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Research Article

Discrimination of multiple sclerosis patients from healthy individuals using a combination of walking ability and blood metallic nanoparticle concentrations

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HIGHLIGHTS

- Aluminum and copper concentrations were correlated negatively with 6MWT performance
- Higher iron concentrations were correlated with enhanced walking ability
- Metallic nanoparticle concentration (NPs) with 6MWT achieved 86.7% accuracy
- The combination of NPs with TUG performance achieved perfect in-sample classification
- NPs with TUG performance can accurately discriminate people with Multiple Sclerosis

ABBREVIATIONS

6MWT	Six-Minute Walk Test
Al	Aluminum
CNS	Central nervous system
Cr	Chromium
Cu	Copper
EDSS	Expanded Disability Status Scale
Fe	Iron
FSS	Fatigue Severity Scale
HC	Healthy control
ICP-OES	Inductively coupled plasma-optical emission spectrometry
Mg	Magnesium
MMSE	Mini-Mental State Exam
MS	Multiple Sclerosis
Ni	Nickel
PwMS	People with MS
Rc^2	Canonical correlation squared
SDMT	Symbol Digit Modality Test
SRDD	Self-reported disease disability
TUG	Time Up and Go
Zn	Zinc

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BACKGROUND: The concentrations of metallic nanoparticles have been associated with symptoms of multiple sclerosis (MS), which may affect the neuronal excitatory-inhibitory balance by depositing on the myelin sheath. A balanced regulation of the cortical–striatal–thalamic–cortical pathway is necessary for optimal walking performance. Alterations in neuronal excitatory-inhibitory balance have the potential to adversely impact walking ability.
AIM: To explore the importance of metallic nanoparticles to walking ability in people with MS (PwMS) and healthy controls (HC).
METHODS: The present study involved 30 individuals, including 19 PwMS (EDSS=2.4±0.3) and 11 HCs. Whole blood samples were collected for the quantification of aluminum, chromium, copper, iron, magnesium, nickel, zinc and total metallic nanoparticles (T-NPs). The participant's walking ability was assessed using the Time Up and Go (TUG) test and the Six-Minute Walk Test (6MWT).
RESULTS: The findings showed negative correlations between 6MWT performance and aluminum ($r=-0.45, p<0.05$) and copper ($r=-0.44, p<0.05$), and positive correlations with iron ($r=0.60, p<0.007$) and T-NPs ($r=0.53, p<0.02$). The correlations were significantly different from the control group using iron, magnesium, and T-NPs concentrations ($Z>2.01; p<0.04$). TUG performance correlated negatively with iron ($r=-0.56, p<0.01$) and T-NPs ($r=-0.53, p<0.02$), with significant differences from the control group using iron and magnesium ($Z>2.11, p<0.04$). The incorporation of T-NPs concentrations and TUG in the discriminant model yielded perfect classification in-sample, while the integration of 6MWT in the model attained 86.7% to discriminate between PwMS and HCs.
INTERPRETATION: The present study demonstrated that the combination of blood metallic nanoparticle concentrations and walking ability can effectively differentiate between PwMS and HCs, and may be suggested as potential biomarkers for MS.

KEYWORDS: Multiple sclerosis | Metals | Biomarkers | Physical disability | TUG | 6MWT

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS) that leads to neurodegeneration and functional disability. It is widely recognized that MS leads to both motor impairments [1,2] and contributes to metabolic dysfunction [3]. Despite the advancements in diagnostic tools and treatment strategies over the past 15 years, the early identification of disease progression remains a significant clinical challenge [4]. Detecting reliable markers that distinguish people with MS (PwMS) from healthy controls (HC) is critical to enhancing individualized treatment and disease management. Among the potential candidates, blood metallic nanoparticle concentrations and walking performance have emerged as promising complementary parameters. For example, the blood concentration of metallic nanoparticles, particularly the iron concentration, has been proposed as a promising biomarker for monitoring cognitive impairment in PwMS [5], which may also be related to motor symptoms and serve to discriminate PwMS from those without the disease.

