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Iron species in cerebrospinal fluid and dementia risk in subjects with mild cognitive impairment: A cohort study

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ABSTRACT

Background: Iron dysregulation has been implicated in the pathogenesis of dementia, since it is an essential nutrient for neuronal function, but also contributes to oxidative stress and neurotoxicity at elevated levels.
Methods: We enrolled 56 individuals with newly-diagnosed mild cognitive impairment (MCI) and followed over a 47-month period to monitor conversion to dementia according to baseline percentage concentrations of cerebrospinal fluid iron species.
Results: In this cohort, 28 participants developed Alzheimer's dementia, 5 frontotemporal dementia, 2 Lewy body dementia, and 2 vascular dementia during the follow-up. Higher Fe-Ferritin was associated with a higher though statistically unstable dementia risk (hazard ratio-HR 1.36 for 10-unit % increase, 95 % confidence interval-CI 0.88–2.11), while Fe-Transferrin was linked to a lower risk (HR 0.65, 95 % CI 0.21–2.08) and inorganic Fe showed little association (HR 1.06, 95 % CI 0.80–1.40). Patterns of association were non-linear: inorganic Fe had a U-shaped association, with reduced risk at 25–40 % and increased risk above 45 %; Fe-Ferritin showed an inverted U-shaped relation with higher risk between 10 % and 20 %; Fe-Transferrin showed almost no relation with dementia risk. When considering conversion to Alzheimer's dementia only, the relation was similarly U-shaped for inorganic Fe and almost null for Fe-Transferrin, while Fe-Ferritin showed a positive relation with risk above 15 %.
Conclusions: Despite the statistical imprecision of the estimates, our study provides novel evidence linking iron species in cerebrospinal fluid to dementia risk in individuals with MCI. These findings also underscore the importance of elemental speciation in dementia research.

1. Introduction

Mild cognitive impairment (MCI) is clinical condition between normal cognitive functioning and clinically overt dementia. This condition has a high risk of progression to dementia, including Alzheimer's dementia (AD) (Sanford, 2017).

Several environmental factors, possibly interacting with genetic determinants, have been investigated for their ability to affect such a progression, including air pollution, social isolation, toxic chemicals and trace elements (Livingston et al., 2024; Urbano et al., 2023; White and Brand, 2019). These latter trace elements generally enter the body through the gut or lung and are distributed in the bloodstream and transported to the brain. Some of them may also reach the central nervous system through the olfactory pathway and cross the blood-brain barrier, thus accumulating in the brain (White and Brand, 2019). Trace elements may be of both nutritional and toxicological relevance and are frequently considered critical for maintaining cognitive function in humans. Several studies have indicated an imbalance in trace

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elements in patients affected by different forms of dementia, suggesting either their role in disease etiology or an effect of the disease itself, and therefore the need for longitudinal studies to reduce the risk of reverse causation (Huat et al., 2019; Liu et al., 2022; Urbano et al., 2022; Vinceti et al., 2019, 2023).

Among trace elements, iron has a key role for both its neurotoxic properties and its key nutritional role (Li et al., 2023a,b). The dietary reference intake for iron varies according to age and sex, and maintenance of iron status is required for most energy and substrate metabolism pathways in the human body (Berger et al., 2022). Accumulation of this element in the brain is a recognized hallmark of aging and could promote ferroptosis. Excess iron storage may increase pro-oxidant reactions and could contribute to neurodegeneration through several mechanisms, including β-amyloid deposition, tau hyperphosphorylation, and neuronal loss (Chen et al., 2023; Gutierre et al., 2025; Li et al., 2023a,b). Nonetheless, a recent randomized clinical trial suggested that lowering iron with deferiprone, a brain-permeable iron chelator, was detrimental in patients with AD (Ayton et al., 2025). One explanation of this constrasting finding might be that the chemical speciation of these elements significantly influences their biological and environmental impact and their health effects. This is particularly notable for trace elements such as selenium and manganese, where different chemical forms exhibit distinct bioavailability and interactions with biological systems (Filippini et al., 2017; Michalke et al., 2007; Vinceti et al., 2013, 2025). Similar findings have been observed for iron, which exists in several forms, including ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions, as well as complexed forms such as heme and non-heme iron (Brittenham and Fairweather-Tait, 2023). However, the role and impact of different iron species in health and disease is far less investigated and understood. Specifically, there is some evidence that Fe^{2+} species can participate in Fenton and Fenton-like reactions to generate hydroxyl radical associated with the pathological progression of AD (Zhao, 2019). With reference to iron-related proteins, higher levels of ferritin were observed in the cerebrospinal fluid (CSF) of AD patients compared to controls (Gong et al., 2023).

In this cohort study, we aimed to assess the concentrations of iron species in subjects diagnosed with MCI, and to assess the related risk of subsequently converting to dementia.

2. Methods

2.1. Study population

We asked to participate in the study subjects without of dementia, but consecutively receiving an MCI diagnosis, either amnestic MCI (single domain or multiple domain) or non-amnestic MCI, from 2008 to 2014 at the Neurology Memory Clinic of Modena University Hospital, Northern Italy (Urbano et al., 2023). MCI diagnoses were made according to established diagnostic criteria (Limongi et al., 2019; Winblad et al., 2004). The study received approval by the Modena Ethics Committee (no. 84/2015) and was carried out in accordance with the principles of the Helsinki declaration. Participants were eligible for inclusion if they provided informed consent, underwent a lumbar puncture for diagnostic purposes, and had no abnormalities on brain imaging or medical history indicative of a vascular cause for their cognitive impairment. Individuals not meeting these criteria were excluded.

Each participant underwent a comprehensive neurological, neuropsychological, and neuroradiological workup, along with lumbar puncture. We also collected data regarding sociodemographic factors, namely sex, date and place of birth, educational attainment, and smoking habits. Inclusion in the study was restricted to those patients who provided written informed consent for the utilization of CSF samples along with their personal data for research purposes. A total of 71 subjects met the eligibility criteria, after the exclusion of 15 of them for unavailability of CSF samples, the study cohort finally consisted of 56 individuals. We then followed-up participants every six months until August 2021 and at each time point classified as non-converters or converters to dementia. Diagnosis of dementia included the form due to AD, frontotemporal dementia (FTD), Lewy body dementia (LBD), or vascular dementia (VaD) (Dubois et al., 2021; Fabrizi et al., 2024; McKeith et al., 2005; Rascovsky et al., 2011). No lumbar puncture was performed at follow-up.

2.2. Analytical determinations

We collected CSF samples through lumbar puncture according to Standard International Procedures for CSF Biobanking in fasting patients (Willemse and Teunissen, 2015) as described in detail elsewhere (Urbano et al., 2022). After the lumbar puncture was performed, CSF samples arrived at the Neuro-Immunology Laboratory within 30 min. from collection. Each sample was then anonymized with an alpha-numeric code and immediately centrifuged at 2500 × *g* for 10 min at controlled room temperature. If analytical determination was not available straightaway after the centrifugation, samples were aliquoted into polypropylene sterile vials and kept frozen at −80°C until testing. Manual sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) method (INNOTEST® β-amyloid(1−42), hTau Ag and phospho-Tau (181 P), Innogenetics, Ghent, Belgium) was used for the quantification of beta 1–42 (Aβ1–42), total (t-tau), and phosphorylated tau (p-tau) proteins. Cut-off values used in the laboratory were the following: t-tau= 350 pg/mL; p-tau= 61 pg/mL; β-amyloid$_{1–42}$= 500 pg/mL (Tondelli et al., 2022).

