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The association of bone, fingernail and blood manganese with cognitive and olfactory function in Chinese workers

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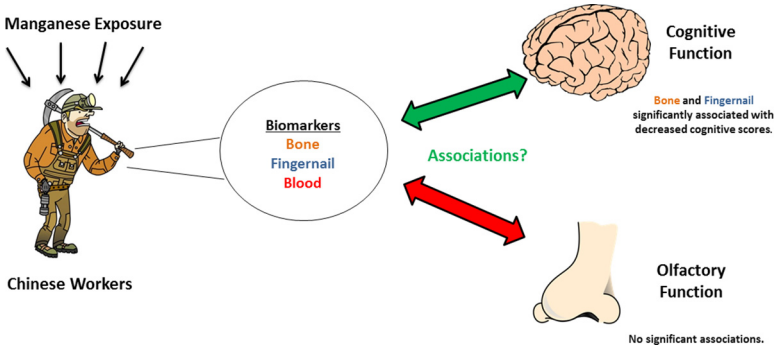
HIGHLIGHTS

- Manganese (Mn) exposure has been related to impaired cognition and olfaction.
- Bone Mn, for cumulative exposure, was determined using neutron activation analysis.
- After adjustment, bone and fingernail Mn were associated with impaired cognition.
- There were no significant associations between biomarkers and olfaction.

GRAPHICAL ABSTRACT



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ABSTRACT

Occupational manganese (Mn) exposure has been associated with cognitive and olfactory dysfunction; however, few studies have incorporated cumulative biomarkers of Mn exposure such as bone Mn (BnMn). Our goal was to assess the cross-sectional association between BnMn, blood Mn (BMn), and fingernail Mn (FMn) with cognitive and olfactory function among Mn-exposed workers. A transportable *in vivo* neutron activation analysis (IVNAA) system was designed and utilized to assess BnMn among 60 Chinese workers. BMn and FMn were measured using inductively coupled plasma mass spectrometry. Cognitive and olfactory function was assessed using Animal and Fruit Naming tests, World Health Organization/University of California-Los Angeles Auditory Verbal Learning Test (AVLT) and the University of Pennsylvania Smell Identification Test (UPSIT). Additional data were obtained via questionnaire. Regression models adjusted for age, education, factory of employment, and smoking status (UPSIT only), were used to assess the relationship between Mn biomarkers and test scores. In adjusted models, increasing BnMn was significantly associated with decreased performance on average AVLT scores

Abbreviations: AVLT, Auditory Verbal Learning Test; BMn, blood manganese; BnMn, bone manganese; CI, confidence interval; DL, detection limit; FMn, fingernail manganese; HMn, hair manganese; HPGe, high purity germanium; IVNAA, *in vivo* neutron activation analysis; Mn, manganese; SD, standard deviation; UPSIT, University of Pennsylvania Smell Identification Test; UPSIT-TC, University of Pennsylvania Smell Identification Test - Traditional Chinese; ZMU, Zunyi Medical University.

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Biomarker
Neutron activation analysis

[β (95% confidence interval (CI)) = −0.65 (−1.21, −0.09)] and Animal Naming scores [β (95% CI) = −1.54 (−3.00, −0.07)]. Increasing FMn was significantly associated with reduced performance measured by the average AVLT [β (95% CI) = −0.35 (−0.70, −0.006)] and the difference in AVLT scores [β (95% CI) = −0.40 (−0.77, −0.03)]. BMn was not significantly associated with any test scores; no significant associations were observed with Fruit Naming or UPSIT tests. BnMn and FMn, but not BMn, are associated with cognitive function in Mn-exposed workers. None of the biomarkers were significantly associated with olfactory function.

1. Introduction

Manganese (Mn) is an essential element that is used in the metabolism of lipids, proteins, and carbohydrates (Andreini et al., 2008); however, overexposure to the metal has been associated with neurotoxicity (Dobson et al., 2004). Workers involved in mining (Myers et al., 2003), welding (Bowler et al., 2006; Bowler et al., 2007), smelting (Cowan et al., 2009a, 2009b), ore-processing (Chia et al., 1993), ferroalloy steel production (Lucchini et al., 1999), dry-cell battery manufacturing (Bader et al., 1999), and pesticide manufacturing (Ferraz et al., 1988) may be at increased risk due to their chronic exposure to elevated Mn.

Occupational studies have identified associations of Mn exposure with cognitive deficits. In a cross-sectional study of 141 Mn oxide and salt producing plant workers, those exposed to Mn had decreased audio-verbal short-term memory (Roels et al., 1987). Decreased general intelligence has also been seen in chronically exposed Mn workers (Hua and Huang, 1991). In a group of 76 former and current chemical industry welders, welders had worse verbal learning, working memory, cognitive flexibility, and visuomotor processing speed when compared to non-exposed controls (Bowler et al., 2003). Welders who have developed parkinsonism due to Mn have experienced progressive cognitive slowing and forgetfulness (Sadek et al., 2003) as well as decreased information processing speed and cognitive flexibility (Bowler et al., 2006). Welders, and other Mn-exposed workers, have also experienced decreases in executive function, sustaining concentration, cognitive flexibility, and working memory (Bowler et al., 2007; Bowler and Lezak, 2015).