Evidence linking MS and metallic nanoparticle concentrations largely has emerged from case–control studies examining biological specimens, including blood, serum, hair, urine, and cerebrospinal fluid [6,7]. As brain metallic nanoparticles are primarily derived from circulating blood, peripheral metallic nanoparticle levels can directly influence their concentrations in the CNS [8]. High exposure to metallic nanoparticles can increase blood-brain barrier permeability through systemic inflammation and oxidative stress, allowing these particles to reach the CNS via the direct (olfactory or trigeminal nerves) and indirect (inhalation and transfer via the blood circulation) pathways [9,10]. Once in the CNS, such nanoparticles induce neuroinflammation, mitochondrial dysfunction, and oligodendrocyte damage, contributing to myelin degeneration and the potential exacerbation of diseases such as MS [11]. Post-mortem studies have demonstrated that, in comparison to age-matched HCs, PwMS exhibit increased metallic nanoparticle deposition in brain regions, including the basal ganglia and motor cortex [12]. Thus, the CNS exhibits heightened sensitivity to metal toxicity, as excessive accumulation on the myelin sheath can activate inflammatory and oxidative stress pathways [13]. Altered metallic nanoparticle levels, particularly of iron, copper, and zinc, have been associated with MS-related neurodegeneration [7,14,15]. While these elements are critical cofactors in neuronal and immune function, supporting myelin synthesis, oligodendrocyte maintenance, and neurotransmitter production, their imbalance may exacerbate oxidative and inflammatory damage within the CNS [16]. Consequently, the quantification of blood metallic nanoparticle concentrations emerges as a cost-effective and practical approach for developing a promising biomarker for MS.

Gait impairments in MS may be indicative of underlying neurophysiological dysfunctions [17], which may be associated with altered metallic nanoparticle concentrations. During walking, a balanced regulation in the cortical–striatal–thalamic–cortical pathway is necessary for motor planning and movement execution [18]. Disruption of the cortical–striatal–thalamic–cortical pathway may result from metal deposition on myelin, thereby impairing neuronal excitatory–inhibitory balance [19] and interfering with walking ability. For instance, impaired motor timing, manifested as slowed cadence and impaired rhythmic stepping, has been associated with disrupted cortical-striatal-thalamic-cortical function. This disruption can lead to spastic gait (i.e., stiff, dragging legs), resulting from inadequate inhibitory control over descending pathways. Additionally, damage to cerebellar-basal ganglia interactions may contribute to imbalance and increased variability during walking [20–22]. Therefore, alterations in this pathway provide a plausible neurophysiological rationale for correlating walking-performance measures with metallic nanoparticle concentrations.

Generally, walking ability is among the most prevalent disabilities, affecting approximately 85% of PwMS [23]. A plethora of assessments are available to evaluate walking ability; however, the Timed Up and Go (TUG) test and the Six-Minute Walk Test (6MWT) have demonstrated robust validity for this purpose. PwMS generally require more time to complete the TUG and cover shorter distances during the 6MWT compared with HCs [23,24]. The TUG has been demonstrated to exhibit a robust correlation with functional mobility[24] and serves as an effective monitoring instrument for disease progression [25], while the 6MWT offers a reproducible and sensitive metric for evaluating habitual walking performance [26,27]. This context suggests a plausible link between metallic nanoparticle dysregulation and reduced walking ability in PwMS, which could serve as an interesting biomarker for MS. Therefore, the present study aims to explore the importance of metallic nanoparticle concentrations to walking ability in PwMS. Specifically, we examined whether the combination of metallic nanoparticle concentrations with walking measures (TUG and 6MWT) could discriminate PwMS from HCs. The following hypothesis was formulated: 1) poorer walking performance—indicated by longer TUG times and shorter 6MWT distances—would be correlated with elevated metallic concentrations in PwMS, and 2) the combination of metallic nanoparticle concentrations and walking ability would discriminate PwMS from HCs more effectively than either parameter alone. As demonstrated in extant literature, the integration of biological markers with functional measures enhances the capacity to differentiate between groups with and without disease, identify early neurodegeneration, and predict clinical progression [28–30]. Consequently, the integration of metallic nanoparticles and gait measurements aligns with a methodological approach that has already been validated within the field, despite the novelty of the specific application of metallic nanoparticles.

METHODS

This study constitutes a secondary analysis of previous studies, employing the participants reported herein [5,31,32]. New research questions and hypotheses from previous studies were stated in the current study.

Figure 1 presents the schematic representation of the methodological framework utilized in this study. The participants were evaluated within a single day. Firstly, blood samples were collected from the participants after an overnight fast to quantify the metallic nanoparticle levels. Following a breakfast comprising fruits, bread, cheese, and juice, the participants underwent a series of clinical tests.

Consequently, the participants underwent walking ability assessments, specifically the TUG and 6MWT. Prior to the evaluation day, the participants were instructed to abstain from any intense or vigorous physical activity for 48 hours and to abstain from consuming any food or liquid, except for water, for 12 hours before the assessment. All measurements were conducted in the morning (from 7:00 am to 12:00 pm) to minimize the influence of the circadian cycle on biological samples.

Figure 1. Schematic representation of the methodology framework. TUG: Time Up and Go; 6MWT: Six-Minute Walk test; PwMS: individuals with Multiple Sclerosis; HC: Healthy Controls.

