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RESEARCH

Serum elementomic analysis indicates a panel of elements related with age

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Abstract

Background: Elementomics, which includes metallic and non-metallic elements, is an emerging and promising research field for human diseases. Researchers are focusing on discovering the relationship between elements and various diseases; however, the changes in element concentrations during the process of aging remain unclear.

Materials and Methods: We performed elementomic analysis in the serum of 70 subjects aged 30 to 96 years using inductively coupled plasma mass spectrometry. The subjects were divided into 7 groups with an age range of 10 years. Random forest was used to estimate the variable importance of elements. Linear regression model and restricted cubic spline analysis were performed to screen for elements individually associated with age. Candidate elements were combined by corresponding multivariate linear regression coefficients to generate a risk score representing their collective effect on age.

Results: Among the 62 detected elements, lithium, boron, calcium, titanium, and selenium were identified as the most important predictors of age. There was an increase in lithium and boron as well as a decrease in calcium and titanium with increasing age. The concentration of selenium was elevated before the age of 60, but decreased thereafter. A formula of element risk score was constructed using the respective coefficients from a multivariate linear regression model for the above five elements. The formula = 4.5522 × lithium + 6.0575 × boron - 4.9990 × calcium - 7.0403 × titanium - 0.8849 × selenium.

Conclusion: Elementomics could be a novel and promising non-invasive biomarker for the assessment of senescence.

Keywords: Elementomics, serum, age, inductively coupled plasma mass spectrometry

Introduction

Aging is an inevitable natural phenomenon characterized by a gradual, time-dependent decline in normal physiological functions [1]. It is predicted that by 2050, the elderly population in China will experience a significant surge, reaching up to 400 million individuals aged over 65 years and 150 million individuals aged over 80 years [2, 3]. Biomarkers of aging are quantifiable parameters that reflect both physiological and pathological aging [4]. To better understand the mechanisms of aging, recent studies have focused on the search for novel biomarkers of aging. In addition to the identified aging-related substances and metabolites, such as total homocysteine in blood and 8-dihydroguanosine in urine [5, 6], there has also been a growing interest in investigating the effects of elements in the progression of aging.

Elementomics is the study of metals, metalloids and their relationships with genomes and proteomes [7]. Metal ions play an important role in regulating protein expression in cells, which is essential for maintaining cellular homeostasis and facilitating detoxification processes [8]. Additionally, a substantial number of protein enzymes rely on metal elements to facilitate their catalytic activities [9]. Current perspectives on elements and disease are mainly concerned with cancer, Alzheimer's disease, Parkinson's

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disease, cardiovascular disease, *etc*. [10]. Patients with Huntington's disease have been found to have elevated circulating levels of iron, chromium, selenium, zinc, and arsenic, as well as decreased levels of antimony, lead, and vanadium [11]. Zinc levels are decreased and cadmium levels are increased in patients with prostate cancer [12-14]. In the cardiovascular system, iron and calcium deficiencies have been identified to play a critical role in the development of heart failure [15]. Previous studies have found that the levels of several elements are increased in the aorta, blood, and other tissues of aged rats compared with young rats [16-18]. Meanwhile, age-related differences in elements have also been observed in the elderly population [19, 20]. However, few studies have investigated the relationship between elementomics and the process of aging. The use of inductively coupled plasma mass spectrometry (ICP-MS) allows the rapid and accurate measurement of more than 60 elements simultaneously [21, 22]. In the present study, we aimed to explore the changes in blood element concentrations in different age groups.

Materials and Methods

Study population

The study population consisted of 70 subjects, aged 30 to 96 years, recruited from the Health Examination Center of Sir Run Run Hospital, Nanjing Medical University. The subjects were divided into 7 groups as a 10-year age range, with 5 males and 5 females in each group. Participants with the following conditions were excluded: (1) acute cardiopulmonary insufficiency; (2) severe renal insufficiency (creatinine clearance rate < 60 mL/min) or severe liver damage (transaminase levels more than twice the normal values); (3) malignant tumors; (4) gastrointestinal diseases; (5) acute or chronic inflammatory diseases; (6) eating disorders or malnutrition; (7) taking any kind of dietary supplements or trace elements before enrollment or during the study; (8) occupationally exposed to pollution or living in a polluted environment. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Sir Run Run Hospital, Nanjing Medical University (approval number 2019-SR-S041). Written informed consent was obtained from each participant.

Laboratory measurements

For hematologic and biochemical parameters, peripheral venous blood samples were collected after at least 8 hours of fasting. Blood samples were centrifuged at 3000 g for 10 minutes to separate the blood into serum and cell fractions within 2 hours. Serum samples were collected and stored at -80°C prior to further analysis. Fasting blood glucose (FBG), alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBil), serum creatinine (SCr), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), albumin (ALB), and globulin (GLOB) were measured using a chemistry analyzer (Olympus AU5400, Chemical Ltd., Japan).

Exposure assessment

Samples were thawed at 4℃ for approximately 1 hour, and then 100 μL of serum was diluted to 1.6 mL with 1% (v/v) HNO_3, internal standards (including 40 ppb lithium, 20 ppb rhodium, 20 ppb indium, and 20 ppb rhenium), and 18 Ω deionized water. For the blank diluent, 100 μL of 18 Ω deionized water was used to replace the sample serum. Sixty-two elements in the serum were analyzed using the iCAP Qc ICP-MS system (Agilent 7700x ICP-MS, USA) of Shanghai Biotree Biotech Co., Ltd. The response of the calibration curve to the measured concentration was evaluated for goodness of fit. The R values of the correlation coefficients of the regression equations are all greater than 0.99, except for Ge, which was 0.98 (Table S1). The limit of detection (LOD) was calculated based on the average of 10 consecutive measurements of the blank diluent. Concentrations of elements below the LOD were imputed as LOD/2. Elements with a detection rate of less than 50% (wolfram and samarium) were categorized as "undetectable" (Table S2). Coefficients of variation varied with limits around 10% and met the required standards. All concentrations showed a right-skewed distribution and were loge transformed prior to statistical analysis.

Statistical analysis

Normality of distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation or median [interquartile range (IQR)]. Differences between age groups were determined using the ANOVA test or the Kruskal-Wallis test for continuous variables, depending on the data distribution. The chi-squared test was used to compare categorical variables presented as frequencies. To identify potential binary covariates, the enrolled participants were divided into two groups according to sex, alcohol consumption, smoking, hypertension, and cerebral infarction. Student t-test was used to compare the difference in age between the two groups. Conditional permutation for variable importance via random forest analysis was used to identify important elements most strongly associated with chronological age. Variables were ranked in descending order based on the importance score with higher scores indicating greater importance. A sliding window of nested random forest was used to calculate out-of-bag (OOB) errors. The element model with the minimum OOB error corresponded to a certain number of elements, which were selected for further analysis. Multivariate linear regression analysis was then performed to explore the potential linear relationship between the concentration of each element and age. Beta and 95% confidence intervals (CIs) were calculated. To further assess the potential nonlinear relationship of individual elements with age, restricted cubic spline was performed adjusting for covariates with 3 knots at the 25th, 50th, and 75th percentiles, with the value at the 50th percentile used as the reference. False discovery

Table 1. Characteristics of the enrolled participants.

