ORIGINAL ARTICLE

Whole blood and serum concentrations of metals in a Swedish population-based sample

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Abstract

Objective. While the potential toxicity of metals in humans is a well-established field of research, there are few studies that examine circulating concentrations of metals in large population-based samples. The aim of this study was to analyze levels of heavy metals and trace elements in both whole blood and serum in an elderly population, and to examine if gender, kidney function, haemoglobin or serum albumin could impact the distribution of metals between whole blood and serum.

Methods. Whole blood and serum samples from 1016 70-year-olds living in Uppsala, Sweden, were analyzed for aluminium, cadmium, cobalt, copper, chromium, mercury, manganese, molybdenum, nickel, lead, and zinc using inductively coupled plasma-sector field mass spectrometry (ICP-SFMS). Distribution between whole blood and serum was evaluated by the ratio between whole blood and serum concentration (B/S-ratio).

Results. Concentrations differed significantly between whole blood and serum measurements for all 11 metals \((p < 0.00001)\). The highest B/S-ratios were found for lead (27), zinc (9), manganese (6), and nickel (4). Copper (0.86), cobalt (0.84), and molybdenum (0.86) showed B/S-ratios \(\leq 1\). Especially the B/S-ratios for chromium, mercury and nickel correlated with kidney function (GFR) \((r = 0.21, 0.21\) and \(0.36\) respectively, \(p < 0.0001)\). Conclusions. The distribution between whole blood and serum varied considerably for different metals. This distribution correlated with physiological factors, mainly with kidney function, for several of the metals.

Key Words: Metals, trace elements, elderly, whole blood, serum

Introduction

Today, toxic metals have been studied extensively. While the acute effects of metal and trace element poisoning are well studied, there are also several indications of long-term effects from non-acute exposure levels [1–8].

Heavy metals and trace elements can be measured in whole blood, serum, urine, hair and other tissues. In blood, metals are distributed between the non-cellular (plasma/serum) and intra-cellular compartment (predominantly erythrocytes). Metals have varying affinity for each compartment, depending on chemical properties. For example, lead (Pb) is known to have a strong affinity for erythrocytes [9], and therefore Pb is measured primarily in whole blood in the clinical setting.

Levels of metals in blood and serum have been studied previously in different samples [10–12]. However, there are few studies that have measured metals in blood and serum in a large population-based cohort, where additional information is available for examining possible connections between the blood/serum distribution and physiological factors. Also, as the age pyramid in Western populations is changing towards the elderly, there is a need to examine metal and trace element levels in an exclusively elderly population.

In this study, we aimed to examine the levels of aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), chromium (Cr), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), and zinc (Zn) in both blood and serum in an elderly population-based cohort in order to establish reference levels in this age group. Furthermore, we aim to investigate if the relative distribution of the metals between blood and serum was related to some clinical characteristics, such as gender, glomerular filtration rate (GFR), haemoglobin and serum albumin levels.
Materials and methods

Subjects

The PIVUS study (Prospective Investigation of the Vasculature in Uppsala Seniors) started in 2001 with the aim to investigate the predictive power of different measurements of endothelial function and arterial compliance. All citizens aged 70 years living in the county of Uppsala (Sweden) were qualified for the study. Of these, subjects were chosen from the register of community residents and were invited in a randomized order. The subjects received an invitation to partake in the study by letter within 2 months of their 70th birthday. The PIVUS study standardized the age at 70 years to have the same age in all subjects, since age is considered an important characteristic in the elderly. Of the 2025 subjects invited, 1016 subjects elected to participate, giving an initial participation rate of 50.1%. Of the subjects, 50.2% were female. Blood was sampled in the morning between 08:00 and 10:00 h, after an overnight fast, no medication or smoking was allowed after midnight. Blood was collected in Vacutainer tubes with EDTA for the whole blood measurements. The serum samples were left for 45–60 min before serum was removed. All the analytes presented were analyzed from frozen (−80°C) serum/whole blood except haemoglobin which was analyzed in the same day from fresh blood.

The participants answered a questionnaire about their medical history, regular medication and smoking habits. Some of the basic characteristics of the sample are given in Supplementary Table I, to be found online at http://informahealthcare.com/doi/abs/10.3109/00365513.2013.864785.

The study was approved by the Ethics Committee of Uppsala University, and the participants gave their informed written consent [1].