Participants

The present study enrolled a total of 30 subjects, including 19 individuals with MS and 11 HCs. All MS participants were diagnosed with the relapse-remitting subtype in accordance with the revised 2017 McDonald criteria [33] and had an Expanded Disability Status Scale (EDSS) [34] score of less than 4.5. Prior to the initiation of data collection, participants had been free from relapses for a period of at least three months, and they were not taking fampridine. Additionally, individuals afflicted with orthopedic, cardiac, oculomotor, cerebellar, respiratory, or other neurological disorders that could potentially influence the experimental protocol, as well as those with cognitive decline (scoring less than 24 points on the Mini-Mental State Exam - MMSE) [35], were excluded from the study. The local Ethics Committee of the University approved the study (CAAE#99191318.0.0000.5398), and all participants provided informed consent to participate.

Clinical Evaluation

All participants were evaluated using the Symbol Digit Modality Test (SDMT) [36] and the MMSE [35]. The objective of these evaluations was twofold: firstly, to assess the cognitive processing speed (SDMT) of the participants, and secondly, to perform a cognitive screening (MMSE) of the participants. Also, the Fatigue Severity Scale (FSS) was employed to assess the severity of perceived fatigue experienced during the previous week [37]. Additionally, the time since onset and last relapse, as well as self-reported disease disability (PDDS) and the level of disability by EDSS, were assessed exclusively for PwMS.

Blood Collection and Quantification of Metal Nanoparticle Concentration

The present study followed the methodology described by de Oliveira et al. [7]. Whole blood samples of 4 mL were collected from all subjects using standard procedures and collected in dry tubes without anticoagulant factors. These samples were subsequently utilized for the quantification of metallic nanoparticles.

Following blood collection, the blood samples were subjected to freeze-drying and subsequently stored at a low temperature until further analysis. Approximately 1.2 mL of the freeze-dried sample was placed into closed vessels, along with 2 mL of hydrogen peroxide and 3 mL of nitric acid. The mixture was then subjected to microwave-assisted digestion, a process that was carried out in accordance with the following steps: Initially, the temperature was elevated to 180°C over a period of 15 minutes. Then, the temperature was maintained at 180°C for an additional 15 minutes. Subsequently, the temperature was reduced to ambient temperature over a period of 15 minutes. Finally, the digested samples were diluted using a 1.2:25 (v:v) ratio and brought up to a final volume of 25 mL with deionized water.

The samples were subsequently analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES) to obtain the concentrations of metallic nanoparticles. During the course of the analysis, a reference blood sample was subjected to digestion under conditions identical to those applied to the study samples. All glassware and instruments were thoroughly cleaned, and stringent precautions were taken to prevent any trace-element contamination of the samples [38]. In addition, blank samples of nitric acid and hydrogen peroxide were incorporated into the analytical method to ensure the reliability of the results. The ICP-OES calibration curve was constructed using five standard solutions with known concentrations of the elements of interest, prepared according to the recommendations for multielement calibration described by Boss & Fredeen [39]. Subsequently, the concentrations of aluminum (Al), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), nickel (Ni), and zinc (Zn) were quantitatively analyzed using ICP-OES. Additionally, the total concentration of all metallic nanoparticles was calculated.

Walking Ability

The quantification of walking ability was carried out using TUG [40] and 6MWT [41]. The TUG test involves the patient rising from a seated position, walking at their fastest possible pace over a distance of 3 meters, executing a 180° turn, and then returning to the initial seated

position. Each participant underwent one trial for practice and two trials for the actual measurement. The mean time (in seconds) from these two trials was documented as the final performance result. The 6MWT was performed in accordance with the guidelines established by the American Thoracic Society [42], and its use in PwMS has been previously documented[43]. The participants were instructed to walk back and forth at the fastest and safest possible speed for a period of six minutes within a 20-meter corridor. Verbal encouragement and a reminder that the minute was over were provided at one-minute intervals. The total distance covered (in meters) during the test was recorded.