Mn overexposure may also result in decreased olfactory function. Mn-exposed Bay Bridge welders had significantly lower olfactory test scores than non-exposed controls (Antunes et al., 2007). When reassessed after 3.5 years following cessation of Mn exposure cessation, a group of 43 confined space welders still had depressed olfactory test scores (Bowler et al., 2011).

Biomarkers such as blood (Roels et al., 1987; Chia et al., 1993; Myers et al., 2003; Bowler et al., 2007), urine (Roels et al., 1992; Lucchini et al., 1995; Cowan et al., 2009a), and toenail (Laohaudomchok et al., 2011; Hassani et al., 2016) have previously been used to assess the relationship between neuropsychological deficits and occupational Mn exposure. However, the utility of these biomarkers has been questioned due to issues such as the short half-life ($t_{1/2}$) of manganese in these biomarkers, particularly blood, as a result of the body's homeostatic control of Mn (Aschner and Aschner, 2005; Menezes-Filho et al., 2009; Zheng et al., 2011; Costa and Aschner, 2015; O'Neal and Zheng, 2015), poor association with external air Mn concentrations (Smith et al., 2007; Laohaudomchok et al., 2011), and risk of external Mn contamination (Laohaudomchok et al., 2011; Reiss et al., 2016). Possibly due to these limitations, these biomarkers have been inconsistent in their results of identifying cognitive neuropsychological symptoms due to Mn.

Bone Mn (BnMn) has been suggested as a biomarker of cumulative Mn exposure as 40% of Mn in the body is stored in bone (Andersen et al., 1999; Arnold et al., 2002) and the $t_{1/2}$ of Mn in human bone is estimated to be approximately 8–9 years (Arnold et al., 2002; O'Neal et al., 2014). Moreover, BnMn in rats has shown to be correlated with Mn levels in the striatum, hippocampus and cerebral spinal fluid, suggesting that BnMn may also reflect brain Mn concentrations (O'Neal et al., 2014).

Researchers from McMaster and Ryerson Universities have previously quantified BnMn among Mn-exposed workers using *in vivo* neutron activation analysis (IVNAA) (Arnold et al., 2002). In a feasibility study utilizing the IVNAA system, BnMn was assessed in a group of 29 welders and 10 controls: researchers from the McMaster and Ryerson groups found that BnMn among welders was significantly higher than among controls (Pejović-Milić et al., 2009). Despite the IVNAA system's capability of successfully quantifying BnMn, its large size prohibits movement. Our research team has developed and validated a transportable neutron generator-based IVNAA system that can be used to assess Mn in the hand bones of participants (Liu et al., 2013, 2017); and recently reported BnMn concentrations in a pilot study of 19 volunteers (Wells et al., 2018) and cross-sectional occupational study of 60 Chinese workers (Liu et al., 2018).

The goal of this study was to present further analyses from our occupational study (Liu et al., 2018; Rolle-McFarland et al., 2018); specifically, to determine whether BnMn, as well as blood and fingernail manganese, are associated with neuropsychological tests of verbal fluency, verbal learning, and olfactory function. To the best of our knowledge, this analysis represents the first study to evaluate the association of BnMn with measures of cognitive and olfactory function.

2. Methods

2.1. Study design and population

This cross-sectional study recruited adult (≥18 years old) male workers from an equipment manufacturing and installation company ($N = 30$) and a ferroalloy smelting facility ($N = 31$) in Zunyi, China. Workers from the manufacturing facility did not work with Mn-related products in their current positions and performed assembling, managerial, and custodial jobs. Initially, the manufacturing facility workers were to act as a control group for the ferroalloy workers. However, upon further inspection of their work histories, some workers from the manufacturing facility had a history of previous Mn-related occupations. Therefore, to account for historical exposure, overall Mn exposure was evaluated on a continuous level among all participants. This allowed us to assess relationships between outcomes and Mn exposure as it increased from no/low levels to higher levels of exposure. One ferroalloy worker did not complete a BnMn measurement and was excluded from all analyses, leaving a total of $N = 60$.

Additional exclusion criteria included: 1) the self-reported presence of non-manganese related cognitive symptoms (*i.e.* from head trauma), active neurological or psychiatric disease, or movement impairments; and 2) participation in other studies involving the use of radiation within the past year. No participants were excluded from analyses for meeting these exclusion criteria. Five participants did not provide a sufficient quantity of fingernail tissue to conduct laboratory analyses and another two participants did not complete the olfactory test, thus these individuals were not included in analyses involving fingernail manganese and/or olfactory function, respectively.

The study was explained to the participants by local study staff prior to their signing an informed consent document. Both the Purdue Biomedical Institutional Review Board and the Zunyi Medical University (ZMU) Ethical Review Board approved this study. Participants visited the new ZMU campus where they completed a BnMn measurement, a

short physical examination, blood and fingernail collection, and a short battery of neurological performance tests. Demographic information was self-reported using a questionnaire administered during the bone manganese assessment. Data collected included age, years of education completed, current drinking status and current smoking status.