Variables	31–40 years old (*n* = 10)	41–50 years old (*n* = 10)	51–60 years old (*n* = 10)	61–70 years old (*n* = 10)	71–80 years old (*n* = 10)	81–90 years old (*n* = 10)	91–100 years old (*n* = 10)	*P*
Age, years	38.0 (32.8–38.3)	47.0 (45.8–47.3)	54.5 (51.0–57.3)	64.5 (63.0–66.5)	74.0 (72.0–76.3)	83.5 (81.8–84.0)	92.5 (91.0–94.3)	0.000
Male, *n* (%)	5 (50.0)	5 (50.0)	5 (50.0)	5 (50.0)	5 (50.0)	5 (50.0)	5 (50.0)	1.000
BMI, kg/m^2	24.2 ± 2.9	22.9 ± 1.4	23.1 ± 2.5	22.9 ± 4.0	25.3 ± 2.5	22.1 ± 1.6	23.6 ± 4.3	0.275
Smokers, *n* (%)	2 (20.0)	2 (20.0)	2 (20.0)	2 (20.0)	0 (0)	1 (10.0)	0 (0)	0.564
Drinkers, *n* (%)	3 (30.0)	1 (10.0)	2 (20.0)	0 (0)	0 (0)	1 (10.0)	1 (10.0)	0.342
Hypertension, *n* (%)	1 (10.0)	2 (20.0)	4 (40.0)	3 (30.0)	6 (60.0)	6 (60.0)	9 (90.0)	0.005
Cerebral infarction, *n* (%)	1 (10.0)	2 (20.0)	2 (20.0)	4 (40.0)	7 (70.0)	7 (70.0)	6 (60.0)	0.014
FBG, mmol/L	5.02 ± 0.34	4.88 ± 0.59	5.35 ± 0.75	5.19 ± 0.67	5.27 ± 0.95	5.14 ± 0.95	4.73 ± 0.34	0.416
ALT, U/L	18.5 (12.5–25.5)	15.1 (13.4–21.6)	13.2 (11.2–24.7)	15.8 (12.7–22.6)	13.4 (11.6–16.7)	15.7 (10.0–22.7)	13.1 (9.0–20.5)	0.741
AST, UL	19.8 (16.0–30.1)	18.3 (15.8–22.5)	18.1 (15.0–20.9)	19.8 (18.0–23.2)	18.7 (15.8–21.4)	20.8 (17.3–26.2)	23.8 (18.0–28.6)	0.311
TBil, μmolL	7.7 ± 2.8	13.1 ± 3.6	11.6 ± 5.5	11.0 ± 5.0	10.9 ± 5.2	9.5 ± 6.1	10.8 ± 3.2	0.255
SCr, μmol/L	59.6 ± 13.1	63.9 ± 12.4	63.9 ± 12.3	60.5 ± 9.6	70.0 ± 15.7	78.2 ± 22.4	70.5 ± 17.6	0.099
BUN, mmol/L	4.49 ± 1.28	5.04 ± 1.25	5.04 ± 1.31	4.93 ± 0.61	5.94 ± 1.01	6.01 ± 1.77	5.07 ± 1.88	0.148
TP, g/L	77.5 (71.4–78.7)	69.1 (65.9–76.3)	72.9 (64.4–80.0)	68.9 (63.8–78.6)	71.5 (65.0–79.0)	64.4 (62.5–70.1)	67.1 (64.8–70.8)	0.106
ALB, g/L	49.2 (47.9–50.8)	47.1 (42.4–48.5)	45.0 (40.8–50.9)	44.7 (41.0–50.2)	45.3 (42.9–46.4)	40.2 (37.2–41.9)	38.8 (34.8–41.5)	0.000
GLOB, g/L	28.6 (23.9–29.6)	22.7 (20.4–25.4)	29.6 (27.8–40.8)	25.2 (21.2–28.2)	26.5 (23.4–29.1)	26.5 (23.7–29.2)	29.8 (24.3–32.7)	0.008
A/G	1.70 (1.68–1.88)	1.91 (1.78–2.23)	1.80 (1.50–2.05)	1.85 (1.60–2.11)	1.70 (1.58–1.85)	1.55 (1.42–1.71)	1.25 (1.18–1.53)	0.000
TC, mmol/L	4.38 (4.03–5.22)	4.55 (4.24–4.96)	5.25 (4.93–5.66)	5.01 (3.97–6.42)	4.52 (4.08–5.18)	4.36 (4.11–4.66)	3.91 (3.22–4.44)	0.018
TG, mmol/L	1.22 (0.83–3.24)	1.08 (0.70–1.53)	1.11 (0.97–2.15)	1.43 (1.08–1.96)	1.01 (0.86–1.68)	0.82 (0.73–1.28)	0.99 (0.77–1.22)	0.159
LDL-C, mmol/L	2.75 ± 0.71	2.91 ± 0.57	2.88 ± 0.68	2.81 ± 0.67	2.75 ± 0.57	2.44 ± 0.61	2.24 ± 0.60	0.183
HDL-C, mmol/L	1.51 ± 0.54	1.54 ± 0.49	1.87 ± 0.77	1.61 ± 0.62	1.53 ± 0.16	1.49 ± 0.21	1.37 ± 0.39	0.454
UA, μmol/L	316.2 (251.5–363.2)	283.0 (209.3–361.4)	337.8 (272.7–360.4)	247.3 (228.9–301.1)	299.5 (233.9–390.3)	273.2 (244.8–334.6)	306.6 (246.7–349.2)	0.628
WBC, 10^9 cells/L	5.45 (5.16–6.76)	5.31 (4.35–6.11)	5.35 (4.55–5.80)	5.14 (4.40–7.12)	5.00 (4.60–6.13)	5.86 (4.02–7.07)	5.45 (4.23–7.19)	0.911
RBC, 10^9 cells/L	4.60 (4.03–4.95)	4.57 (4.21–4.68)	4.41 (4.13–5.05)	4.72 (4.55–4.84)	4.78 (4.21–4.95)	3.94 (3.52–4.31)	3.82 (3.67–4.05)	0.001
HCT, L/L	40.9 ± 3.6	41.2 ± 1.9	41.2 ± 5.0	41.9 ± 3.0	41.6 ± 3.4	37.0 ± 4.7	35.9 ± 2.4	0.001
Hb, g/L	137.5 (127.0–150.3)	135.0 (130.8–144.5)	142.5 (131.5–148.3)	138.5 (127.3–141.5)	141.5 (124.5–148.5)	119.0 (109.5–132.5)	121.0 (117.8–127.3)	0.005
PLT, 10^9 cells/L	239.7 ± 49.1	187.3 ± 44.9	216.0 ± 75.5	224.1 ± 43.0	174.9 ± 55.8	189.7 ± 50.4	194.1 ± 50.2	0.097
MONO, 10^9 cells/L	0.34 ± 0.07	0.33 ± 0.07	0.36 ± 0.11	0.40 ± 0.08	0.35 ± 0.07	0.44 ± 0.11	0.61 ± 0.23	0.000
MONO, %	5.85 (5.33–6.90)	6.80 (5.45–7.25)	6.11 (5.48–7.51)	7.20 (6.02–8.51)	6.85 (6.28–7.41)	7.30 (6.78–9.73)	9.90 (9.28–11.10)	0.000
LYMPH, 10^9 cells/L	1.83 ± 0.34	1.84 ± 0.42	1.94 ± 0.82	1.72 ± 0.55	1.49 ± 0.41	1.42 ± 0.66	1.45 ± 0.61	0.209
LYMPH, %	32.4 ± 7.3	35.5 ± 5.1	34.1 ± 8.0	31. 3± 9.6	29.2 ± 6.5	25.4 ± 7.6	25.5 ± 8.1	0.018