Statistical Analysis

Statistical analyses were conducted using SPSS Statistics software (IBM Corporation, Armory, N.Y.), with a significance level set at 0.05. The normality of the data was assessed using the Shapiro-Wilk test. For parametric parameters (age, SDMT score, Cu, Fe, Zn, total metallic nanoparticle concentrations, and 6MWT distance), independent sample t-tests were used to compare the groups. The remaining values (MMSE, FSS scores, Al, Cr, Mg, and Ni concentrations, and TUG) were compared using the Mann-Whitney test. To examine the relationship between serum metallic nanoparticle concentrations and functional performance, we used Pearson correlation coefficients separately for the MS group and the healthy control group. The r-values were classified as small (> 0.01 and < 0.29), medium (> 0.30 and < 0.49), and large (> 0.50) [44]. Finally, multivariate discriminant analysis was applied to assess the ability to distinguish between PwMS and the HC [45]. Initially, a discriminant analysis was executed individually for each metallic nanoparticle concentration and walking ability test. Subsequently, the discriminant analysis was performed by combining metallic nanoparticle concentrations with TUG and 6MWT, separately. These analyses were accompanied by the canonical correlation squared (Rc^2), interpreted as an effect size measure that represents the proportion of the total variance in the discriminant function explained by group differences. The Rc^2-values were classified as negligible (< 0.01), small (> 0.011 and < 0.09), medium (> 0.091 and < 0.24) and large (> 0.25) [46]. To determine whether the strength of these associations differed between groups, we used the Fisher Z transformation, considering the normality and null heteroscedasticity of the data [47,48].

RESULTS

Table 1 presents the group characteristics, clinical evaluation scores, and walking ability performance (TUG and 6MWT). All PwMS were under medication treatment, with six using fingolimod hydrochloride, six using monoclonal antibody, five using dimethyl fumarate, one using interferon beta-1a, and one using azathioprine. In addition, seven PwMS were undergoing vitamin D supplementation.

There were no statistically significant differences between the groups in terms of age, body mass, height, and MMSE score. PwMS demonstrated higher fatigue scores in the FSS, exhibited poorer performance on the SDMT, required a longer time to complete the TUG test, and covered a shorter distance in the 6MWT when compared to HCs.

Table 1. Means, standard deviations, and minimum-maximum values (in brackets) of characteristics, clinical evaluation scores and walking ability in individuals with Multiple Sclerosis (PwMS) and healthy controls (HC). The last column presents the p-values for the group comparisons. *Indicates significant differences between groups.

	PwMS	HC	*p*-value
Group characteristics			
Sex F/M	12/7	9/2	-
Age (years)	33±2 (18 – 49)	31±2 (22 – 42)	0.553
Body mass (kg)	73.5±3.3 (51.1 – 94.6)	69.1±3.4 (56.8 – 88.2)	0.445
Height (m)	1.68±0.02 (1.55 – 1.89)	1.68±0.02 (1.56 – 1.84)	0.165
Clinical evaluations			
FSS (score)	30.3±2.2 (19.0 – 49.0)	20.3±0.8 (17.0 – 26.0)	0.000*
MMSE (score)	29.0±0.2 (28.0 – 30.0)	29.6±0.3 (27.0 – 30.0)	0.498
SDMT (score)	47.9±3.5 (4.0 – 65.0)	61.1±2.3 (49.0 – 73.0)	0.005*
EDSS (score)	2.4±0.3 (1.0 – 4.5)	-	-
Walking ability			
TUG (s)	8.60±0.66 (4.88 – 14.05)	5.85±0.29 (4.25 – 7.69)	0.005*
6MWT (m)	452.0±31.6 (160.0 – 680.0)	609.4±34.4 (470.0 – 815.0)	0.005*

Note. F: Female; M: Male; FSS: Fatigue Severity Scale; MMSE: Mini-Mental State Exam; SDMT: Symbol Digit Modality Test; EDSS: Expanded Disability Status Scale; TUG: Time Up and Go; 6MWT: Six-Minute Walk test.

Table 2 displays the concentrations of metallic nanoparticles observed in PwMS and HC. PwMS demonstrated significantly higher levels of Cu, Fe, and Zn compared to HC. Furthermore, the total concentration of metallic nanoparticles was elevated in PwMS.

Table 2. Means, standard deviations, and minimum-maximum values (in brackets) for each metallic nanoparticle concentration in individuals with Multiple Sclerosis (PwMS) and healthy controls (HC). The last row displays the total value of metallic nanoparticle concentrations. The last column

presents the p-values for the group comparisons. *Indicates significant differences between groups.

Metal	PwMS	HC	*p*-value
Al (μg/L)	7.70±0.59 (4.39-16.18)	7.05±0.51 (4.66-10.29)	0.589
Cu (μg/L)	1.01±0.10 (0.44-1.78)	0.97±0.06 (0.58-1.25)	0.022*
Cr (μg/L)	0.42±0.03 (0.29-0.65)	0.41±0.03(0.31-0.55)	0.219
Fe (μg/L)	300.55±8.90 (212.99-363.58)	267.01±14.02 (173.68-330.72)	0.047*
Mg (μg/L)	27.89±1.37 (20.03-41.77)	26.84±1.71 (20.04-35.92)	0.582
Ni (μg/L)	0.38±0.12 (0.04-1.69)	0.44±0.22 (0.04-2.51)	0.682
Zn (μg/L)	3.15±0.21 (1.63-4.58)	2.43±0.19 (1.58-3.24)	0.026*
Total (μg/L)	337.95±8.51 (258.36-400.34)	302.7±13.5 (213.57-364.08)	0.033*

Note. Al: aluminum; Cr: chromium; Cu: copper; Fe: iron; Mg: magnesium; Ni: nickel; Zn: zinc; Total: sum of all concentrations.