2.2. Manganese sample collection and analysis

A transportable *in vivo* neutron activation analysis (IVNAA) system, previously described in detail (Liu et al., 2013, 2018), was used to determine participants' BnMn. Briefly, a participant's right hand and arm were washed with soap and water and then wiped with a 50% alcohol solution. The participant's right hand was irradiated for 10 min in order to excite ^{55}Mn atoms in the hand bone to ^{56}Mn. After a 5-minute break, the participant was seated and asked to place their hand in a high purity germanium (HPGe) detection system. The HPGe system collected characteristic Mn γ ray signals (847 keV) over the span of an hour. Mn γ ray counts were used to calculate BnMn concentrations based on a pre-existing calibration curve created from Mn-doped bone-equivalent hand phantoms. A Mn/Ca γ ray ratio was calculated to account for variation in counting geometry, neutron flux, as well as hand-palm beam attenuation. The detection limit (DL) for this method can reach 0.64 μg Mn per g bone for a 30 minute measurement with on HPGe detector (Liu et al., 2017). Nineteen participants (31.7%) had BnMn concentrations <DL for the transportable IVNAA and $N = 13$ (21.7%) of these measurements were negative. It is possible to obtain negative values when the true BnMn concentrations were close to zero; this is also seen in measurement of bone lead (Park et al., 2009). Prior work on bone lead measurements recommended retaining values <DL, including negative values, in analyses as this will decrease bias and increase analytical efficiency (Kim et al., 1995; Park et al., 2009). Therefore, all BnMn concentrations were retained for analyses.

Toenail Mn, which reflects approximately the past 7–12 months of exposure, has been previously used in several studies (Grashow et al., 2014; Hassani et al., 2016; Laohaudomchok et al., 2011). We learned that workers in our study population wore open-toed shoes to work, which could result in substantial external contamination of toenails; therefore, we collected fingernail samples instead of toenails. Methods to collect and analyze fingernails were adapted from Kile et al. (2007). Fingernails grow at about twice the rate of toenails; thus, it is estimated that they reflect the prior 3–6 months of exposures (Viana et al., 2014; Yaemsiri et al., 2010). After washing with soap and water, nail samples were collected from participants' 10 fingers using titanium dioxide nail clippers and stored in Ziploc bags at room temperature. Fingernail samples were cleaned using an ultrasonic water bath filled with a solution of 1% Triton X-100 (Sigma-Aldrich Inc., USA), rinsed with deionized water, and dried at 60 °C. This cleaning procedure was completed twice, prior to digestion in ultrapure nitric acid (Sigma-Aldrich Inc., USA) at 200 °C.

After digestion, samples were analyzed for Mn using inductively coupled plasma-mass spectrometry (ICP-MS) at Purdue University's Campus-Wide Mass Spectrometry Center with a Thermo Fisher ELEMENT 2 (ThermoFinnigan/FinniganMAT, San Jose, CA, Bremen, Germany). Samples were run in multiple batches with results given in ppb. These values were converted to μg/g for analysis as this measure takes the fingernail mass into account. Detection limits (DLs) were calculated for values prior to transformation into μg/g and were calculated separately for each batch. This resulted in a range of DLs for fingernail Mn (1.31–3.97 ppb). For the purpose of this study, we considered any concentration below 3.97 ppb to be below the DL. Normally, an imputation method such as $^{DL}/_{\sqrt{2}}$ would be used to impute values below the DL. However, because seventeen (28.3%) measurements were < DL, the imputation of values could introduce additional bias. The samples below the DL still had detectable concentrations which were larger than blank samples. Therefore we addressed these in a manner similar to BnMn and retained concentrations for samples <DL in statistical analyses. To ensure that these results were robust, we conducted analyses using FMn classified into tertiles as well as a continuous variable.

Trained study staff collected whole blood samples using standard collection protocols using trace-metal free vacutainers (Becton-Dickinson, USA). Samples were stored at −20 °C prior to being shipped on dry ice to the Chinese Centers for Disease Control and Prevention in Beijing, China where they were analyzed for Mn using ICP-MS as described previously (Zhang et al., 2015). The DL for BMn was 0.11 μg/L; all 60 samples were above the detection limit.

2.3. Cognitive and olfactory assessments

A group of trained research assistants fluent in both Mandarin and English conducted individual neuropsychological assessments on participants. All assessments were previously validated and used in Chinese populations, described in more detail below. The test battery included 1) Animal Naming; 2) Fruit Naming; 3) World Health Organization/University of California Los Angeles Auditory Verbal Learning Test (AVLT); and 4) University of Pennsylvania Smell Identification Test – Traditional Chinese version (UPSIT-TC).

