) by cancer site.

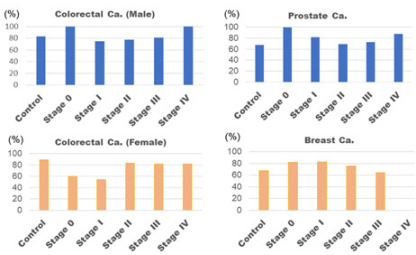

Figure 3: Predicting value (%) when substituting validation data into discriminant derived from training data analysis.

To date, several studies have used multivariate analysis (discriminant analysis or logistic regression analysis) to consider the relationship between trace elements in blood and cancer. Nakayama et al. [45] has shown that multivariate discriminant analysis of metallothionein, Cu and Zn levels in serum of patients with chronic hepatitis, cirrhosis and liver cancer can be classified with 80%-90% sensitivity. Wu et al. [46] examined 13 elements in the blood of 25 breast cancer patients, 43 benign breast cancer patients, and 26 healthy subjects. Co, Ni, and Al were significantly higher concentrations in breast cancer patients. It is reported that Cd, Mn, Fe, Cr and Zn were identified by logistic regression analysis using the stepwise method, and discrimination was possible with a sensitivity of 96% or more. Yasuda et al. [30] performed logistic regression analysis and multiple linear regression analysis using 24 elements from hair of 124 solid cancer patients and 86 healthy persons. As a result, AUC=0. 918 and I, As, Zn, Fe, Na, Se, K and Mn were identified.

Conclusion

These studies were conducted in order to compare cancer patients with healthy persons and to identify elements related to cancer development, and no mention regarding an imbalance of trace elements is mentioned. In this study, all measured elements were used as data for identification, and "the risk of cancer" could be estimated by the difference in the balance. From these results, risk of experiencing colorectal cancer and prostate cancer in men and colorectal cancer and breast cancer in women can be estimated with high probability based on one blood sample.

Currently, research aimed at risk diagnosis for lifestyle related diseases including cancer in other areas are underway, and progress in research for practical use has been made. By placing the results of this research in practical use, risk assessment of lifestyle-related diseases (referred to as Metallo-Balance) is possible. Through annual blood collection to monitor serum trace elements, health support for many persons is possible. In addition, improvement in risk management is expected to increase public awareness and wellness.

Acknowledgements

This study was conducted together with Chiba Cancer Center, Kanagawa Cancer Center and RENATECH Co., Ltd. by the support of the Ministry of Economy, Trade, and Industry of Japan (Accepted number 2831412036). For blood specimen of controls, we gained support from the Platform of Supporting Cohort Study and Biospecimen Analysis, J-MICC Research, (Kenji Wakai, Chief investigator, Professor of Preventive Medicine, Nagoya University Graduate School of Medicine) of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Grants

The support of the Ministry of Economy, Trade, and Industry of Japan (Accepted number 2831412036)

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The authors declare no conflicts of interest associated with this manuscript.

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