2.3. Iron speciation

We performed iron speciation analysis alongside selenium speciation (Vinceti et al., 2017). For iron species determination, a Knauer 1100 Smartline inert Series gradient HPLC system was equipped with an ion exchange column AS-11 (250 ×4 mm I.D.) from Thermo Fischer Scientific Inc. (Sunnyvale, California, USA). Undiluted CSF samples (20 μL) were determined in duplicate. The mobile phase consisted of eluent A (3.33 mM Tris-HAc buffer, 5 % methanol, pH 8.0) and eluent B (10 mM Tris-HAc buffer, 500 mM ammonium acetate, 5 % Methanol, pH 8.0). Gradient elution was as reported in (Vinceti et al., 2017) as % eluent B, in brief: 0–3 min 0 %; 3–10 min 0–40 %; 10–23 min 40–55 %; 23–26 min 55–57 %; 26–28 min 57–100 %; 28–52 min 0 −100 %. Flow rate was constant at 0.8 mL/min. The column effluent was directed to ICP-DRC-MS. The experimental settings for ICP-DRC-MS (NexIon 300 D, Perkin Elmer) were: radio frequency power: 1250 W; plasma gas flow: 15 L Ar/min; auxiliary gas flow: 1.05 L Ar/min; nebulizer gas flow: 0.92 L Ar/min; daily optimized, dwell time 300 ms; the monitored iron isotopes were ^{56}Fe and ^{57}Fe. DRC reaction gas: CH4 reaction at 0.58 mL/min; DRC rejection parameter q: 0.6. Data files from iron chromatograms were processed with Peakfit™ software for peak area evaluation. Example of iron chromatogram is reported in Supplemental Figure S1. For each sample, iron peaks were monitored and their peak area determined as % from total iron. Peak area % particularly were calculated for iron species identified by standard match, i.e., for Inorganic Fe (10.6 min), Fe-Transferrin (17.0 min), and Fe-Ferritin (33.8 min).

2.4. Data analysis

We used descriptive statistics with median and interquartile range (IQR) and number and percentage (%) to report results. We used boxplots for graphical comparison of baseline iron species peak area % of total iron divided by main subjects' characteristics, namely sex, age (<65 and ≥65 years), education (elementary school, middle school, and high school or higher), and subsequent diagnosis at follow-up (stable MCI, converted to AD, FTD, LB and VaD). We then estimated correlation between iron species peak % using the Pearson correlation coefficient matrix expressed as a heatmap.

We performed linear and spline regression analyses, this latter using restricted cubic splines with three knots at fixed percentiles (10th, 50th, and 90th) to assess the non-linear association between iron species peak % and neurodegeneration and amyloidosis biomarkers (β-amyloid$_{1–42,}$ t-tau and p-tau).

We then computed hazard ratios (HRs) and 95 % confidence interval (CI) of MCI conversion to dementia using Cox proportional hazards in multivariable models using both linear (by 10 %-unit increase) and non-linear (by restricted cubic spline model with three knots at fixed 10th, 50th, and 90th percentiles).

For this purpose, we defined person-time at risk as the time range between the first MCI diagnosis and the date in which dementia diagnosis was made, or the last follow-up date (August 2021), whichever occurred earlier. To allow for different baseline hazard functions across different sex groups (i.e., male and female), we stratified the analyses by sex adding the option 'strata' in the Cox regression command. To reduce the effect of the outliers by assigning them a lower weight, we performed winsorization for Fe-Ferritin at the highest value observed, i.e., 50.84 %. We adjusted for age at entry as continuous variable, educational attainment, and smoking status. We also added alternatively selenate and selenoprotein P as adjustment covariates given their previously described positive association with dementia risk in the study population (Urbano et al., 2022; Vinceti et al., 2017, 2023). Finally, we added neurodegeneration and amyloidosis biomarkers (β-amyloid$_{1–42}$, t-tau and p-tau) to the main model to take into account also the relation between such biomarkers.

We performed the analysis in the overall population to estimate risk of all dementia, and risk of AD only.

We used the "correlate", "heatplot", "mkspline", "regress", "stcox", "stset", and "winsor" Stata routines (version 18.0, Stata Corp., College Station, TX, 2023) to carry out all analyses.

3. Results

We eventually recruited 56 MCI subjects, 30 males and 26 females with a median age of 66.2 years, during the study period. Of the 56 subjects 28 converted to AD, 5 developed FTD, 2 LBD, and 2 vascular dementia. Median follow-up duration corresponded to 48 months for MCI subjects (interquartile range, IQR 35–54 months) and 37 months for dementia converters (IQR 28–48 months). Subjects converting to dementia did not substantially differ from non-converters in terms of age, sex, or educational attainment level. T-tau and p-tau were found to be higher in subjects converting to dementia compared to non-converters, while β-amyloid$_{1–42}$ concentrations were lower in converters compared to non-converters (Table 1).

Based on diagnosis at follow-up, baseline inorganic Fe was higher in converters compared to non-converters, while baseline Fe-Transferrin and Fe-Ferritin were slightly lower in converters compared to non-converters (Table 1 and Fig. 1). Concentrations of the three baseline iron species investigated according to sex, age, educational attainment level are reported in Fig. 1.

In Pearson's correlation analysis, inorganic Fe was inversely associated with both Fe-Transferrin and Fe-Ferritin, while the latter were positively correlated each other (Fig. 2). In spline regression analyses evaluating the association with biomarkers of neurodegeneration and amyloidosis, inorganic Fe was inversely U-shaped associated with amyloid levels, also showing a U-shaped association with tau proteins, with midpoints detected at peaks of 35 %. Fe-Transferrin and Fe-Ferritin were not associated and positively associated with β-amyloid$_{1–42}$ levels, respectively. These forms were also inversely associated with tau proteins, although an entirely linear inverse correlation was observed for Fe-Ferritin, while for Fe-Transferrin the inverse relation existed only up to the peak of 5 % (Fig. 3).

In linear analysis about iron species, we found a HR of converting to dementia for Fe-Ferritin slightly above the null (HR 1.03, 95 % CI 0.99–1.07), and slightly below for Fe-Transferrin (HR 0.96, 95 % CI 0.85–1.09). Inorganic Fe was not associated with risk of converting to dementia (HR 1.00, 95 % CI 0.98–1.03). When adjusting for potential confounders, we observed almost no association between the three compounds and dementia risk (HR 1.01, 95 % CI 0.98–1.04 for Inorganic Fe; HR: 0.97, 95 % CI 0.85–1.10 for Fe-Transferrin; HR: 1.02, 95 % CI 0.98–1.07 for Fe-Ferritin).

In spline regression analysis, we found different and generally non-linear patterns of associations between the three iron species investigated and the risk of dementia (Fig. 4). Inorganic Fe exhibited a U-shaped association with dementia risk, with lower risk observed for the range 25–40 % of total iron, and increased risk above 45 %. Conversely, Fe-Transferrin showed almost null relation with dementia risk. For Fe-

Table 1
Characteristics of included subjects at baseline and distribution of iron species.