Note: A/G = albumin-globulin ratio; ALB = albumin; ALT = alanine transaminase; AST = aspartate aminotransferase; ASMI = appendicular skeletal muscle mass index; BMI = body mass index; BUN = blood urea nitrogen; CA-199 = carbohydrate antigen 199; FBG = fasting blood glucose; GLOB = globulin; Hb = hemoglobin; HCT= hematocrit; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; LYMPH = lymphocyte; MONO = monocyte; PLT = platelet; RBC = red blood cell; SCr = serum creatinine; TBil = total bilirubin; TC = total cholesterol; TG = triglyceride; TP = total protein; UA = uric acid; WBC = white blood cell.

rate (FDR) was used to control for multiple comparisons, and *P* after FDR ≤ 0.05 was considered statistically significant. Pairwise interaction analyses of linear regression were performed to examine the correlations among 5 elements. Pearson correlation analysis was used to evaluate the relationships between selected elemental concentrations and laboratory indices. We also constructed an elemental risk score (ERS) to develop a potential approach for assessing elemental age. The ERS was calculated as the sum of the standardized concentrations of the selected elements multiplied by their respective coefficients. The formula was ERS = $\sum_{K=1}^{K} \beta_k E_k$, where β_k denotes the respective coefficient, E_k denotes the element standardized concentrations, and K denotes the number of elements. Statistical analyses were performed using R software, version 3.5.1 (The R Foundation for Statistical Computing) and SPSS 22.0 (IBM, Chicago, USA).

Results

Study participant characteristics

The demographic characteristics of the study population are shown in Table 1. Of the 70 participants, 35 (50.0%) were men and 35 (50.0%) were women. The mean age was 65.5 years and the median age was 65.2 years. There were no significant differences in sex, body mass index (BMI), smoking, and alcohol consumption among the different age groups. In contrast, the prevalence of hypertension and cerebral infarction was higher in subjects older than 70 years. Regarding biochemical parameters, the levels of ALB, A/G, and TC were lower in subjects older than 70 years. In addition, there were significant decreases in red blood cell (RBC), hematocrit (HCT), hemoglobin (Hb), and lymphocyte percentage (LYMPH) in subjects older than 80 years.

Elementomics profiling

We quantified 62 elements by ICP-MS, including 5 alkali metals, 5 alkaline earth metals, 5 metalloids, 24 transition metals, 6 post-transition metals, 14 lanthanoids, 2 actinoids, and 1 other nonmetal (Figure 1A). The distributions of elemental exposure levels and the heatmap of different age groups are shown in Table S2 and Figure S1, respectively. OOB error analysis showed the top 13 elements that might be associated with age (Figure 1B-C). Further linear regression analysis and restricted cubic spline

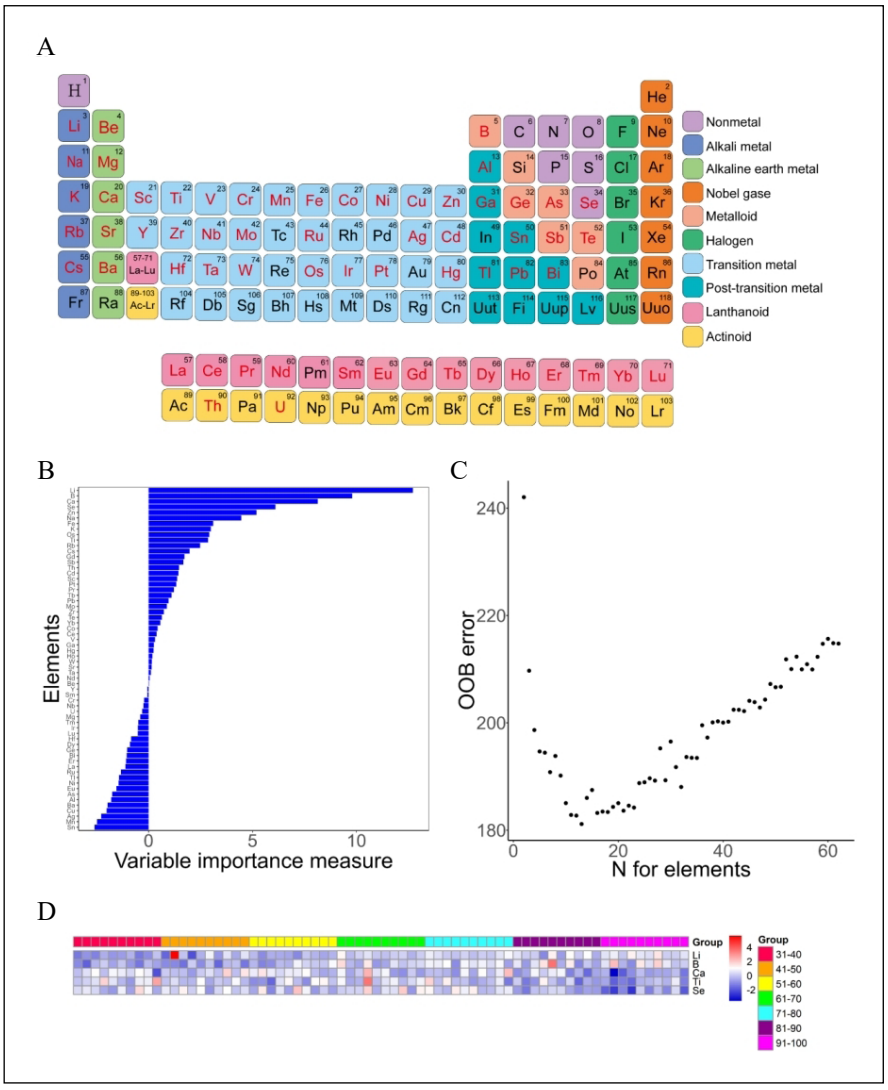

Figure 1. Periodic table of elements and elements identified by random forest. (**A**) Periodic table of elements, with detected elements marked in red. (**B**) Elements identified by random forest. The bar chart illustrates the variable importance measures of 62 elements. (**C**) The scatter plot illustrates the out-of-bag errors of the detected elements. The minimum OOB error corresponds to the first 13 elements. (**D**) Heatmap of 5 selected elements in different age groups.