The Animal and Fruit Naming tests are designed to assess verbal fluency (Tombaugh et al., 1999; Bowler and Lezak, 2015) and have been used in Chinese populations (Chiu et al., 1997; Lee et al., 2002). For these tests, participants were asked to name as many animals or fruits as possible in 1 min. Scores were reported as the number of correct words stated by the participant in the time limit; a higher score indicated better verbal fluency.

The AVLT is a test of verbal learning and retention (Bowler et al., 2018; Maj et al., 1993), which has also been previously used among Chinese (Zhou and Jia, 2009). Participants were given 15 common words and asked to repeat the list of words during five acquisition (learning) trials. After the five acquisition trials, a new list of 15 words (an interference list) was given and participants were asked to repeat the new list of words back to the trained research assistant (trial 6). Finally, participants were asked to repeat as many words from the original acquisition list in a post-interference recall trial (trial 7). Several results from the AVLT were reported including the number of average correct words recalled across trials 1–5 (average) and the score from trial five minus the score from trial one (difference); these scores indicate the number of words learned over the five trials. Scores from trial 6 and trial 7, indicating new learning after distraction as well as longer-term retention, respectively, are also represented. For both trials 6 and 7 the number of correct words was reported as well as the number of intrusions (words that did not belong to the respective list) are reported. Higher AVLT average, difference, trial 6, or trial 7 scores indicate better verbal learning and retention; whereas, a lower intrusion score indicates better verbal learning and retention.

The UPSIT-TC test is an assessment of olfactory function intended for use in Chinese populations (Doty et al., 1984; Jiang et al., 2010; Jiang et al., 2014; Jiang and Liang, 2016). This is a multiple choice, scratch and sniff test consisting of four booklets each containing 10 odors. Participants release each scent by scratching scent pads embedded on each page using the tip of a pencil. They indicate the correct odor by selecting one of the four choices. The UPSIT-TC score is calculated as the total number of correct odors selected. Thus, a higher UPSIT-TC score indicates better olfactory function.

2.4. Statistical analyses

All statistical analyses were completed using Stata 13.1 (College Station, Texas). A p-value ≤0.05 was considered statistically significant. We recruited workers from two different factories with the expectation that workers in the ferroalloy factory would have higher exposures compared to those from the manufacturing factory, which was observed (Liu et al., 2018). However, current analyses are based on individual

biomarker measurements rather than group exposure comparisons because individual biomarker concentrations reflect all potential exposure sources, not just those from the workplace. Additionally, several manufacturing workers reported previous employment at the ferroalloy factory; thus variations in cumulative exposure are not completely explained by current factory. All manganese biomarkers were lognormally distributed; therefore, median and interquartile ranges were used in descriptive statistics and natural log transformations were used on the biomarker variables prior to their inclusion in regression models. A constant of 5.99 was added to all BnMn concentrations so that all values were positive prior to the log transformation (Atkinson, 1994). Although the addition of a constant affects specific estimates of BnMn concentrations, it does not affect the results of statistics investigating the correlation or association of BnMn with other variables.

We completed descriptive statistics, including univariate and bivariate comparisons of all exposure, outcome, and confounder variables. Results from bivariate comparisons of each variables compared to tertiles of BnMn are presented. Adjusted regression models were created to assess the association between Mn biomarkers with cognitive and olfactory test scores. Mn biomarkers were modeled as both a natural-log transformed continuous variable and tertiles. Covariate selection for adjusted models was based on Spearman correlations, model coefficients of determination, and previous literature (Bowler et al., 2007). Covariates included in the adjusted models were age (continuous), years of completed education (continuous), and current factory of employment (ferroalloy/manufacturing). Olfactory models were also adjusted for current smoking status (yes/no). Current factory of employment was retained as a covariate to account for any sampling differences between the manufacturing and ferroalloy facilities even though participants were assessed as a whole group.

Although we incorporated multiple cleaning steps in our protocol, it is possible for either our nail samples or BnMn measurements to be affected by external contamination from Mn-containing dirt on the nails and/or hands. Therefore, a post-hoc sensitivity analysis was conducted to assess whether any bias may have been introduced by contamination of FMn or BnMn samples. FMn was established using a handheld K-X-ray fluorescence device before and after the first ultrasonic water bath cleaning. The difference between pre- and post-cleaning FMn concentrations was calculated and used as an indicator of participants with potentially high external contamination on their fingernails or hands. A kernel density plot was created to visualize the distribution of these differences (Supplementary Fig. 1). Most the samples ($N = 54$; 88.5%) had differences of <7000 μg/g. Another distinct group of samples ($N = 7$; 11.5%) had differences that were >7000 μg/g, suggesting possible external contamination. Sensitivity analyses were conducted by rerunning multivariable linear regressions without these 7 samples (Supplementary Tables 1 and 2).

3. Results

Population characteristics stratified by BnMn tertiles are reported in Table 1. For the entire population ($N = 60$) mean (standard deviation (SD)) age and years of education were 47.4 (7.9) and 10.0 (3.9) years, respectively. On average, participants had been in their current position for 9.0 years (SD = 6.8). Current factory of employment was borderline significant ($p = 0.07$) across BnMn tertiles; it was more likely that those in the highest tertile of BnMn exposure worked in the ferroalloy factory. More than half of the study population reported being current smokers ($N = 46$; 76.7%).