	Baseline (All, n = 56)		Stable (MCI) at follow-up (n = 19)		Converters at follow-up (n = 37)	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Age	66.2	62.8–70.8	64.5	59.4–70.2	66.2	62.8–70.8
Education (years)	8	5–13	8	5–13	8	8–13
	N	**%**	**N**	**%**	**N**	**%**
Age (categories)						
< 65 years	24	42.9	10	52.6	14	37.8
≥ 65 years	32	57.1	9	47.4	23	62.2
Sex						
Males	30	53.6	11	57.9	19	51.4
Females	26	46.4	8	42.1	18	48.7
Education (categories)						
Elementary school	18	32.1	9	47.4	9	24.3
Middle school	16	28.6	3	15.8	13	35.1
High school or higher	22	39.3	7	36.8	15	40.5
Smoking						
Yes	7	12.5	1	5.3	6	16.2
No	49	87.5	18	94.7	31	83.8
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
t-tau (pg/mL)	372.0	220.0–619.5	255.0	164.0–370.0	482.0	255.0–699.0
p-tau (pg/mL)	69.0	49.5–88.0	53.3	46.0–82.0	83.0	56.0–105.0
β-amyloid$_{1–42}$ (pg/mL)	596.0	448.5–797.5	789.0	521.0–1021.0	513.0	417.0–686.0
Iron species (Peak area %)						
Inorganic Fe	36.1	29.2–43.8	30.1	27.0–39.4	38.2	29.3–47.3
Fe-Transferrin	1.2	0.4–2.7	1.7	0.9–2.9	1.0	0.3–2.5
Fe-Ferritin	12.0	8.2–15.5	12.1	6.8–14.9	11.9	8.3–15.6

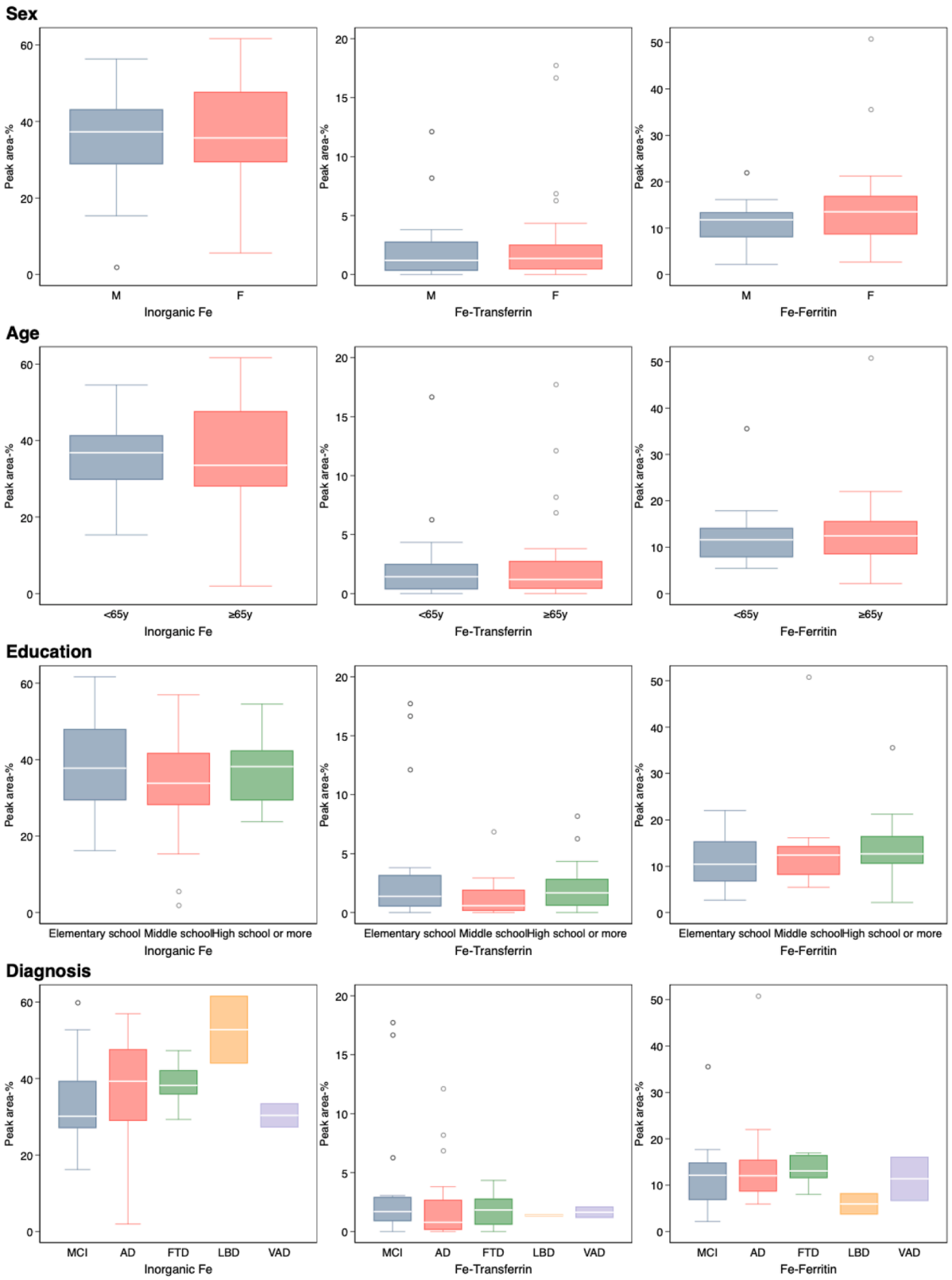

Fig. 1. Boxplots of baseline iron species peak area % of total iron (Fe) according to sex, age, education, and subsequent diagnosis at follow-up (n = 56 overall; 19 mild cognitive impairment - MCI, 28 Alzheimer's dementia - AD, 5 frontotemporal dementia - FTD, 2 Levy body dementia - LBD, 2 vascular dementia VAD).

Ferritin a slight indication of an inverted U-shaped relation emerged, with a peak in risk observed between 10 % and 20 % of Fe-Ferritin (Fig. 4). When excluding subjects converting to dementia other than AD (9 subjects), inorganic Fe was still U-shaped associated with risk of AD, although the curve indicated a less steep increase in risk at higher exposure levels, as compared with the overall dementia outcome. Similarly, the relation was almost null for Fe-Transferrin, while Fe-Ferritin showed a positive association with AD risk above 15 %.