analysis identified 5 age-associated elements, including lithium (Li) [β (95 %CI) = 0.448 (14.02, 36.32), $P < 0.001$, P_{linear} FDR < 0.001], boron (B) [β (95 %CI) = 0.289 (3.04, 20.02), $P = 0.009$, P_{linear} FDR = 0.022], calcium (Ca) [β (95 %CI) = -0.271 (-189.72, -30.69), $P = 0.007$, P_{linear} FDR = 0.022], titanium (Ti) [β (95 %CI) = -0.332 (-49.44, -12.11), $P = 0.002$, P_{linear} FDR = 0.010] and selenium (Se) [β (95 %CI) = -0.296 (-38.12, -8.06), $P = 0.003$, $P_{nonlinear}$ FDR = 0.008] (Table S3). With increasing age, the levels of Li and B increased, whereas the levels of Ca and Ti decreased (Figure 1D and Figure 2A-D). Interestingly, the concentration of Se was elevated before 60 years of age, but decreased thereafter (Figure 1D and Figure 2E). Concentrations of 5 identified elements in different age groups are presented (Table S4). The interaction analysis was performed to explore the possible interactive effects of Li, B, Ca, Ti, Se on the association with age. However, no significant correlation was observed among the above five elements (Table S5).

Correlation between candidate elements and laboratory measurements

Pearson correlation analysis showed that the concentration of serum Ca was positively correlated with the levels of total protein ($r = 0.584$, $P = 0.000$), ALB ($r = 0.551$, $P = 0.001$). The concentration of serum Se was positively correlated with the level of HCT ($r = 0.472$, $P = 0.020$) (Table S6).

Construction of ERS

After fitting a multivariate linear regression between age and 5 candidate elements, the age prediction formula was established as the ERS. The ERS was calculated as the sum of the standardized concentrations of the selected elements multiplied by their respective coefficients (Table S7) as follows: ERS=4.5522 × Li + 6.0575 × B - 4.9990 × Ca - 7.0403 × Ti - 0.8849 × Se, which means that higher ERS represents older age.

Discussion

The identification of reliable biomarkers of aging is one of the major research goals in geroscience. Although numerous studies have investigated the role of age-related biomarkers in degenerative diseases [23], the relationship between elementomics and senescence has received little attention. To address this gap, our study measured comprehensive serum elemental exposure profiles to investigate the association of elemental changes with age.

Aging is an inevitable process that affects all organisms over time and occurs at multiple levels, including the molecular, cellular, organ, and organismal levels [24]. It has long been accepted that aging can be attributed to the accumulation of reactive oxygen species (ROS), DNA damage, mitochondrial dysfunction, impaired antioxidant

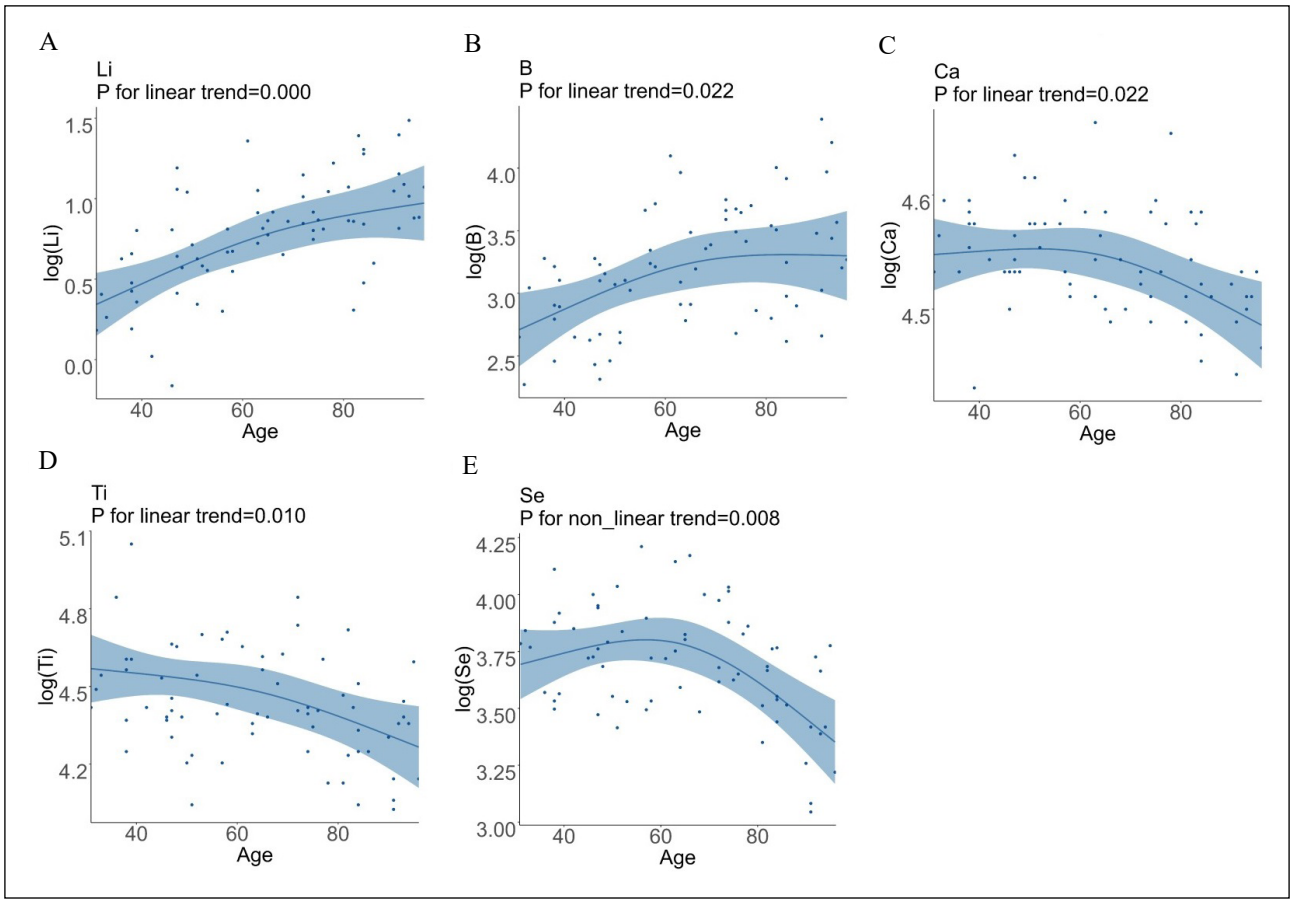

Figure 2. Relationship between age and concentrations of 5 identified elements. Multiple linear regression graphs and restricted cubic spline curves were used to analyze the correlation between age and concentration of Li (**A**), B (**B**), Ca (**C**), Ti (**D**), and Se (**E**).