Figure 2 illustrates the correlation between metallic nanoparticle concentrations and walking ability (TUG and 6MWT) for PwMS and HC. The 6MWT performance exhibited a negative correlation with Al concentrations (r = - 0.45, $p < 0.05$) and Cu concentrations (r = - 0.44, $p < 0.05$) and a positive correlation with Fe (r = 0.60, $p < 0.007$) and total metallic nanoparticle concentration (r = 0.53, $p < 0.02$) in PwMS (Figure 2A). The correlations were significantly different between groups for Fe (Z = 2.41; $p = 0.02$), Mg (Z = - 2.08; $p = 0.04$) and total metallic nanoparticle concentrations (Z = 2.01; $p = 0.04$). Furthermore, the TUG test exhibited a negative relationship with Fe (r = - 0.56, $p < 0.01$) and total metallic nanoparticle concentration (r = - 0.53, $p < 0.02$). For HCs (Figure 2.B), Mg exhibited a negative correlation with TUG performance (r = -0.63, $p < 0.05$). The correlations were significantly different between groups for Fe (Z = -2.11; $p = 0.04$) and Mg (Z = 2.13; $p = 0.03$). All correlations were moderate (> 0.3) or large (> 0.5).

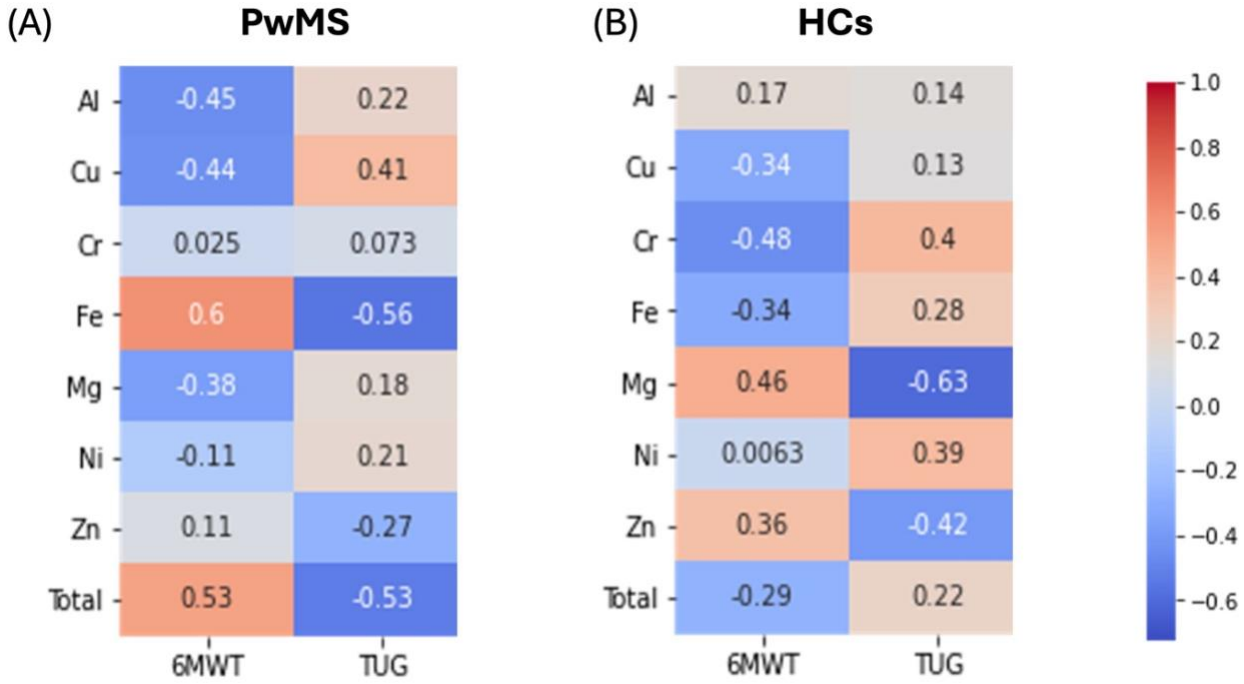

Figure 2. Correlation among metallic nanoparticle concentrations and walking capacity (6MWT and TUG) in individuals with Multiple Sclerosis (PwMS) and healthy controls (HC). Color intensity represents correlation strength and direction, from strong negative (blue) to strong positive (red). *TUG: Time Up and Go; 6MWT: Six-Minute Walk test; Al: aluminum; Cr: chromium; Cu: copper; Fe: iron; Mg: magnesium; Ni: nickel; Zn: zinc; Total: total metallic nanoparticle concentrations.*