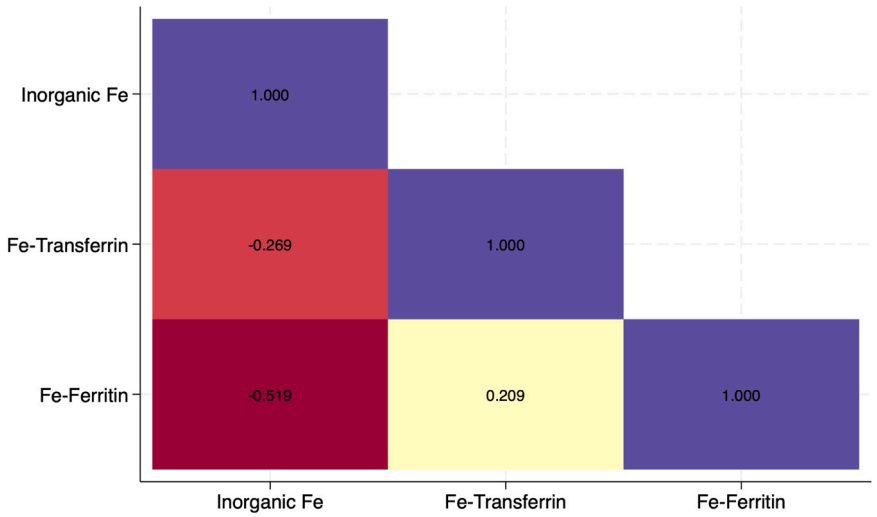

Fig. 2. Pearson's correlation heatmap of baseline iron species.

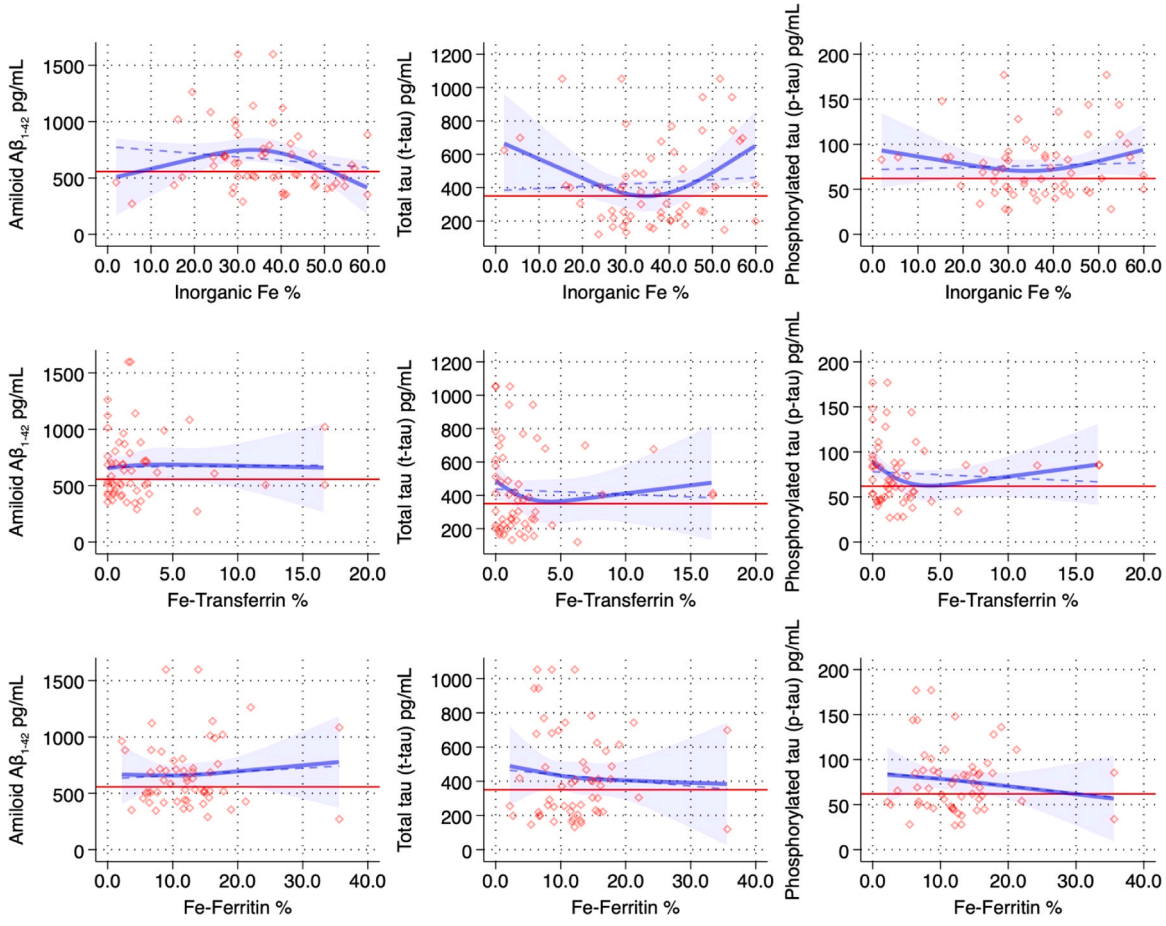

Fig. 3. Regression analyses of the association between baseline iron species and Alzheimer's dementia pathology biomarkers. The solid blue line represents spline regression analysis with 95 % confidence interval in the blue shaded area. Dashed blue line represent regression analysis assuming a linear model. Red diamonds represent individual observations. Analyses are adjusted for age, sex, educational attainment and smoking status. Red line represents cut-off laboratory values.

We performed additional analyses by alternatively adding selenate and selenoprotein P as adjustment covariates, given their previously described association with dementia risk in the study population. While we did not find substantially different results in the model including selenate (Supplemental Figure S2), the analysis adjusted for selenoprotein P concentrations showed a much steeper positive association between Fe-Transferrin and Fe-Ferritin with AD risk, as compared to the less adjusted model (Supplemental Figure S3). Incorporation of β-amyloid$_{1-42}$, t-tau and p-tau levels did not substantially alter the association with the exception of p-tau and conversion to AD, with steeper positive association for Fe-Transferrin and Fe-Ferritin (Supplemental Figures S4-S6).