defense, damaged autophagy process, loss of proteostasis, and telomere shortening, *etc*. [25]. During the aging process, trace and macroelements, which are critical regulators of metabolic and physiological pathways, are altered and can influence oxidative and inflammatory processes [23, 26]. For example, some trace elements (such as Se, Zn, Mn) have the ability to reduce oxidative damage or enhance repair capacity by acting as essential cofactors for antioxidant enzymes and the different types of glutathione peroxidases, and therefore may play a crucial role in the aging process [27, 28]. Our study identified 5 elements as the most important predictors of chronological age. Our analyses showed that 4 elements had a linear relationship with age. Serum concentrations of Li and B appeared to be positively associated with increasing age, while Ca and Ti showed a decrease with age. Se levels were elevated before 60 years of age and showed a decline thereafter. The highlight of this study is that we constructed an integrated predictive model including Li, B, Ca, Ti, and Se to generate ERS, which could potentially serve as a tool for predicting senility.

Essential trace elements and major elements play an indispensable role in maintaining human health by participating in various metabolic processes and signaling pathways [29]. Among these elements, Se is a trace element essential for several metabolic processes, including thyroid hormone metabolism, antioxidant defense systems, and immune function [30]. Se is absorbed in the form of organic (selenomethionine and selenocysteine) or inorganic (selenate and selenite) by erythrocytes from the gastrointestinal tract and subsequently transported by plasma to tissues and cells [31]. A previous study showed that serum selenium levels were positively correlated with hematocrit (P <0.001, r = 0.215) in 1000 older adults [32]. Consistently, we found that Se concentration was positively correlated with the level of HCT, suggesting that the absorption of Se decreases with aging. Our results showed that Se concentration was increased before 60 years of age, but decreased thereafter. One study reported that centenarians (91-110 years) had lower Se levels compared with elderly subjects (60-90 years) [33]. However, another study showed that centenarians (≥ 100 years) had much higher Se levels compared with elderly controls [34]. The discrepancy may be attributed to the different comorbidity status between different study populations, as decreased Se levels have been associated with cognitive decline and hyperglycemia in the elderly [35, 36]. Interestingly, a previous study found that Se concentration was increased in the thyroids of old men, accompanied by a decrease in B and Li, indicating a redistribution of trace elements with aging [37]. Therefore, these results may indicate the importance of Se supplementation at an appropriate age with an appropriate dose. Further studies are needed to explore the exact effects and the underlying mechanism of Se on the aging process.

Similar to Se, B is absorbed from the gastrointestinal tract and excreted in the urine [38]. Boron has been shown to be beneficial for bone growth and maintenance, central nervous system function, and regulation of inflammatory response [39]. Our study found a positive correlation between aging and serum B concentration. In men, serum B increased and reached a plateau between 50 and 69 years of age, followed by a gradual increase after 70 years of age. In women, a gradual increase in serum B concentration was observed up to the age of 70 years. Our results were consistent with previous studies showing a positive correlation between age and blood B levels [40, 41]. Although studies have shown some benefits of higher B status in the immune system, embryonic development, brain function, liver development, osteoporosis, cancer therapy, and wound healing, high-dose boron showed opposite effects [38]. Therefore, additional data are needed to elucidate the appropriate supplemental amount of B for human health as well as its mechanism of action.

Li has been used for decades to treat mood disorders, including bipolar disorder, anxiety, and depression [42]. A previous study in a Japanese population found that the concentration of Li in drinking water was associated with lower mortality [43]. Here, we showed that Li levels decreased with increasing age. Consistent with this, another study reported that younger subjects (mean age = 13.4 years) had a lower brain-to-serum Li concentration ratio than adults (mean age = 37.3 years) with bipolar disorder, indicating a decrease in serum Li in the adults [44]. Recent preclinical studies have shown that lithium can protect against various forms of oxidative and xenobiotic stress, thereby extending the lifespan of fission yeast, *C. elegans*, and *drosophila* [45-49]. However, the precise effects of Li as a biomarker and intervention for human aging need to be further confirmed.

As a heavy metal, Ti can enter the bloodstream from the respiratory and gastrointestinal tracts, as well as from titanium-containing implants [50]. Once in the blood, Ti quickly dissociates and transforms into titanium dioxide, which is then transported to various tissues throughout the body by a protein called serum transferrin [51]. Within cells, Ti can increase the production of ROS, leading to oxidative stress, apoptosis, and the induction of inflammation [52]. Accumulation of Ti in the nervous system may contribute to pathological damage and the development of degenerative diseases, including Alzheimer's disease and Parkinson's disease [53, 54]. In aged rats, titanium dioxide nanoparticles promoted the disruption of the blood-brain barrier, resulting in neuronal dysfunction and cognitive impairment, suggesting a detrimental effect of increased Ti on the development of age-related diseases [55]. However, here we found that Ti levels decreased with increasing age. In the body, Ti is rapidly eliminated from the blood and accumulates in tissues with a highly perfused reticuloendothelial system, such as the liver and spleen [56-58]. Therefore, we hypothesized that decreased circulating Ti concentration may be attributed to increased ectopic accumulation of Ti in aging organs such as the brain. Future studies are needed to investigate the distribution of Ti in the body and its effects on the function of different organs during the aging process.