Summary statistics for Mn biomarkers and outcome measures are reported in Table 2, again stratified by BnMn tertile. Both the average AVLT ($p = 0.02$) and UPSIT-TC ($p = 0.05$) tests had significant decreases in scores associated with increasing BnMn tertiles. Both FMn ($p < 0.01$) and BnMn ($p < 0.01$) were also significantly different across BnMn tertiles with increasing concentrations as BnMn tertiles increase.

Adjusted regression models, assessing the relationship between the Mn biomarkers and test scores, are reported in Table 3 (continuous ln (Mn) variables) and Table 4 (tertiles of Mn). Increasing ln(BnMn) was significantly associated with decreased Animal Naming scores [β (95% CI) = −1.54 (−3.00, −0.07)] and decreased average AVLT scores [−0.65 (−1.21, −0.09)]. There was a decrease in average AVLT scores comparing those in the third tertile of BnMn to those in the lowest tertile [−1.18 (−2.31, −0.04)]. Those in the second BnMn tertile had lower scores for AVLT Trial 6 compared to those in the lowest BnMn tertile [−1.27 (−2.44, −0.08)]. Trial 6 intrusions increased for both the second [0.49 (0.04, 0.93)] and third [0.53 (0.07, 0.99)] tertile of BnMn when compared to the lowest tertile. Increasing ln(FMn) was also significantly associated with lower average AVLT [β (95% CI) = −0.35 (−0.70, −0.006)] and lower AVLT difference scores [−0.40 (−0.77, −0.03)]. BMn was not significantly associated with any of the cognitive or olfactory test scores.

Results from our sensitivity analysis where $N = 7$ individuals with the strongest likelihood of FMn or BnMn contamination were removed from regression models is shown in the Supplementary material (Supplementary Tables 1 and 2). Results were similar to analyses including the entire population, although fewer comparisons achieved statistical significance. There was still a significant association between increasing FMn and decreased average AVLT [β (95% CI) = −0.44 (−0.80, −0.07)]. The second tertile of BnMn was still associated with decreased AVLT Trial 6 scores [−1.25 (−2.46, −0.04)].

4. Discussion

The purpose of this study was to determine whether BnMn, as well as FMn and BMn, were associated with decreased cognitive and olfactory performance in a population of Chinese workers. Our results from adjusted regression models suggest that BnMn and FMn are associated with decreased performance on several measures of cognitive function,

Table 1
Population characteristics, stratified by bone manganese tertile.

Variable	Category	Total population	Bone manganese			*p*-Value
			≤0.6 μg/g	0.7 to 5.1 μg/g	≥5.2 μg/g	
Age, years	29 to 43	17 (28.3)	7 (35.0)	4 (20.0)	6 (30.0)	0.43
	44 to 52	28 (46.7)	10 (50.0)	10 (50.0)	8 (40.0)	
	>52	15 (25.0)	3 (15.0)	3 (15.0)	6 (30.0)	
Education, years	0 to 8	18 (30.0)	9 (45.0)	3 (15.0)	6 (30.0)	0.41
	9 to 13	30 (50.0)	7 (35.0)	13 (65.0)	10 (50.0)	
	>13	12 (20.0)	4 (20.0)	4 (20.0)	4 (20.0)	
Factory	Manufacturing	30 (50.0)	13 (65.0)	11 (55.0)	6 (30.0)	0.07
	Ferroalloy	30 (50.0)	7 (35.0)	9 (45.0)	14 (70.0)	
Current smoker	No	14 (23.3)	5 (25.0)	5 (25.0)	4 (20.0)	0.91
	Yes	46 (76.7)	15 (75.0)	15 (75.0)	16 (80.0)	

Values are *N* (%); $N = 60$; $N = 20$ in each tertile of bone manganese. *p*-Values are based on one-way ANOVA comparing results by bone manganese tertiles.

Table 2
Average neuropsychological test scores and manganese biomarker concentrations, stratified by bone manganese tertile.