Measurements of GFR, serum albumin and haemoglobin

After sampling, analytes were centrifuged in a refrigerated centrifuge and stored at −80°C until analysis. Measurements of creatinine, albumin and haemoglobin were performed on an Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, IL, USA). For albumin, reagents (no. 7D54-20) were supplied by Abbott Laboratories. Creatinine reagents (no. 14.3600.01) were supplied by Synermed International (Westfield, Ind., USA). Glomerular filtration rate was estimated using the Four-variable Modification of Diet in Renal Disease Study Equation [13].

Analysis in whole blood

All 11 elements (Al, Cd, Co, Cu, Cr, Hg Mn, Mo, Ni, Pb and Zn) were determined in whole blood. The analysis was performed using inductively coupled plasma-sector field mass spectrometry, ICP-SFMS, after microwave assisted digestion with nitric acid [14] according to a method accredited for 10 of the 11 elements tested, Al being unaccredited.

Analysis in serum

The analysis was performed using inductively coupled plasma-sector field mass spectrometry, ICP-SFMS, following digestion with nitric acid [14]. In brief, 0.25–1 mL concentrated HNO₃ (SP grade) was added to 50–200 μL aliquots of serum and microwave-assisted, closed-vessel digestion was performed at 300 W power for 30 min. After cooling, sample digests were diluted with deionized water in order to provide uniform 50-fold dilution and acid strength (1.4 M). For quality assurance, a set of method blanks, serum control materials and replicate samples were prepared with each analytical batch of approximately 70 samples. All measurement solutions were spiked with indium (In) at 2 μg/L and measurements were performed using combination of internal standardization and external calibration. Further details on operational conditions and measurement parameters can be found elsewhere [15,16].

All above testing followed the stringent QA/QC demands required by the Swedish National Accreditation Body, SWEDAC, in accordance with ISO 17025. The performing laboratory, ALS Scandinavia AB in Luleå, is registered with the U.S. Food and Drug Administration (FDA) and has a certificate of Good Manufacturing Practice (GMP) compliance issued by the Swedish Medical Products Agency. The performing laboratory had no knowledge of the clinical/final application of the samples provided to them for analysis.

Statistical analysis

Since all of the metals (except Al) were found to be non-normally distributed, differences in concentrations between whole blood and serum were determined by Wilcoxon signed-rank test. The B/S-ratio was obtained by dividing the whole blood with the serum concentration of metals at the individual level. Relationships between pairs of variables (such as when relating whole blood to serum concentrations, or the B/S-ratio to clinical characteristics such as GFR etc.) were determined by Spearman’s rank correlation. In Table IV, both the mean and median for the B/S-ratio were given to facilitate comparison vs. other published studies. Since altogether 61 statistical tests were performed, we set the level of significance at 0.05/61 = 0.00082 according to the Bonferroni-correction.
Results

Whole blood and serum concentrations

There was a significant difference between concentrations in whole blood and serum for all of the measured trace elements and heavy metals ($p < 0.00001$). Only Cu (15.1 vs. 12.9 μmol/L) and Mo (15.2 vs. 11.2 μmol/L) had higher concentrations in serum samples than in blood. Co showed the lowest overall absolute concentrations, and its distribution was found to be similar in blood and serum (see Table I for details). The measurement of Cd in serum fell below the detection limit for the analysis in the majority of the samples and was subsequently not further evaluated.

Correlation between blood and serum levels

Six of the measured metals (Co, Cr, Cu, Hg, Mo and Ni) showed a significant correlation between blood and serum ($p < 0.0001$, see Table II for details).

Blood vs. serum ratio in relation to physiological factors

The whole blood to serum concentration ratio (B/S-ratio) was determined as a measure of the distribution between red blood cells and serum, by dividing blood and serum concentrations. The highest B/S-ratios were found for lead (27), zinc (9), manganese (6), and nickel (4). Copper (0.86), cobalt (0.84) and molybdenum (0.86) showed B/S-ratios $< 1$.

Gender

Cu, Pb and Zn showed significantly higher B/S-ratios in males than in females (see Table III for details).

GFR

The B/S-ratio was inversely correlated with GFR for Cu, Hg, Ni, Pb and Zn ($p < 0.0001$). Cr showed the opposite relationship ($p < 0.0001$), and was the only element whose B/S-ratio correlated positively with GFR (Table III).

Haemoglobin

A significant relationship between the B/S-ratio and haemoglobin was seen only for Zn ($p < 0.0001$).