Ca plays a vital role in skeletal and muscle maintenance, hormone secretion, nerve impulse transmission, and vas-

cular integrity and activity [59]. Ca is mainly absorbed in the small intestine, and calcium homeostasis is maintained by parathyroid hormone and calcitonin [60]. Ca^{2+} serves as a multifunctional second messenger that fine-tunes a variety of physiological and pathological processes within the cell [61]. Changes in Ca levels and the Ca^{2+} signaling pathway have also been associated with accelerated aging [61]. A variety of senescence stimuli (oxidative stress, telomere attrition, genotoxic agents, *etc*.) can stimulate Ca^{2+} influx through cell membrane calcium channels [62-64]; however, along with Ca^{2+} overload, inefficient coupling of mitochondrial respiration can result in the production of excess reactive oxygen species, leading to mitochondrial damage, DNA damage, and ultimately apoptosis [63]. Several studies have suggested that serum $1,25(OH)_2D$ levels decrease with age, accompanied by impaired Ca absorption, leading to a defect in intestinal Ca absorption [65-67], which may explain our finding of decreased serum Ca levels with age. In addition, our finding of a positive correlation between calcium concentration and total protein as well as serum albumin is consistent with previous studies [68]. Calcium supplementation has been recommended for the prevention of age-related diseases such as osteoporosis [69]. However, the adverse effects of calcium supplementation have also been reported, especially the formation of kidney stones [69]. Therefore, further studies are needed to explore the appropriate dosage of Ca that may have the ability of anti-aging.

There are several potential limitations to this study. First, the number of subjects was insufficient and limited to the area of Jiangsu Province, which may limit the universality of the study results. To improve the quality of research, it is recommended to increase the sample size and recruit people from different locations for further study. Second, our study did not take into account the dietary structure, eating habits, nutritional status, and drinking water source of the participants, which may lead to relatively inaccurate results. Therefore, a more comprehensive further study is needed to confirm our findings. Third, it was difficult to exclude the possibility of causality bias due to the cross-sectional nature of this study. In addition, due to the complex biological and molecular mechanisms involved in aging, it may be difficult to identify a single biomarker as a valid measure of healthy aging. Therefore, it is of utmost importance to conduct prospective studies to validate the effect of our ERS model in reflecting aging. Finally, our findings were not externally validated. The ERS needs to be validated by future well-designed experimental studies before it can be used as an indicator of aging in other studies.

Conclusions

This study identified five age-related elements in serum and constructed an ERS that may reflect the chronological age. Our findings emphasize the importance of assessing the changes in elements as well as their effects on the aging process.

Declarations

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Author Contributions: Wei Gao and Xiang Lu conceived and designed the study. Kai-Yue Jia and Yan-Ru Li carried out the experiments. Hui-Xian Sun and Can Zhao analyzed and interpreted the data. Kai-Yue Jia and Hui-Xian Sun drafted the manuscript. Wei Gao, Xiang Lu, and Yan-Ru Li revised the manuscript. All authors read and approved the final version of the manuscript.

Data availability: The data will be available from the corresponding author upon reasonable request.

Ethics approval: The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Sir Run Run Hospital, Nanjing Medical University (approval number 2019-SR-S041).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

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Table S1. Correlation coefficients in calibration curves.

Element	Abbreviation	R	Element	Abbreviation	R	Element	Abbreviation	R
Lithium	7Li	0.9999	Selenium	78Se	0.9993	Gadolinium	157Gd	0.9999
Beryllium	9Be	0.9994	Rubidium	85Rb	0.9998	Terbium	159Tb	0.9999
Boron	11B	0.9996	Strontium	88Sr	0.9995	Dysprosium	163Dy	0.9999
Sodium	23Na	0.9989	Yttrium	89Y	0.9988	Holmium	165Ho	0.9998
Magnesium	24Mg	0.9990	Zirconium	90Zr	0.9995	Erbium	166Er	0.9997
Aluminum	27Al	0.9991	Niobium	93Nb	0.9813	Thulium	169Tm	0.9998
Potassium	39K	0.9999	Molybdenum	95Mo	0.9994	Ytterbium	172Yb	0.9999
Calcium	44Ca	0.9994	Ruthenium	101Ru	1	Lutetium	175Lu	0.9998
Scandium	45Sc	0.9995	Silver	107Ag	0.9999	Hafnium	178Hf	1
Titanium	47Ti	0.9996	Cadmium	111Cd	0.9990	Tantalum	181Ta	0.9982
Vanadium	51V	0.9986	Tin	118Sn	0.9999	Wolfram	182W	0.9998
Chromium	52Cr	0.9998	Antimony	121Sb	0.9997	Osmium	189Os	1
Manganese	55Mn	0.9998	Tellurium	125Te	1	Iridium	193Ir	1
Iron	56Fe	0.9995	Cesium	133Cs	1	Platinum	195Pt	1
Cobalt	59Co	0.9996	Barium	137Ba	0.9998	Hydrargyrum	202Hg	0.9996
Nickel	60Ni	1	Lanthanum	139La	0.9999	Thallium	205Tl	0.9994
Copper	63Cu	1	Cerium	140Ce	0.9999	Lead	208Pb	0.9999
Zinc	66Zn	0.9996	Praseodymium	141Pr	0.9999	Bismuth	209Bi	0.9998
Gallium	71Ga	0.9999	Neodymium	146Nd	0.9999	Thorium	232Th	1
Germanium	72Ge	0.9784	Samarium	147Sm	0.9999	Uranium	238U	1
Arsenic	75As	0.9999	Europium	151Eu	0.9999			

Table S2. Quality control and statistical summary of element exposure levels in serum samples.

Abbreviation	Unit	LOD	*N* (%) of Conc.<LOD	RSD (%)	Median (Q1–Q3)
7Li	μg/L	0.0004	0	1.25	2.255 (1.785-2.843)
9Be	μg/L	0.0005	5 (7.14%)	1.17	0.020 (0.010-0.030)
11B	μg/L	0.4200	0	0.73	24.720 (17.238-34.703)
23Na	mg/L	0.3400	0	0.05	3260.000 (3167.000-3353.250)
24Mg	μg/L	0.0220	0	0.09	20883.500 (20148.250-21661.750)
27Al	μg/L	0.0280	0	1.71	116.210 (85.750-213.000)
39K	mg/L	1.7000	0	0.70	310.320 (173.752-513.530)
44Ca	mg/L	0.8100	0	0.05	93.331 (91.214-97.321)
45Sc	μg/L	0.0010	0	1.42	1.115 (0.708-1.693)
47Ti	μg/L	0.0140	0	0.25	82.314 (74.110-99.251)
51V	μg/L	0.0015	0	0.27	4.795 (3.663-5.913)
52Cr	μg/L	0.0110	0	1.42	2.385 (1.685-3.483)
55Mn	μg/L	0.0031	0	0.62	14.350 (6.508-21.581)
56Fe	μg/L	0.0810	0	0.39	1199.531 (827.243-1493.250)
59Co	μg/L	0.0006	1 (1.42%)	0.55	0.171 (0.118-0.232)
60Ni	μg/L	0.0030	0	0.60	3.195 (2.703-4.185)
65Cu	μg/L	0.0055	0	0.18	997.521 (937.253-1120.511)
66Zn	μg/L	0.1500	0	0.37	917.21 (794.510-1070.250)
71Ga	μg/L	0.0005	2 (2.86%)	0.77	0.050 (0.038-0.091)
72Ge	μg/L	0.0055	9 (12.85%)	0.87	0.033 (0.010-0.060)
75As	μg/L	0.0025	8 (11.42%)	1.02	0.432 (0.128-0.815)
78Se	μg/L	0.2500	0	0.24	41.322 (34.175-47.125)
85Rb	μg/L	0.0010	0	0.60	285.511 (182.752-419.250)
86Sr	μg/L	0.0008	0	0.24	42.623 (36.721-47.950)
89Y	μg/L	0.0002	6 (8.57%)	0.97	19.422 (0.310-28.051)
90Zr	μg/L	0.0004	0	0.81	8.951 (3.420-17.230)
93Nb	μg/L	0.0017	0	0.73	0.620 (0.408-0.913)
95Mo	μg/L	0.0150	0	0.39	2.121 (1.623-2.725)
101Ru	μg/L	0.0013	7 (10.00%)	0.77	0.020 (0.010-0.030)