Variable	*N*	Total Population	Bone Manganese			
			≤0.6 μg/g	0.7 to 5.1 μg/g	≥5.2 μg/g	*p*-Value
Animal Naming[a]	60	15.8 (5.0)	16.5 (5.1)	16.2 (4.7)	14.5 (5.1)	0.22
Fruit Naming[a]	60	11.3 (3.2)	11.4 (3.2)	11.9 (3.6)	10.5 (2.8)	0.38
Average AVLT[a]	60	8.5 (2.2)	9.1 (2.2)	8.9 (1.6)	7.5 (2.4)	0.02
AVLT[a]	60	4.5 (2.0)	4.8 (2.0)	4.6 (2.1)	4.0 (2.0)	0.25
AVLT Trial 6[a]	60	4.6 (1.9)	5.4 (2.2)	4.1 (1.4)	4.4 (1.9)	0.12
AVLT Trial 6 intrusions[a]	60	0.5 (0.7)	0.2 (0.4)	0.6 (0.7)	0.6 (0.9)	0.13
AVLT Trial 7[a]	60	8.8 (3.4)	9.5 (3.7)	8.7 (2.4)	8.1 (3.8)	0.18
AVLT Trial 7 intrusions[a]	60	0.4 (0.7)	0.5 (0.8)	0.4 (0.6)	0.3 (0.8)	0.38
UPSIT-TC[a]	58	20.2 (5.8)	21.7 (7.1)	21.0 (4.7)	18.0 (5.2)	0.05
Blood manganese, μg/L[b]	60	14.1 (4.0)	13.2 (2.9)	14.3 (4.0)	14.4 (6.4)	0.11
Fingernail manganese, μg/g[b]	55	13.5 (58.5)	3.8 (14.4)	9.2 (32.9)	71.6 (319.7)	<0.01
Bone manganese, μg/g[b]	60	2.6 (7.2)	−0.8 (2.6)	2.6 (2.5)	18.5 (21.1)	<0.01

AVLT = World Health Organization/University of California Los Angeles Auditory Verbal Learning Test; UPSIT-TC = University of Pennsylvania Smell Identification Test – Traditional Chinese. The *p*-value is based on an unadjusted linear regression of the neuropsychological test *versus* bone manganese tertile.
[a] Mean (standard deviation).
[b] Median (interquartile range).

but not olfactory function. Additionally, we found no evidence of an association between BMn and cognitive or olfactory test scores. Similar trends occur when assessing the relationship between cognitive function and lead exposure; bone lead tends to be more consistently associated with cognitive decline than blood lead suggesting that cognitive decline may be associated with long-term exposure, but not necessarily recent exposure (Stewart et al., 1999; Shih et al., 2006).

To the best of our knowledge, this is the first study that has assessed the relationship between BnMn, a biomarker of cumulative manganese exposure, with cognitive or olfactory tests in a human population. In a previous study assessing the kinetics of Mn in bone, BnMn in rats was associated with Mn concentrations in both the hippocampus and striatum, two parts of the brain that play an important role in cognition (O'Neal et al., 2014). In our study, BnMn was significantly associated with decreased scores for both AVLT and Animal Naming, two well-established tests of verbal memory and fluency function. Verbal memory tests like the AVLT have been associated with hippocampal impairment (Vyhnalek et al., 2014) whereas verbal fluency tests, like the Animal Naming test, have been associated with decreased striatal matter (Ellfolk et al., 2014). Thus, it is plausible that our observed results of BnMn's association with decreased verbal memory and fluency may be related to the accumulation of Mn in the striatum and hippocampus.

FMn in our study was associated with decreased AVLT scores, suggesting an association between increasing Mn and lower verbal learning and retention. Associations between nail Mn and cognitive decline have been reported elsewhere. In a previous study, toenail Mn was associated with decline in several cognitive tests assessing domains such as visual and spatial working memory in a group of Mn-exposed welders and smelters (Hassani et al., 2016). The association between Mn in nails and cognitive decline has also been seen in an environmental population. In a group of 89 environmentally exposed adults in Brazil, increasing FMn was significantly correlated with decreasing visual working memory (Viana et al., 2014). Mn in nails has been associated with Mn accumulation in striatum and midbrain of rat models (Sriram et al., 2012), suggesting that Mn in nail samples is also reflective of brain Mn concentrations.

Hair Mn (HMn) has been suggested as a similar biomarker to nail Mn (Laohaudomchok et al., 2011). In the previously mentioned environmental study of Mn-exposed adults in Brazil, both FMn and HMn were associated with decline in several cognitive domains including visual working memory, IQ, and cognitive flexibility (Viana et al., 2014). In an environmental study assessing Mn exposure in a group of Mexican adults, HMn was associated with decreasing olfactory function in the adults with the more exposed individuals having lower olfactory scores; however, these relationships were not significant (Guarneros et al., 2013). A similar trend was seen in our study where total FMn was associated with a non-significant decline in olfactory test scores. When compared to the lowest tertile of FMn, individuals in the middle tertile of FMn were associated with an increase in olfactory scores whereas individuals in the highest tertile of FMn were associated with a decrease in olfactory scores.

Although BMn in our study was associated with decline in some cognitive functions as well as the olfactory outcome, none of these relationships were significant. In a study of approximately 700 alloy plant

Table 3
β (95% confidence interval) from adjusted linear regression models comparing continuous, natural-log transformed manganese biomarkers with cognitive and olfactory test scores.