Albumin

The B/S-ratio for Cu was positively correlated with serum albumin levels, while this ratio for Hg showed an inverse correlation ($r = -0.11$).

Discussion

The absolute concentrations of metals and trace elements were generally higher in whole blood than in
Cu concentration for each element. Zinc; GFR, glomerular filtration rate; B/S-ratio, Blood vs. serum ratio, which was calculated as whole blood concentration divided by serum concentration for each element.

Table III. Relationships between the ratio between whole blood and serum measurements (B/S-ratio) for the different metals and trace elements and gender (0 for males and 1 for females), GFR, haemoglobin and serum albumin. Relationships obtained by Spearman’s rho.

<table>
<thead>
<tr>
<th>Element</th>
<th>Gender</th>
<th>Spearman’s rho</th>
<th>p-value</th>
<th>GFR</th>
<th>Spearman’s rho</th>
<th>p-value</th>
<th>Haemoglobin</th>
<th>Spearman’s rho</th>
<th>p-value</th>
<th>Albumin</th>
<th>Spearman’s rho</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Al</td>
<td>0.05</td>
<td>0.08</td>
<td>−0.08</td>
<td>0.0068</td>
<td>−0.06</td>
<td>0.05</td>
<td>−0.01</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
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<td>0.20</td>
<td>0.06</td>
<td>0.05</td>
<td>0.01</td>
<td>0.80</td>
<td>−0.01</td>
<td>0.78</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cr</td>
<td>−0.01</td>
<td>0.99</td>
<td>0.21</td>
<td>&lt;0.0001*</td>
<td>0.02</td>
<td>0.39</td>
<td>0.05</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>−0.11</td>
<td>0.0002*</td>
<td>−0.15</td>
<td>&lt;0.0001*</td>
<td>−0.01</td>
<td>0.91</td>
<td>0.17</td>
<td>&lt;0.0001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>−0.10</td>
<td>0.015</td>
<td>−0.21</td>
<td>&lt;0.0001*</td>
<td>0.04</td>
<td>0.11</td>
<td>−0.11</td>
<td>−0.003*</td>
<td></td>
<td></td>
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<tr>
<td>Mn</td>
<td>0.02</td>
<td>0.38</td>
<td>−0.07</td>
<td>0.012</td>
<td>0.01</td>
<td>0.73</td>
<td>0.01</td>
<td>0.76</td>
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<tr>
<td>Mo</td>
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<td>0.99</td>
<td>−0.04</td>
<td>0.17</td>
<td>−0.01</td>
<td>0.82</td>
<td>0.02</td>
<td>0.41</td>
<td></td>
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<td></td>
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<tr>
<td>Ni</td>
<td>−0.05</td>
<td>0.10</td>
<td>−0.36</td>
<td>&lt;0.0001*</td>
<td>−0.04</td>
<td>0.31</td>
<td>0.06</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>−0.11</td>
<td>0.0002*</td>
<td>−0.20</td>
<td>&lt;0.0001*</td>
<td>0.09</td>
<td>0.0045</td>
<td>−0.02</td>
<td>0.48</td>
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<tr>
<td>Zn</td>
<td>−0.15</td>
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<td>−0.28</td>
<td>&lt;0.0001*</td>
<td>0.15</td>
<td>&lt;0.0001*</td>
<td>−0.07</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Al, aluminium; Co, cobalt; Cr, chromium; Cu, copper; Hg, mercury; Mn, manganese; Mo, molybdenum; Ni, nickel; Pb, lead; Zn, zinc; GFR, glomerular filtration rate; B/S-ratio, Blood vs. serum ratio, which was calculated as whole blood concentration divided by serum concentration for each element.

∗Critical p-value was set at < 0.00082.

serum, the exceptions being Cu and Mo. Kidney function was the major physiological variable related to the distribution between the red blood cell and serum compartment.

Reference ranges

Previous studies have been performed in order to establish reference values in both whole blood and serum for metals. In the present study, the mean Al levels in serum (0.424 μmol/L) were above previously proposed reference ranges [17]. Consumption of antacids might previously have been more prevalent in the elderly and could possibly have contributed in part to the Al levels [17,18]. Haemodialysis patients may also accumulate Al in the course of treatment. At the time of sampling there was one subject in the cohort undergoing haemodialysis, and was therefore excluded from the study. Furthermore, once integrated into cortical bone, aluminium half-life is roughly 29 years [19], so exposition earlier in life will most probably influence Al levels in the cohort. For the other metals measured in the present study, mean (and median) values were within previously established reference ranges [10,20–22].