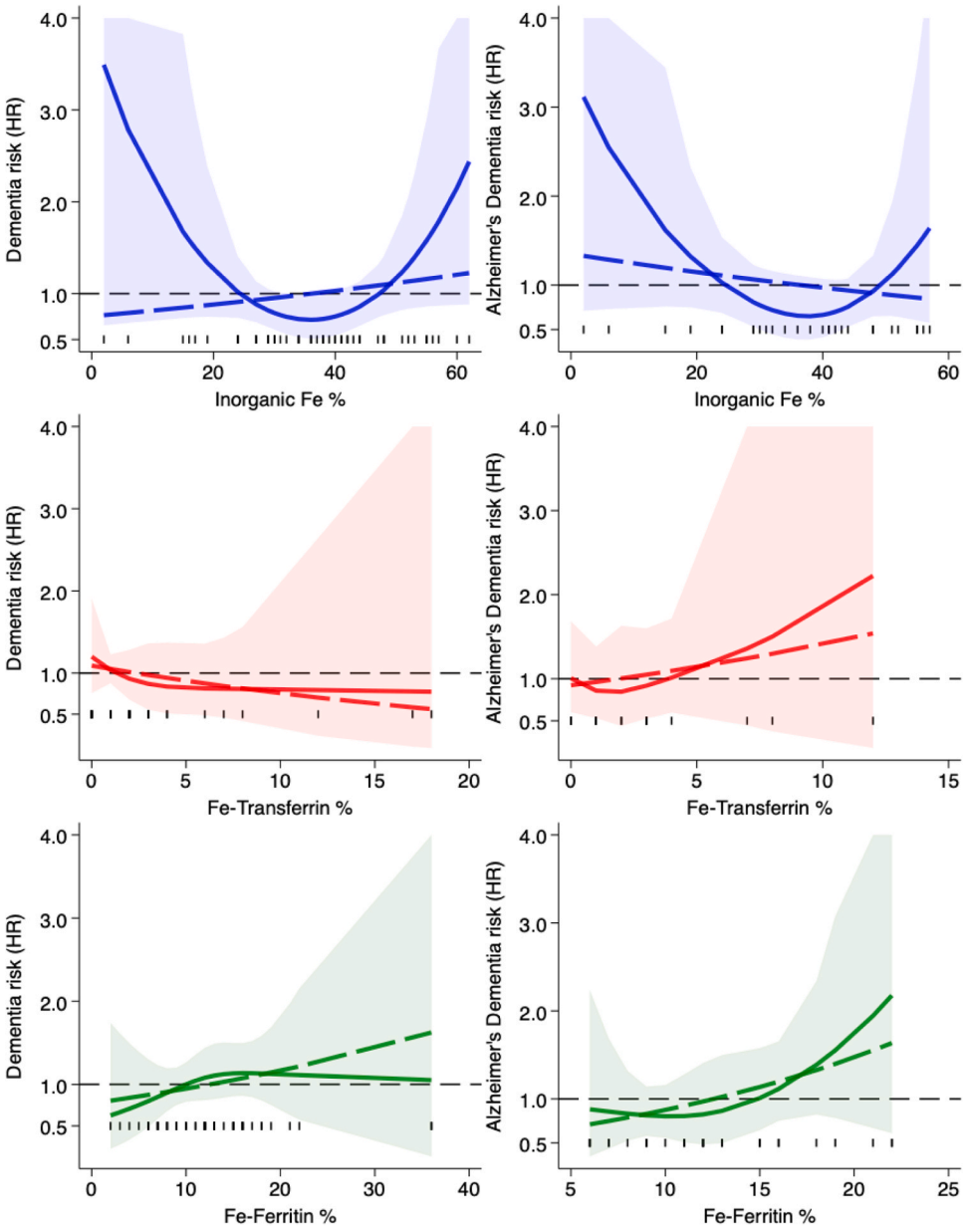

Fig. 4. Regression analysis of the association between baseline iron species and risk of developing any dementia (left panels) or Alzheimer's dementia (AD – right panels). The solid line indicates hazard ratio (HR) and the shaded areas the 95 % confidence intervals. Dashed line represents regression analysis assuming a linear model. Analyses are adjusted for age, sex, educational attainment and smoking status. Black line represents null risk (HR=1). Black vertical lines represent individual values of Fe species.

4. Discussion

In this study, we found that inorganic Fe had a U-shaped association with subsequent incidence of overall dementia and AD, while Fe-Ferritin levels predicted conversion to AD only at the highest levels of exposure. Transferrin and ferritin are key proteins involved in iron transport and storage, respectively (Daru et al., 2017; Zeng et al., 2023). In our study, both species were positively correlated, but also showing a negative correlation with inorganic Fe. This latter iron species is less bioavailable compared to its protein-bound forms. Notably, inorganic Fe^{2+} has the potential to generate reactive oxygen species, leading to oxidative stress (Dignass et al., 2018) and therefore can cause harmful effects on human brain. Iron is vital for brain functioning, including myelination, oxidative metabolism, and neurotransmitter synthesis.

Diminished iron concentrations have been associated with poorer cognitive ability during late adolescence (Larsen et al., 2020). However, too high iron levels may be neurotoxic and brain iron accumulation during aging is one of the first biochemical hallmarks identified in AD (Yasar et al., 2024). Even relatively low levels of iron can accelerate dementia progression, actively contributing to progression of the neurodegenerative process once it has started (Ayton et al., 2021). Studies investigating the relation between iron and dementia have yielded very conflicting results, suggesting that both low and high iron status, as assessed through dietary intake or blood levels, can influence cognitive health (Shi et al., 2019). In a recent study, low iron intake was associated with higher incidence of young-onset dementia, whilst excessive iron intake was linked to higher dementia risk above 65 years (Liu et al., 2023). Moreover, in a recent trial reducing brain iron levels with deferiprone, iron reduction appeared to be detrimental and accelerated cognitive decline in individuals with AD (Ayton et al., 2025). However, these studies could be affected by exposure misclassification, as dietary iron intake does not necessarily reflect circulating iron levels,

being its absorption highly variable and influenced by several factors, while the human organism lacks an active mechanism for iron excretion (EFSA Panel on Dietetic Products and Allergies, 2015).

Most studies measured brain iron through magnetic resonance imaging, while few used CSF levels to assess iron status (Bartzokis et al., 2007). In two previous studies longitudinally assessing the association between CSF ferritin and AD outcomes, ferritin concentrations were related to worst cognitive performance and predicted MCI conversion to AD. A strong positive association between ferritin and apolipoprotein E levels was also detected (Ayton et al., 2015; Diouf et al., 2019). In our study, high Fe-Ferritin predicted AD risk, but not overall dementia risk. This discrepancy might suggest that Fe-Ferritin dysregulation may be more specifically linked to AD pathology rather than dementia as a broader category. However, to date, no epidemiological study has systematically examined specific iron compounds in relation to the differential association with AD or overall dementia risk. With regards to the association with AD biomarkers, higher Fe-Ferritin % demonstrated a slight positive relation with amyloid β_{1-42} levels and a negative correlation with tau proteins measured at baseline (Fig. 3). This latter result needs further investigation, given the contrasting findings obtained from the longitudinal assessment, where Fe-ferritin was selectively associated with AD risk.

With reference to Fe-Transferrin, we did not find any association with either overall dementia or AD risk, which is somewhat contrasting with a previous study, in which higher plasma transferrin levels were associated with a steeper cognitive decline in MCI and AD groups (Guan et al., 2020). Such a discrepancy might be due to the different matrix used (CSF versus plasma) to assess iron status. However, in the sensitivity analysis adjusting for selenoprotein P, previously found to be positively associated with dementia risk in this study cohort (Urbano et al., 2022; Vinceti et al., 2023), we found a positive association between Fe-Transferrin and AD risk (Supplemental Figure S3), suggesting an independent role of this iron species in predicting disease risk. Future studies should, however, further explore the interaction between selenium species and selenoproteins, especially selenoprotein P and iron species. Investigating how selenoprotein P relates to iron metabolism could provide information on their potential synergistic or antagonistic roles in modulating oxidative stress.

In this study, we also observed a U-shaped association of inorganic Fe with AD biomarkers and dementia risk. This suggests that both too low and too high levels of this iron form may be harmful, with reference to dementia risk. Inadequate inorganic Fe levels may correspond to insufficient availability for chemical oxidation to Fe^{3+} and incorporation into ferritin, i.e., the primary intracellular iron reservoir. Conversely, too high levels might exert adverse effects due to their pro-oxidant properties, particularly oxidative stress, a key mechanism involved in cognitive decline and neurodegeneration (Bai et al., 2022; Ionescu-Tucker and Cotman, 2021).