Outcome	Blood manganese	Fingernail manganese	Bone manganese
Animal Naming[a]	0.009 (−4.68, 4.69)	−0.77 (−1.66, 0.11)[d]	−1.54 (−3.00, −0.07)[c]
Fruit Naming[a]	−1.06 (−4.42, 2.30)	−0.29 (−0.96, 0.38)	−0.40 (−1.49, 0.69)
Average AVLT[a]	−1.15 (−2.93, 0.62)	−0.35 (−0.70, −0.006)[c]	−0.65 (−1.21, −0.09)[c]
Difference AVLT[a]	−0.68 (−2.58, 1.22)	−0.40 (−0.77, −0.03)[c]	−0.33 (−0.94, 0.28)
AVLT Trial 6[a]	−0.02 (−1.95, 0.91)	−0.07 (−0.47, 0.33)	−0.19 (−0.82, 0.43)
AVLT Trial 6 intrusions[a]	0.08 (−0.65, 0.82)	0.07 (−0.08, 0.22)	0.12 (−0.12, 0.35)
AVLT Trial 7[a]	−0.88 (−4.10, 2.34)	−0.39 (−1.03, 0.26)	−0.46 (−1.50, 0.58)
AVLT Trial 7 intrusions[a]	0.09 (−0.65, 0.83)	−0.04 (−0.19, 0.12)	−0.19 (−0.42, 0.05)
UPSIT-TC[b]	−1.19 (−6.55, 4.18)	−0.48 (−1.47, 0.51)	−1.45 (−3.13, 0.22)[d]

AVLT = World Health Organization/University of California Los Angeles Auditory Verbal Learning Test; UPSIT-TC = University of Pennsylvania Smell Identification Test – Traditional Chinese.
[a] $N = 60$, except for fingernail manganese models, where $N = 55$. Models adjusted for age (continuous), years of completed education (continuous), and current factory of employment (ferroalloy/manufacturing).
[b] $N = 58$, except for fingernail manganese models, where $N = 53$. Model adjusted for age (continuous), years of completed education (continuous), and current factory of employment (ferroalloy/manufacturing) and current smoking status (yes/no).
[c] p value ≤0.05.
[d] p value >0.05 but ≤0.1.

Table 4
β (95% confidence interval) from adjusted linear regression models comparing tertiles of manganese biomarkers with cognitive and olfactory test scores.

Outcome	Blood manganese		Fingernail manganese		Bone manganese	
	Tertile 2, 12.4 to 15.3 μg/L	Tertile 3, ≥15.5 μg/L	Tertile 2, 2.2 to 27.7 μg/g	Tertile 3, ≥32.6 μg/g	Tertile 2, 0.7 to 5.1 μg/g	Tertile 3, ≥5.2 μg/g
Animal Naming[a]	1.21 (−1.80, 4.23)	−1.01 (−4.03, 2.02)	−1.94 (−5.74, 1.86)	−3.73 (−8.77, 1.32)	−0.89 (−3.79, 2.01)	−2.52 (−5.53, 0.50)[d]
Fruit Naming[a]	1.81 (−0.22, 3.85)[d]	−1.41 (−3.46, 0.63)	−2.31 (−5.09, 0.48)	−3.33 (−7.03, 0.37)[d]	0.30 (−1.78, 2.39)	−1.40 (−3.57, 0.76)
Average AVLT[a]	−0.12 (−1.27, 1.02)	−1.00 (−2.15, 0.15)[d]	−0.57 (−2.06, 0.91)	−1.71 (−3.68, 0.27)[d]	−0.11 (−1.20, 0.99)	−1.18 (−2.31, −0.04)[c]
Difference AVLT[a]	−0.54 (−1.78, 0.70)	−0.53 (−1.77, 0.72)	0.09 (−1.55, 1.73)	−0.39 (−2.57, 1.79)	−0.23 (−1.42, 0.97)	−0.80 (−2.04, 0.44)
AVLT Trial 6[a]	0.49 (−0.74, 1.73)	−0.62 (−1.86, 0.62)	0.98 (−0.70, 2.66)	0.31 (−1.92, 2.54)	−1.27 (−2.44, −0.08)[c]	−0.65 (−1.88, 0.57)
AVLT Trial 6 intrusions[a]	−0.17 (−0.64, 0.31)	0.11 (−0.37, 0.59)	0.14 (−0.49 0.78)	0.47 (−0.38, 1.31)	0.49 (0.04, 0.93)	0.53 (0.07, 0.99)[c]
AVLT Trial 7[a]	0.29 (−1.81 2.39)	−0.88 (−2.98, 1.23)	−1.75 (−4.48, 0.97)	−2.53 (−6.14, 1.09)	−0.71 (−2.75, 1.33)	−0.94 (−3.05, 1.18)
AVLT Trial 7 intrusions[a]	−0.15 (−0.63, 0.33)	−0.09 (−0.57, 0.40)	0.45 (−0.18, 1.08)	0.06 (−0.78, 0.90)	−0.09 (−0.56, 0.38)	−0.12 (−0.61, 0.37)
UPSIT-TC[b]	−0.30 (−3.81, 3.21)	0.02 (−341, 3.46)	1.89 (−2.20, 5.98)	−0.82 (−6.33, 4.69)	−1.02 (−4.33, 2.28)	−3.11 (−6.55, 0.33)[d]

AVLT = World Health Organization/University of California Los Angeles Auditory Verbal Learning Test; UPSIT-TC = University of Pennsylvania Smell Identification Test – Traditional Chinese. Tertile 1 for each biomarker is the referent group.