Figure 3 presents the classification success (number of correctly classified and misclassified cases) of each group of the discriminant analysis. Despite the significant power to discriminate the groups using Fe (Wilks' λ = 0.861; $p = 0.043$; $Rc^2 = 0.138$ [Medium]), Zn (Wilks' λ = 0.841; $p = 0.029$; $Rc^2 = 0.158$ [Medium]), and the sum of metallic nanoparticle concentrations (Wilks' λ = 0.835; $p = 0.026$; $Rc^2 = 0.165$ [Medium]), the misclassifications were greater than 23.3% using the metallic nanoparticle concentrations individually (Figure 3A). Analogous findings were ascertained through the implementation of TUG (Wilks' λ = 0.751; $p = 0.005$; $Rc^2 = 0.249$ [Large]) and 6MWT performance (Wilks' λ = 0.732; $p = 0.003$; $Rc^2 = 0.267$ [Large]), which exhibited 26.7% of misclassifications.

All discriminant models employing the combination of metallic nanoparticle concentration and 6MWT distance exhibited statistical significance (Wilks' λ > 0.497; $p < 0.032$; $Rc^2 > 0.268$ [Large]), resulting in misclassifications exceeding 26.7% (Figure 3B).

When the metallic nanoparticle concentration was combined with TUG, all models proved to be significant (Wilks' $\lambda > 0.497$; $p < 0.021$; $Rc^2 = 0.249$ [Large]), with miscalculations ranging from 0% to 26.7% (Figure 3C). Specifically, the most effective model incorporated total metallic nanoparticle concentration and TUG (Wilks' $\lambda = 0.404$; $p = 0.005$; $Rc^2 = 0.596$ [Large]), resulting in accurate classification of all individuals. The discriminant function of this model accounted for 100% of the total variance and exhibited a high correlation value (0.772). Positive discriminant function coefficients were observed for TUG (0.382) and concentrations of Al (0.283), Cr (14.116), Fe (0.002), Mg (0.058), and Zn (1.314). The analysis revealed negative discriminant coefficients for Cu (- 2.113) and Ni (- 1.388). The constant value was also found to be negative (- 14.052). The centroids for the PwMS and HC groups were 0.894 and - 1.544, respectively.

DISCUSSION

The present study was conducted to investigate the relationship between blood metallic nanoparticle concentrations and walking ability. Additionally, the investigation sought to determine whether combining blood metallic nanoparticle concentrations with walking ability could serve as a means to differentiate between PwMS and HC. The primary finding of our study indicated that the combination of total metallic nanoparticle concentration with TUG performance attained perfect classification in-sample. The combination of total metallic nanoparticle concentration with 6MWT performance achieved 86.7% accuracy. This study builds upon prior research by demonstrating that blood levels of metallic nanoparticles in combination with walking ability have the potential to serve as a biomarker for distinguishing between PwMS and healthy individuals, which could facilitate monitoring in clinical settings. However, the validity of this potential should be confirmed by subsequent studies. In addition, our first hypothesis was partially confirmed. The findings of the study demonstrated a negative correlation between Al and Cu concentrations and 6MWT performance, with higher concentrations corresponding to poorer walking performance (lower distance covered). However, higher Fe concentrations were correlated with enhanced walking ability (increased distance covered in the 6MWT and the reduced time in the TUG test), contradicting our first hypothesis.

Higher metallic nanoparticle concentration and worse walking ability in PwMS: is there a correlation?

PwMS required a greater amount of time to complete the TUG test and covered a shorter distance in the 6MWT compared to HCs. The inferior performance in both TUG and 6MWT observed in this study is consistent with prior research demonstrating substandard walking performance in PwMS [43]. A decline in performance during the TUG and the 6MWT in PwMS has been associated with various factors in MS. For example, while the balance confidence and muscle strength functional test (i.e., functional stair test) explained 75% of the 6MWT variance [49], muscle weakness of the plantar flexors has been linked to worse performance on the 6MWT [50]. However, factors as falls [51] and MS motor symptoms may contribute to a prolonged time to complete the TUG in PwMS. More specifically, Lorefice et al. [52] found correlations between performance on the TUG test and its sub-phases with white and gray matter in PwMS. These factors that have been identified as contributors to the impaired performance in walking ability tests among PwMS may be indicative of an advanced stage of neurodegeneration initiated by an imbalance of metallic nanoparticles.