Strengths of this study include the longitudinal design, allowing us to provide stronger evidence for causality as compared to cross-sectional studies, and the speciation analysis of iron, a major difference from studies based only on overall iron exposure, consistently with what observed for other elements such as copper, zinc, and selenium (Ajsuvakova et al., 2020; Filippini et al., 2023; Urbano et al., 2024; Willkommen et al., 2018). In addition, we used a key matrix for the assessment of exposure to iron species, CSF, a compartment more appropriate and relevant for central nervous system disease compared with blood levels. In fact, while serum is the most used noninvasive assessment of iron levels, CSF levels are more likely to accurately reflect iron transport within the central nervous system (CNS) and there is evidence for using CSF biomarkers as surrogate indicators of iron metabolism and neuronal injury in the brain (Ayton et al., 2023). Concerning limitations, our study had a limited sample size, a factor reducing the statistical precision of the risk estimates, especially in fully adjusted models. In addition, we were unable to measure iron during the follow-up, a limitation due to the challenges of performing repeated lumbar punctures. Finaly, we obtained only indirect measurement (peak area % of total iron in CSF) of iron and iron species, instead of absolute concentrations, caused primarily by methodical parameter which had been optimized for the prioritized, parallel-performed selenium speciation analysis.

In conclusion, our results suggest that higher CNS inorganic Fe content is associated with higher risk of converting to dementia in subjects with MCI, while Fe-Ferritin and Fe-Transferrin seemed to have no association with the disease. However, with reference to conversion to AD, also Fe-Ferritin exceeding 15 % of the total iron concentrations may be associated with a higher risk. The possible causal or simply predictive role of dementia onset by some iron species warrants further investigation.

CRediT authorship contribution statement

Tommaso Filippini: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Marco Vinceti:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Teresa Urbano:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Annalisa Chiari:** Writing – review & editing, Supervision, Resources, Methodology. **Bernhard Michalke:** Writing – review & editing, Methodology, Formal analysis. **Carlotta Malagoli:** Writing – review & editing, Data curation. **Manuela Tondelli:** Writing – review & editing, Resources. **Roberta Bedin:** Writing – review & editing, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2025.06.006.

Data availability

The datasets supporting the conclusions of this article are included within the article and available from the corresponding upon reasonable request.

References

Ajsuvakova, O.P., Tinkov, A.A., Willkommen, D., Skalnaya, A.A., Danilov, A.B., Pilipovich, A.A., Aschner, M., Skalny, A.V., Michalke, B., Skalnaya, M.G., 2020. Assessment of copper, iron, zinc and manganese status and speciation in patients with Parkinson's disease: a pilot study. J. Trace Elem. Med. Biol. 59, 126423. https://doi.org/10.1016/j.jtemb.2019.126423.

Ayton, S., Barton, D., Brew, B., Brodtmann, A., Clarnette, R., Desmond, P., Devos, D., Ellis, K.A., Fazlollahi, A., Fradette, C., Goh, A.M.Y., Kalinowski, P., Kyndt, C., Lai, R., Lim, Y.Y., Maruff, P., O'Brien, T.J., Rowe, C., Salvado, O., Schofield, P.W., Spino, M., Tricta, F., Wagen, A., Williams, R., Woodward, M., Bush, A.I., 2025. Deferiprone in Alzheimer disease: a randomized clinical trial. JAMA Neurol. 82 (1), 11–18. https://doi.org/10.1001/jamaneurol.2024.3733.

Ayton, S., Faux, N.G., Bush, A.I., Alzheimer's Disease Neuroimaging, I., 2015. Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. Nat. Commun. 6, 6760. https://doi.org/10.1038/ncomms7760.

Ayton, S., Janelidze, S., Kalinowski, P., Palmqvist, S., Belaidi, A.A., Stomrud, E., Roberts, A., Roberts, B., Hansson, O., Bush, A.I., 2023. CSF ferritin in the clinicopathological progression of Alzheimer's disease and associations with APOE and inflammation biomarkers. J. Neurol. Neurosurg. Psychiatry 94 (3), 211–219. https://doi.org/10.1136/jnnp-2022-330052.

Ayton, S., Portbury, S., Kalinowski, P., Agarwal, P., Diouf, I., Schneider, J.A., Morris, M. C., Bush, A.I., 2021. Regional brain iron associated with deterioration in Alzheimer's disease: a large cohort study and theoretical significance. Alzheimers Dement. 17 (7), 1244–1256. https://doi.org/10.1002/alz.12282.

Bai, R., Guo, J., Ye, X.Y., Xie, Y., Xie, T., 2022. Oxidative stress: the core pathogenesis and mechanism of Alzheimer's disease. Ageing Res. Rev. 77, 101619. https://doi.org/10.1016/j.arr.2022.101619.

Bartzokis, G., Tishler, T.A., Lu, P.H., Villablanca, P., Altshuler, L.L., Carter, M., Huang, D., Edwards, N., Mintz, J., 2007. Brain ferritin iron may influence age- and gender-related risks of neurodegeneration. Neurobiol. Aging 28 (3), 414–423. https://doi.org/10.1016/j.neurobiolaging.2006.02.005.

Berger, M.M., Shenkin, A., Schweinlin, A., Amrein, K., Augsburger, M., Biesalski, H.K., Bischoff, S.C., Casaer, M.P., Gundogan, K., Lepp, H.L., de Man, A.M.E., Muscogiuri, G., Pietka, M., Pironi, L., Rezzi, S., Cuerda, C., 2022. ESPEN micronutrient guideline. Clin. Nutr. 41 (6), 1357–1424. https://doi.org/10.1016/j.clnu.2022.02.015.

Brittenham, G.M., Fairweather-Tait, S., 2023. Iron. Adv. Nutr. 14 (5), 1241–1243. https://doi.org/10.1016/j.advnut.2023.06.011.

Chen, L.L., Fan, Y.G., Zhao, L.X., Zhang, Q., Wang, Z.Y., 2023. The metal ion hypothesis of Alzheimer's disease and the anti-neuroinflammatory effect of metal chelators. Bioorg. Chem. 131, 106301. https://doi.org/10.1016/j.bioorg.2022.106301.

Daru, J., Colman, K., Stanworth, S.J., De La Salle, B., Wood, E.M., Pasricha, S.R., 2017. Serum ferritin as an indicator of iron status: what do we need to know? Am. J. Clin. Nutr. 106 (6), 1634S–1639S. https://doi.org/10.3945/ajcn.117.155960.

Dignass, A., Farrag, K., Stein, J., 2018. Limitations of serum ferritin in diagnosing iron deficiency in inflammatory conditions. Int J. Chronic Dis. 2018, 9394060. https://doi.org/10.1155/2018/9394060.

Diouf, I., Fazlollahi, A., Bush, A.I., Ayton, S., Alzheimer's disease Neuroimaging, I., 2019. Cerebrospinal fluid ferritin levels predict brain hypometabolism in people with underlying beta-amyloid pathology. Neurobiol. Dis. 124, 335–339. https://doi.org/10.1016/j.nbd.2018.12.010.

Dubois, B., Villain, N., Frisoni, G.B., Rabinovici, G.D., Sabbagh, M., Cappa, S., Bejanin, A., Bombois, S., Epelbaum, S., Teichmann, M., Habert, M.O., Nordberg, A., Blennow, K., Galasko, D., Stern, Y., Rowe, C.C., Salloway, S., Schneider, L.S., Cummings, J.L., Feldman, H.H., 2021. Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. Lancet Neurol. 20 (6), 484–496. https://doi.org/10.1016/S1474-4422(21)00066-1.