Continued

Abbreviation	Unit	LOD	*N* (%) of Conc.<LOD	RSD (%)	Median (Q1–Q3)
107Ag	μg/L	0.0010	5 (7.14%)	2.06	0.035 (0.021-0.080)
111Cd	μg/L	0.0010	1 (1.42%)	0.62	0.070 (0.051-0.103)
118Sn	μg/L	0.0046	5 (7.14%)	1.45	0.371 (0.132-3.695)
121Sb	μg/L	0.0007	0	0.41	4.923 (3.678-6.118)
125Te	μg/L	0.0300	12 (17.14%)	0.89	0.515 (0.121-0.901)
133Cs	μg/L	0.0001	0	0.37	0.992 (0.831-1.293)
137Ba	μg/L	0.0019	0	0.71	5.661 (4.150-6.821)
139La	μg/L	0.0002	0	0.40	0.175 (0.148-0.223)
140Ce	μg/L	0.0003	0	0.39	0.312 (0.241-0.380)
141Pr	μg/L	0.0001	2 (2.86%)	0.47	0.040 (0.030-0.051)
146Nd	μg/L	0.0003	0	0.44	0.145 (0.108-0.18)
147Sm	μg/L	0.0003	38 (64.29%)	1.53	0 (0-0.020)
151Eu	μg/L	0.0001	10 (14.29%)	0.78	0.010 (0.010-0.020)
157Gd	μg/L	0.0003	2 (2.86%)	0.56	0.080 (0.050-0.120)
159Tb	μg/L	0.0001	8 (11.43%)	0.72	0.010 (0.010-0.020)
163Dy	μg/L	0.0002	2 (2.86%)	0.67	0.065 (0.040-0.113)
165Ho	μg/L	0.0001	7 (10.00%)	0.74	0.020 (0.011-0.030)
166Er	μg/L	0.0004	5 (7.14%)	0.67	0.050 (0.030-0.071)
169Tm	μg/L	0.0025	19 (27.14%)	0.93	0.010 (0.001-0.011)
172Yb	μg/L	0.0002	1 (1.42%)	0.63	0.040 (0.031-0.070)
175Lu	μg/L	0.0002	34 (48.57%)	1.28	0.011 (0-0.012)
178Hf	μg/L	0.0001	0	0.86	2.580 (1.908-4.055)
181Ta	μg/L	0.0008	0	0.52	0.181 (0.140-0.255)
182W	μg/L	0.0030	48 (68.57%)	2.77	0.002 (0.002-0.343)
189Os	μg/L	0.0004	0	2.08	0.025 (0.020-0.040)
193Ir	μg/L	0.0001	0	0.93	0.021 (0.020-0.033)
195Pt	μg/L	0.0005	1 (1.42%)	0.73	0.080 (0.031-0.130)
201Hg	μg/L	0.0180	0	0.34	1.795 (1.580-2.201)
205Tl	μg/L	0.0005	0	1.73	0.300 (0.201-0.690)
208Pb	μg/L	0.0013	0	2.10	1.791 (1.365-2.325)
209Bi	μg/L	0.0002	0	1.45	0.065 (0.040-0.158)
232Th	μg/L	0.0003	0	1.73	0.435 (0.188-1.228)
238U	μg/L	0.0003	6 (8.57%)	1.06	0.040 (0.010-0.061)

Note: Conc: concentration; LOD: limit of detection, calculated as 3 times the average of 10 consecutive measurements of the blank diluent; RSD: relative standard deviation to evaluate stability of the measurements.

Table S3. Results of multiple linear regression analysis and restricted cubic spline regression of the association of age with elements.

Element	*β*	95%CI	*P*	P_{linear} FDR	$P_{nonlinear}$	$P_{nonlinear}$ FDR
1Li	0.448	(14.02, 36.32)	0.000	0.000	0.183	0.362
3B	0.289	(3.04, 20.02)	0.009	0.022	0.118	0.362
4Na	-0.168	(-161.39, 13.74)	0.097	0.140	0.153	0.362
7K	0.216	(0.67, 12.22)	0.029	0.064	0.264	0.381
8Ca	-0.271	(-189.72, -30.69)	0.007	0.022	0.021	0.136
10Ti	-0.332	(-49.44, -12.11)	0.002	0.010	0.507	0.599
14Fe	-0.192	(-18.09, 0.82)	0.073	0.118	0.109	0.362
18Zn	-0.200	(-30.69, 0.58)	0.059	0.109	0.195	0.362
22Se	-0.296	(-38.12, -8.06)	0.003	0.013	0.001	0.008
23Rb	0.130	(-2.62, 11.86)	0.207	0.270	0.572	0.620
35Cs	0.085	(-6.94, 17.01)	0.404	0.437	0.227	0.368
43Gd	-0.105	(-9.74, 3.1)	0.305	0.361	0.379	0.492
54Os	-0.014	(-3.65, 3.2)	0.895	0.895	0.686	0.686

Note: Linear regression analysis and restricted cubic spline regression adjusted for three covariates including smoking, hypertension, and cerebral infarction.

Table S4. Concentrations of 5 identified elements in different age groups.