Correlation between blood and serum levels

Bárány et al. [11,23] found correlations between blood and serum levels for Co (r = 0.21), Cu (r = 0.62) and Hg (r = 0.52), findings being similar to those in the present study. However, Bárány et al. also found significant correlations for Pb and Zn (r = 0.15, and r = 0.17), which were not seen in the present sample. It should be noted that this previous study was conducted in adolescents, most probably influencing both exposure and relevant physiology compared to the present elderly sample. However, a comparison is potentially interesting since the studies by Bárány were conducted on a population within the same country as the sample presented in this article.

Cr levels were found to be inversely correlated between blood and serum. One explanation might be that different ionic forms of Cr are present in the measurement performed and that those are differently distributed between whole blood and serum. While Cr(III) is an essential nutrient, Cr(IV) is highly toxic. Additional testing, for example with ion chromatography, could elucidate this.

The whole blood to serum concentration ratio

Of the 11 measured elements, only Cu and Mo had higher concentrations in serum than blood. Cu is an essential trace element for humans and has a high affinity for albumin and ceruloplasmin [24]. After oral intake, Cu is initially taken up in the gut and bound to albumin and other proteins in plasma. Cu then enters the liver where some is excreted through bile (depending on copper load). The remaining portion is bound to ceruloplasmin and re-enters the circulation to reach destination tissues [25]. The low B/S ratio might be a result of a high protein binding in serum. Minoia et al. [10] observed a higher B/S ratio for Cu than in the present sample (1.24 vs. 0.86 in the PIWUS group), while the B/S ratio from other studies are more similar to our finding (see Table IV for an overview of previous literature). Perhaps the consistency of Cu distribution seen between these studies could be explained by Cu having its own specific distribution system, involving both ceruloplasmin as well as other specialized pathways. An essential nutrient could be expected to be strongly governed by homeostatic mechanisms.

Mo binds to α2-macroglobulins in plasma in the form of molybdate. However, the metal also readily binds to the protein spectrin on the erythrocyte [26]. The blood to serum ratio is similar to an earlier description in the literature [20].
Blood and serum concentrations of metals

Whole blood to serum ratio in relation to other factors

The higher blood to serum ratios of Cu, Hg, Pb and Zn in men could be explained by males generally having higher haemoglobin levels than females (143 [SD 10] vs. 132 [9], p < 0.0001). This is especially true for Zn since 75–88% of total blood Zn is bound to erythrocytes, mainly to carbonic anhydrase [27]. The blood to serum ratio for Zn was also positively correlated with haemoglobin levels. Erythrocyte zinc concentration and carbonic anhydrase activity themselves depend on the haematocrit and the haemoglobin concentration. Furthermore, serum Zn and blood haemoglobin have previously been shown to be positively correlated [28], and Zn was the only metal for which the blood to serum ratio was significantly correlated to Hb levels in the present sample. The B/S ratios for Cu, Hg, Ni, Pb and Zn were inversely correlated with GFR. The serum/plasma fraction is the one filtered in the glomeruli, and therefore these metals might accumulate in erythrocytes in subjects with poor kidney function. On the other hand, the B/S-ratio for Cr correlated positively with GFR. Cr was also the only metal to show an inverse correlation between blood and serum levels. We have not been able to explain these unique features of Cr.

It would be interesting to see further studies of metal levels compared to kidney function. Possibly CystatinC or inohexol clearance could give even more accurate descriptions of GFR levels in subjects.

The finding that albumin levels correlated positively with the B/S-ratio for Cu seems counterintuitive taking into account that it strongly binds to ceruloplasmin (and albumin) in human plasma [25,29]. Our finding could perhaps instead reflect a better nutrient status in subjects with higher albumin levels. In that case, the better micronutrient status might yield a different distribution of Cu given that Cu is filtered in the glomeruli. The B/S-ratio for Hg was found to be negatively correlated with albumin levels. Kidney function can be impaired following exposure to mercuric salts and can cause albuminuria [30]. Other than that, there seemed to be little information about any relationship between Hg and albumin.

Conclusion

The distribution between whole blood and serum varied considerably for different metals. This distribution correlated with physiological factors, mainly with kidney function, for several of the metals.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Supplementary material available online

Supplementary Table I.