[a] $N = 60$, except for fingernail manganese models, where $N = 55$. Models adjusted for age (continuous), years of completed education (continuous), and current factory of employment (ferroalloy/manufacturing).

[b] $N = 58$, except for fingernail manganese models, where $N = 53$. Model adjusted for age (continuous), years of completed education (continuous), and current factory of employment (ferroalloy/manufacturing) and current smoking status (yes/no).

[c] p value ≤0.05.

[d] p value >0.05 but ≤0.1.

workers, there were no significant associations between BMn and any of the cognitive assessments (Bast-Pettersen et al., 2004). Similar results were also observed in an environmentally-exposed population. In a study assessing Mn exposure in 288 adults in Mexico, BMn was not significantly associated with any neuropsychological tests of cognitive function (Solís-Vivanco et al., 2009). Although not significant, one interesting result was that those in the highest tertile of BMn were associated with increasing UPSIT scores when compared to the lowest tertile. Similar results occurred in a study conducted by Antunes et al. (2007) in a group of bridge welders. Welders with the higher BMn levels had higher UPSIT scores than those with lower BMn levels.

However, our results for BMn are in contradiction with other previous research. In occupational studies, BMn has previously been associated with decreased visual and spatial working memory (Hassani et al., 2016); decreased IQ, verbal learning, and immediate memory in a group of Bay Bridge welders (Bowler et al., 2007), and lower memory scores among ferroalloy workers compared to controls (Lucchini et al., 1999). Additionally, a pilot study conducted on an environmentally-exposed population saw significant associations between cognitive performance and BMn concentrations >15 μg/L (Santos-Burgoa et al., 2001). The differences in results between studies could be due to the relatively short $t_{1/2}$ of Mn in blood due to the homeostatic control of Mn by absorption and liver excretion in the body (Aschner and Aschner, 2005; Menezes-Filho et al., 2009; Costa and Aschner, 2015). This natural process can lead to substantial sample variability (Baker et al., 2014). Additionally, these cross-sectional studies assessed different populations, utilized several different cognitive tests, and incorporated different covariates in models; all of which can influence study results.

There are several limitations of this study. First, the cross-sectional design of the study limits our ability to establish temporality. However, cross-sectional studies can be valuable in generating hypotheses and data to be tested in future longitudinal studies. Additionally, our use of BnMn, a cumulative biomarker correlated with the prior 15–16 years of exposure (Rolle-McFarland et al., 2018), strengthens the hypothesis that long-term exposures likely play a major role in the association of Mn with cognitive function.

Another limitation of our study was the relatively small population size which could reduce study power. However, participants in this study had very high Mn exposure, which in turn would tend to increase study power. At the same time, including a population with high Mn exposure limits the extent to which our results can be extrapolated to those with lower, particularly environmental, Mn exposure. Future studies will need to be conducted to address whether these associations are also observed in populations with lower Mn exposure.

The small study sample also influenced our decision to retain BnMn and FMn values, below their respective DLs, for our statistical analyses. Keeping values below the DL allowed us to reduce potential bias from imputation of the data. Additionally, there is the possibility that Mn-containing dust on participants' hands could have influenced the FMn and BnMn concentrations. However, we feel that this is unlikely due to the rigorous cleaning protocols we undertook in this study. Participants washed their hands before cutting fingernails, and cleaned them a second time prior to collection of BnMn data. Cut fingernails were also thoroughly cleaned, twice, prior to ICP-MS analysis. Overall, results from our sensitivity analysis, where the participants who were most at risk of having contaminated measurements were removed, were generally similar to our main results (Supplementary Table 1). Fewer of the comparisons in the sensitivity analysis were statistically significant; however this could be due to the smaller sample size. Though we cannot completely rule out the possibility of contamination of some samples, our sensitivity analysis suggests that this is not likely to invalidate our main results.

This study also has several strengths. Use of the IVNAA system to measure BnMn is a considerable strength of this study as this technology allowed our team to quantify cumulative manganese exposure in an occupational population. Advantages of this technology are that it summarizes exposures from multiple sources and does not rely on self-reported data. Another strength of this study was that we utilized multiple measures of Mn exposure to allow direct comparison between our work and previous literature on cognitive and olfactory impacts of manganese exposure. In addition, we selected cognitive and olfactory tests that have been widely used in environmental epidemiology studies as well as validated for use in Chinese-speaking populations. Utilizing English and Mandarin bilingual research assistants both fluent in English and Mandarin helped to further strengthen the study by reducing communication issues between study staff and participants.

5. Conclusions

Overall, our results suggest increased concentrations of BnMn and FMn, but not BMn, are associated with reduced performance on cognitive function tests. They also suggest that none of the biomarkers are associated with reduced performance on tests of olfactory function. Future work should assess the association between BnMn and motor function as Mn exposure has also been associated with decreases in motor function and the development of the motor disease manganism.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.208.

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