Variables	31–40 years old (*n* = 10)	41–50 years old (*n* = 10)	51–60 years old (*n* = 10)	61–70 years old (*n* = 10)	71–80 years old (*n* = 10)	81–90 years old (*n* = 10)	91–100 years old (*n* = 10)	*P*
Li, μg/L	1.52 (1.28–1.89)	2.07 (1.39–3.00)	1.83 (1.65–1.98)	2.37 (2.14–2.59)	2.00 (2.25–2.92)	2.37 (1.77–3.62)	2.89 (2.42–3.39)	0.451
B, μg/L	18.1 (13.5–22.9)	14.3 (11.6–23.9)	23.6 (19.1–31.0)	26.5 (18.4–37.7)	37.3 (27.2–39.7)	29.5 (17.7–51.4)	31.8 (23.6–56.5)	0.047
Ca, mg/L	96.5 (93.0–98.3)	93.5 (93.1–97.3)	97.0 (92.8–97.5)	94.1 (90.0–98.2)	93.1 (91.8–98.3)	91.5 (88.8–97.3)	90.5 (86.5–92.3)	0.010
Ti, μg/L	95.1 (82.2–106.8)	82.5 (79.8–96.0)	82.5 (67.1–108.5)	93.5 (79.5–102.8)	82.1 (75.3–103.5)	73.3 (67.3–88.0)	76.0 (61.8–81.3)	0.051
Se, μg/L	43.2 ± 8.9	44.7 ± 6.7	42.7 ± 12.1	46.8 ± 10.7	46.0 ± 7.5	36.1 ± 4.9	30.9 ± 8.0	0.001

Table S5. Pairwise interaction of 5 identified elements.

Element	$P_{interaction}$
Li*B	0.2869
Li*Ca	0.9687
Li*Ti	0.7234
Li*Se	0.666
B*Ca	0.7232
B*Ti	0.0676
B*Se	0.6741
Ca*Ti	0.4234
Ca*Se	0.1067
Ti*Se	0.7531

Table S6. Correlation between 5 identified elements and laboratory measurements.

Element	Laboratory measurement	*r*	*P* value	*P* Bonferroni
Ca	TP, g/L	0.584	0.000	0.000
Ca	ALB, g/L	0.551	0.000	0.001
Se	HCT, L/L	0.472	0.000	0.020
Se	RBC, 109 cells/L	0.440	0.000	0.076
Ca	HCT, L/L	0.441	0.000	0.081
Ca	Hb, g/L	0.430	0.000	0.123
Ca	RBC, 109 cells/L	0.415	0.000	0.211
Se	MONO, %	-0.411	0.000	0.221
Ti	LYMPH, %	0.388	0.001	0.539
Se	Hb, g/L	0.380	0.001	0.643
Ca	MONO, %	-0.374	0.002	0.840

Note: ALB = albumin; *r* = correlation coefficient; Hb = hemoglobin; HCT= hematocrit; LYMPH = lymphocyte; MONO = monocytes; RBC = white blood cell; TP = total protein.

Table S7. Correlation between 5 identified elements and laboratory measurements.

Element	*β*	95%CI	*P*
Li	4.552	(0.99, 8.12)	0.013
B	6.058	(2.43, 9.67)	0.001
Ca	-4.999	(-8.29, -1.71)	0.004
Ti	-7.040	(-10.43, -3.65)	0.001
Se	-0.885	(-4.37, 2.60)	0.613

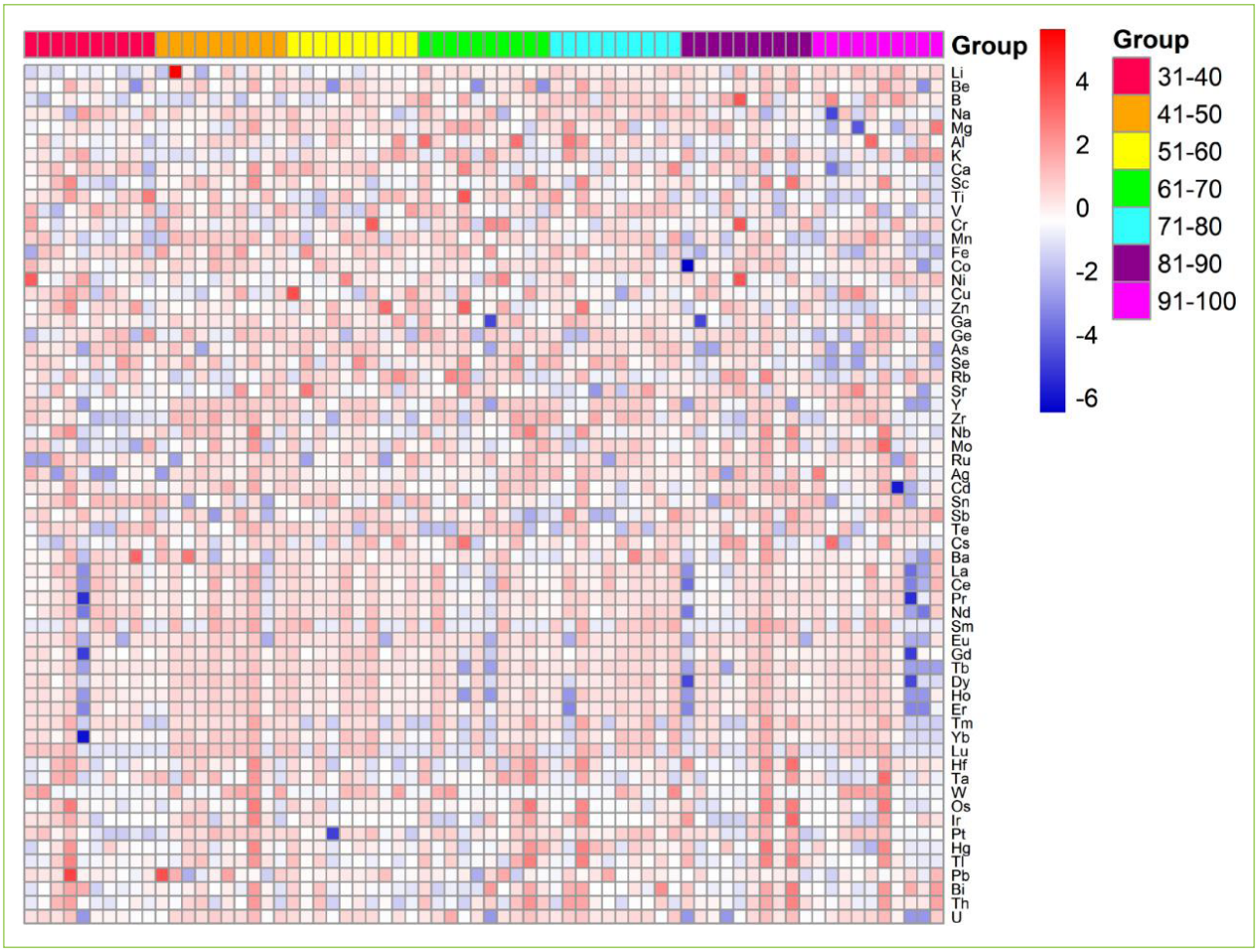

Figure S1. Heatmap of detected elements. Participants were divided into 7 groups as 10 years span of age.