PwMS also exhibited higher concentrations of Cu, Fe, Zn, and total metallic nanoparticles [7,14,15]. While metals are essential for optimal brain function, an imbalance may be detrimental [13]. It has been demonstrated that elevated concentrations of metallic nanoparticles can accumulate in brain tissue, including the myelin sheath, and that this accumulation may contribute to nerve damage and MS progression [16]. Several studies have identified an association between the presence of aberrant metallic nanoparticle levels in blood and other bodily fluids, including cerebrospinal fluid and urine, and the development of MS [7,53]. This imbalance is related to reduced walking ability seen in PwMS. However, the exact relationship between metallic nanoparticle levels and walking performance in this study was not straightforward, suggesting a more complex link between metal regulation and walking ability in MS. While higher concentrations of Al and Cu were correlated with worse performance on the 6MWT, higher concentrations of Fe and total metallic nanoparticles were related with better performance on the TUG test (shorter test duration) and the 6MWT (longer distance walked). Consequently, the relationship between metallic nanoparticle concentrations and diminished walking ability in PwMS remains to be fully elucidated and should be the focus of future research endeavors.

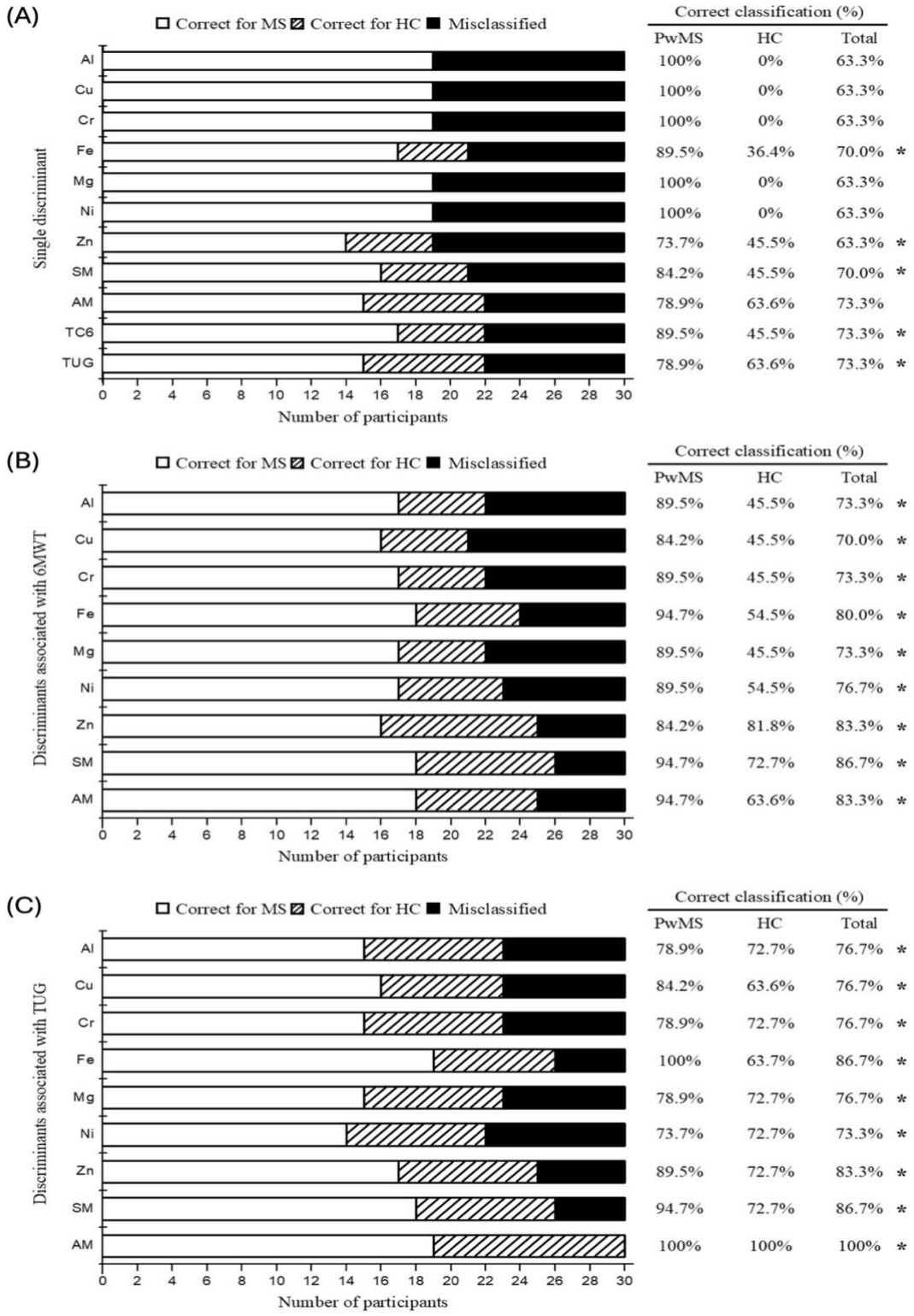

Figure 3. A) Single discriminant results for each metallic nanoparticle concentration and walking test (6MWT and TUG). B) Discriminant association between metallic nanoparticle concentration and 6MWT performance. C) Discriminant association between metallic nanoparticle concentration and TUG performance. **Significant models* (*p* < 0.05). *TUG: Time Up and Go; 6MWT: Six-Minute Walk test; Al: aluminum; Cr: chromium; Cu: copper; Fe: iron; Mg: magnesium; Ni: nickel; Zn: zinc; SM: total metallic nanoparticle concentrations; AM: all metallic nanoparticle concentrations at the same time*.

Metallic nanoparticle concentration combined with walking ability performance may be a biomarker to discriminate MS individuals

The findings of this study demonstrated that the combination of all metallic nanoparticle concentrations with 6MWT enhanced the discriminative power between the groups under study by 86.7%. The most salient and promising finding was the perfect classification in-sample to discriminate PwMS from HC when performance on TUG was combined with all metallic nanoparticle concentrations. This combination can be regarded as a possibility for MS. This assessment can complement MS progression monitoring, which often relies on a combination of clinical assessment, magnetic resonance imaging findings, and cerebrospinal fluid analysis—procedures that can be time-consuming and expensive [54]. Furthermore, continuous monitoring of MS is imperative in light of the disease's relapsing nature and the variability among patients. MS is distinguished by its intermittent periods of remission and exacerbation, which underscores the necessity for continuous evaluation to facilitate timely therapeutic adjustments and management [55]. This biomarker is of particular interest due to its integration of biological MS effects, as measured by blood levels and motor disturbances. However, it is