EFSA Panel on Dietetic Products and Allergies, 2015. Scientific opinion on dietary reference values for iron. EFSA J 13 (10), 4254. https://doi.org/10.2903/j.efsa.2015.4254.

Fabrizi, E., Ancidoni, A., Locuratolo, N., Piscopo, P., Della Gatta, F., Salemme, S., Pani, S. M., Marconi, D., Vignatelli, L., Sagliocca, L., Caffarra, P., Secreto, P., Guaita, A., Stracciari, A., Vanacore, N., Lacorte, E., Guideline Working, G., 2024. The Italian guideline on diagnosis and treatment of dementia and mild cognitive impairment. Age Ageing 53 (11), afae250. https://doi.org/10.1093/ageing/afae250.

Filippini, T., Michalke, B., Grill, P., Malagoli, C., Malavolti, M., Vescovi, L., Sieri, S., Krogh, V., Cherubini, A., Maffeis, G., Lucchini, R., Ferrante, M., Vinceti, M., 2017. Determinants of serum manganese levels in an Italian population. Mol. Med. Rep. 15 (5), 3340–3349. https://doi.org/10.3892/mmr.2017.6379.

Filippini, T., Urbano, T., Grill, P., Malagoli, C., Ferrari, A., Marchesi, C., Natalini, N., Michalke, B., Vinceti, M., 2023. Human serum albumin-bound selenium (Se-HSA) in serum and its correlation with other selenium species. J. Trace Elem. Med. Biol. 79, 127266. https://doi.org/10.1016/j.jtemb.2023.127266.

Gong, L., Sun, J., Cong, S., 2023. Levels of iron and iron-related proteins in Alzheimer's disease: a systematic review and meta-analysis. J. Trace Elem. Med. Biol. 80, 127304. https://doi.org/10.1016/j.jtemb.2023.127304.

Guan, J., Wang, P., Lu, L., Zhao, G., 2020. Association of plasma transferrin with cognitive decline in patients with Mild Cognitive Impairment and Alzheimer's Disease. Front. Aging Neurosci. 12, 38. https://doi.org/10.3389/fnagi.2020.00038.

Gutierre, R.C., Rocha, P.R., Graciani, A.L., Coppi, A.A., Arida, R.M., 2025. Tau, amyloid, iron, oligodendrocytes ferroptosis, and inflammaging in the hippocampal formation of aged rats submitted to an aerobic exercise program. Brain Res. 1850, 149419. https://doi.org/10.1016/j.brainres.2024.149419.

Huat, T.J., Camats-Perna, J., Newcombe, E.A., Valmas, N., Kitazawa, M., Medeiros, R., 2019. Metal toxicity links to Alzheimer's disease and neuroinflammation. J. Mol. Biol. 431 (9), 1843–1868. https://doi.org/10.1016/j.jmb.2019.01.018.

Ionescu-Tucker, A., Cotman, C.W., 2021. Emerging roles of oxidative stress in brain aging and Alzheimer's disease. Neurobiol. Aging 107, 86–95. https://doi.org/10.1016/j.neurobiolaging.2021.07.014.

Larsen, B., Bourque, J., Moore, T.M., Adebimpe, A., Calkins, M.E., Elliott, M.A., Gur, R. C., Gur, R.E., Moberg, P.J., Roalf, D.R., Ruparel, K., Turetsky, B.I., Vandekar, S.N., Wolf, D.H., Shinohara, R.T., Satterthwaite, T.D., 2020. Longitudinal development of brain iron is linked to cognition in youth. J. Neurosci. 40 (9), 1810–1818. https://doi.org/10.1523/JNEUROSCI.2434-19.2020.

Li, N., Duan, Y.H., Chen, L., Zhang, K., 2023b. Iron metabolism: an emerging therapeutic target underlying the anti-Alzheimer's disease effect of ginseng. J. Trace Elem. Med. Biol. 79, 127252. https://doi.org/10.1016/j.jtemb.2023.127252.

Li, K., Li, A., Mei, Y., Zhao, J., Zhou, Q., Li, Y., Yang, M., Xu, Q., 2023a. Trace elements and Alzheimer dementia in population-based studies: a bibliometric and meta-analysis. Environ. Pollut. 318, 120782. https://doi.org/10.1016/j.envpol.2022.120782.

Limongi, F., Noale, M., Bianchetti, A., Ferrara, N., Padovani, A., Scarpini, E., Trabucchi, M., Maggi, S., Group, M.C.I.W., 2019. The instruments used by the Italian centres for cognitive disorders and dementia to diagnose mild cognitive impairment (MCI). Aging Clin. Exp. Res. 31 (1), 101–107. https://doi.org/10.1007/s40520-018-1032-8.

Liu, J., Chen, Y., Lu, X., Xu, X., Bulloch, G., Zhu, S., Zhu, Z., Ge, Z., Wang, W., Shang, X., He, M., 2023. The association between dietary iron intake and incidence of dementia in adults aged 60 years or over in the UK Biobank. Nutrients 15 (2), 260. https://doi.org/10.3390/nu15020260.

Liu, F., Zhang, Z., Zhang, L., Meng, R.N., Gao, J., Jin, M., Li, M., Wang, X.P., 2022. Effect of metal ions on Alzheimer's disease. Brain Behav. 12 (3), e2527. https://doi.org/10.1002/brb3.2527.

Livingston, G., Huntley, J., Liu, K.Y., Costafreda, S.G., Selbæk, G., Alladi, S., Ames, D., Banerjee, S., Burns, A., Brayne, C., Fox, N.C., Ferri, C.P., Gitlin, L.N., Howard, R., Kales, H.C., Kivimäki, M., Larson, E.B., Nakasujja, N., Rockwood, K., Samus, Q., Shirai, K., Singh-Manoux, A., Schneider, L.S., Walsh, S., Yao, Y., Sommerlad, A., Mukadam, N., 2024. Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. Lancet 404 (10452), 572–628. https://doi.org/10.1016/S0140-6736(24)01296-0.

McKeith, I.G., Dickson, D.W., Lowe, J., Emre, M., O'Brien, J.T., Feldman, H., Cummings, J., Duda, J.E., Lippa, C., Perry, E.K., Aarsland, D., Arai, H., Ballard, C.G., Boeve, B., Burn, D.J., Costa, D., Del Ser, T., Dubois, B., Galasko, D., Gauthier, S., Goetz, C.G., Gomez-Tortosa, E., Halliday, G., Hansen, L.A., Hardy, J., Iwatsubo, T., Kalaria, R.N., Kaufer, D., Kenny, R.A., Korczyn, A., Kosaka, K., Lee, T., Lees, A., Litvan, I., Londos, E., Lopez, O.L., Minoshima, S., Mizuno, Y., Molina, J.A., Mukaetova-Ladinska, E.B., Pasquier, F., Perry, R.H., Schulz, J.B., Trojanowski, J.Q., Yamada, M., Consortium on DLB, 2005. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 65 (12), 1863–1872. https://doi.org/10.1212/01.wnl.0000187889.17253.b1.

Michalke, B., Halbach, S., Nischwitz, V., 2007. Speciation and toxicological relevance of manganese in humans. J. Environ. Monit. 9 (7), 650–656. https://doi.org/10.1039/b704173j.

Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., van Swieten, J.C., Seelaar, H., Dopper, E.G., Onyike, C.U., Hillis, A.E., Josephs, K.A., Boeve, B.F., Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.L., Rosen, H., Prioleau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N., Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S., Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L., 2011. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 134 (Pt 9), 2456–2477. https://doi.org/10.1093/brain/awr179.

Sanford, A.M., 2017. Mild cognitive impairment. Clin. Geriatr. Med. 33 (3), 325–337. https://doi.org/10.1016/j.cger.2017.02.005.

Shi, Z., Li, M., Wang, Y., Liu, J., El-Obeid, T., 2019. High iron intake is associated with poor cognition among Chinese old adults and varied by weight status-a 15-y longitudinal study in 4852 adults. Am. J. Clin. Nutr. 109 (1), 109–116. https://doi.org/10.1093/ajcn/nqy254.

Tondelli, M., Salemme, S., Vinceti, G., Bedin, R., Trenti, T., Molinari, M.A., Chiari, A., Zamboni, G., 2022. Predictive value of phospho-tau/total-tau ratio in amyloid-negative Mild Cognitive Impairment. Neurosci. Lett. 787, 136811. https://doi.org/10.1016/j.neulet.2022.136811.

Urbano, T., Chiari, A., Malagoli, C., Cherubini, A., Bedin, R., Costanzini, S., Teggi, S., Maffeis, G., Vinceti, M., Filippini, T., 2023. Particulate matter exposure from motorized traffic and risk of conversion from mild cognitive impairment to dementia: an Italian prospective cohort study. Environ. Res. 222, 115425. https://doi.org/10.1016/j.envres.2023.115425.

Urbano, T., Filippini, T., Malavolti, M., Fustinoni, S., Michalke, B., Wise, L.A., Vinceti, M., 2024. Adherence to the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet and exposure to selenium species: a cross-sectional study. Nutr. Res. 122, 44–54. https://doi.org/10.1016/j.nutres.2023.12.002.

Urbano, T., Vinceti, M., Mandrioli, J., Chiari, A., Filippini, T., Bedin, R., Tondelli, M., Simonini, C., Zamboni, G., Shimizu, M., Saito, Y., 2022. Selenoprotein P concentrations in the cerebrospinal fluid and serum of individuals affected by amyotrophic lateral sclerosis, mild cognitive impairment and Alzheimer's dementia. Int. J. Mol. Sci. 23 (17), 9865. https://doi.org/10.3390/ijms23179865.

Vinceti, M., Chiari, A., Eichmuller, M., Rothman, K.J., Filippini, T., Malagoli, C., Weuve, J., Tondelli, M., Zamboni, G., Nichelli, P.F., Michalke, B., 2017. A selenium species in cerebrospinal fluid predicts conversion to Alzheimer's dementia in persons with mild cognitive impairment. Alzheimers Res. Ther. 9 (1), 100. https://doi.org/10.1186/s13195-017-0323-1.

Vinceti, M., Michalke, B., Malagoli, C., Eichmuller, M., Filippini, T., Tondelli, M., Bargellini, A., Vinceti, G., Zamboni, G., Chiari, A., 2019. Selenium and selenium species in the etiology of Alzheimer's dementia: the potential for bias of the case-control study design. J. Trace Elem. Med. Biol. 53, 154–162. https://doi.org/10.1016/j.jtemb.2019.03.002.

Vinceti, M., Solovyev, N., Mandrioli, J., Crespi, C.M., Bonvicini, F., Arcolin, E., Georgoulopoulou, E., Michalke, B., 2013. Cerebrospinal fluid of newly diagnosed amyotrophic lateral sclerosis patients exhibits abnormal levels of selenium species including elevated selenite. Neurotoxicology 38, 25–32. https://doi.org/10.1016/j.neuro.2013.05.016.

Vinceti, M., Urbano, T., Chiari, A., Filippini, T., Wise, L.A., Tondelli, M., Michalke, B., Shimizu, M., Saito, Y., 2023. Selenoprotein P concentrations and risk of progression from mild cognitive impairment to dementia. Sci. Rep. 13 (1), 8792. https://doi.org/10.1038/s41598-023-36084-6.

Vinceti, M., Urbano, T., Filippini, T., Bedin, R., Simonini, C., Soraru, G., Trojsi, F., Michalke, B., Mandrioli, J., 2025. Changes in cerebrospinal fluid concentrations of selenium species induced by tofersen administration in subjects with amyotrophic lateral sclerosis carrying SOD1 gene mutations. Biol. Trace Elem. Res. 203 (4), 2355–2364. https://doi.org/10.1007/s12011-024-04311-4.

White, R.F., Brand, C., 2019. Cognitive disorders in adults. Neurotoxicant Exposures., in: Press, O.U. (Ed.) The Oxford handbook of adult cognitive disorders. New York. doi:10.1093/oxfordhb/9780190664121.013.11.

Willemse, E.A., Teunissen, C.E., 2015. Biobanking of cerebrospinal fluid for biomarker analysis in neurological diseases. Adv. Exp. Med. Biol. 864, 79–93. https://doi.org/10.1007/978-3-319-20579-3_7.

Willkommen, D., Lucio, M., Schmitt-Kopplin, P., Gazzaz, M., Schroeter, M., Sigaroudi, A., Michalke, B., 2018. Species fractionation in a case-control study concerning Parkinson's disease: Cu-amino acids discriminate CSF of PD from controls. J. Trace Elem. Med. Biol. 49, 164–170. https://doi.org/10.1016/j.jtemb.2018.01.005.

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L.O., Nordberg, A., Backman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., de Leon, M., DeCarli, C., Erkinjuntti, T., Giacobini, E., Graff, C., Hardy, J., Jack, C., Jorm, A., Ritchie, K., van Duijn, C., Visser, P., Petersen, R.C., 2004. Mild cognitive impairment–beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J. Intern. Med. 256 (3), 240–246. https://doi.org/10.1111/j.1365-2796.2004.01380.x.

Yasar, M.T., Bush, A.I., Ayton, S., Agarwal, P., Agrawal, S., Bennett, D.A., Schneider, J.A., Arfanakis, K., 2024. Elevated iron levels in the older adult brain are associated with higher R2 relaxation rate in gray matter and more white matter hyperintensities: an ex-vivo MRI, pathology and mass spectrometry study. Alzheimers Dement 20 (S9), e093668. https://doi.org/10.1002/alz.093668.

Zeng, X., Meng, X., Wang, Y., Li, S., Liao, Q., Shan, Z., Huang, J., Shi, J., Wang, L., Chen, H., Hu, X., Rong, X., 2023. A reference interval study of ferritin and transferrin levels in donors from two blood centers. Exp. Ther. Med. 25 (5), 218. https://doi.org/10.3892/etm.2023.11916.

Zhao, Z., 2019. Iron and oxidizing species in oxidative stress and Alzheimer's disease. Aging Med. 2 (2), 82–87. https://doi.org/10.1002/agm2.12074.