imperative to exercise caution when interpreting the findings of this study. The statistical discriminant analysis is susceptible to overfitting the data, particularly when it reports 100% classification accuracy without implementing cross-validation or external validation. While the exploratory nature of our analysis renders our findings valuable, it is important to acknowledge the potential for significant interpretive overreaches. Furthermore, a longitudinal study encompassing a more substantial population is required to validate the findings of the present study and to integrate them into clinical practice.

CONCLUSION

The study's findings indicated a correlation between blood metallic nanoparticle concentrations and walking ability, particularly as assessed by the TUG test. It can be concluded that the combination of metallic nanoparticle concentration with TUG performance can accurately discriminate PwMS from HC.

Despite the study's strengths, it is important to acknowledge its limitations. First, the measurement of metallic nanoparticle concentrations was conducted exclusively in blood, excluding cerebrospinal fluid and brain tissue. While the relationship between blood and brain metallic nanoparticle concentrations has been demonstrated [8,56], no such measurements were made in the present study. On the other hand, the intricacies and invasiveness of measuring brain metal should be considered [57]. Consequently, the utilization of a blood test as a biomarker for metallic nanoparticle concentrations is more practical than the use of brain tissue. Secondly, the study's modest sample size constrained the extent of the findings and strong claims. The main objective of our study was not the development of a robust predictive model through discriminant analysis, but rather the exploratory investigation of the associations between the variables and, more crucially, the comparison of the strength of these correlations between the PwMS and control groups. To validate and increase the reliability of these findings, especially the results obtained with discriminant analysis, replication in an independent and substantially larger cohort is imperative. In addition, our sample included individuals with EDSS scores below 4.5 who may not have motor disabilities, but rather disabilities in other functional systems affected by MS. This characteristic of the sample should be acknowledged when interpreting the findings. Third, we did not control dietary habits, medication intake, disease-modifying therapy, menstrual status, and environmental metal exposure, which may be confounders for metallic nanoparticle concentrations. In addition, while TUG and 6MWT have been demonstrated to have robust validity for walking ability in PwMS [26,40], we did not assess more detailed gait parameters, such as spatial-temporal parameters, which may limit the interpretability of walking ability in MS. Finally, future studies, based on exploratory findings of correlation differences, should employ Multiple Regression Analysis or Analysis of Covariance to formally control for the effect of essential covariates such as age, sex, and treatment types, thus ensuring a more precise and causal understanding of the observed associations with functional performance.

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