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MAGNETIC RESONANCE

Amyotrophic lateral sclerosis with upper motor neuron predominance: diagnostic accuracy of qualitative and quantitative susceptibility metrics in the precentral gyrus

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Abstract

Objective The study aims at comparing the diagnostic accuracy of qualitative and quantitative assessment of the susceptibility in the precentral gyrus in detecting amyotrophic lateral sclerosis (ALS) with predominance of upper motor neuron (UMN) impairment.

Methods We retrospectively collected clinical and 3T MRI data of 47 ALS patients, of whom 12 with UMN predominance (UMN-ALS). We further enrolled 23 healthy controls (HC) and 15 ALS Mimics (ALS-Mim). The Motor Cortex Susceptibility (MCS) score was qualitatively assessed on the susceptibility-weighted images (SWI) and automatic metrics were extracted from the quantitative susceptibility mapping (QSM) in the precentral gyrus. MCS scores and QSM-based metrics were tested for correlation, and ROC analyses.

Results The correlation of MCS score and susceptibility skewness was significant (Rho = 0.55, $p < 0.001$). The susceptibility SD showed an AUC of 0.809 with a specificity and positive predictive value of 100% in differentiating ALS and ALS Mim versus HC, significantly higher than MCS (Z = −3.384, p-value = 0.00071). The susceptibility skewness value of −0.017 showed specificity of 92.3% and predictive positive value of 91.7% in differentiating UMN-ALS versus ALS mimics, even if the performance was not significantly better than MCS (Z = 0.81, $p = 0.21$).

Conclusion The MCS and susceptibility skewness of the precentral gyrus show high diagnostic accuracy in differentiating UMN-ALS from ALS-mimics subjects. The quantitative assessment might be preferred being an automatic measure unbiased by the reader.

Clinical relevance statement The clinical diagnostic evaluation of ALS patients might benefit from the qualitative and/or quantitative assessment of the susceptibility in the precentral gyrus as imaging marker of upper motor neuron predominance.

Key Points

- *Amyotrophic lateral sclerosis diagnostic work-up lacks biomarkers able to identify upper motor neuron involvement.*
- *Susceptibility-weighted imaging/quantitative susceptibility mapping–based measures showed good diagnostic accuracy in discriminating amyotrophic lateral sclerosis with predominant upper motor neuron impairment from patients with suspected motor neuron disorder.*
- *Susceptibility-weighted imaging/quantitative susceptibility mapping–based assessment of the magnetic susceptibility provides a diagnostic marker for amyotrophic lateral sclerosis with upper motor neuron predominance.*

Francesco Lo Russo and Valeria Elisa Contarino contributed equally to the study.

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Abbreviations

ALS	Amyotrophic lateral sclerosis
ALSFRS-R	ALS Functional Rating Scale-Revised
ALS-mim	ALS-mimicking diseases
c-ALS	ALS patients with no clinically defined predominance
HC	Healthy controls
LMN	Lower motor neurons
LMN-ALS	LMN-predominant ALS
MCS	Motor Cortex Susceptibility
QSM	Quantitative susceptibility mapping
SMD	Suspected motor neuron disease
SuscKurt	Susceptibility kurtosis
SuscMean	Susceptibility mean
SuscMedian	Susceptibility median
SuscSD	Susceptibility standard deviation
SuscSkew	Susceptibility skewness
SWI	Susceptibility-weighted images
UMN	Upper motor neuron
UMN-ALS	UMN-predominant ALS

Introduction

Amyotrophic lateral sclerosis (ALS) is a highly lethal progressive neurodegenerative disorder of unknown etiology, characterized by degeneration of both upper (UMN) and lower motor neurons (LMN) [1]. The discovery of clinical, biological, genetic, radiological, and neurophysiological biomarkers of ALS is currently one of the main challenges [2]. The ideal biomarker should shorten the diagnostic delay and improve the accuracy, thus reducing the number of misdiagnosed cases [3].

Currently, the diagnosis is based on the latest revision of the El Escorial criteria, relying on clinical examination to assess signs of UMN impairment during a long period of time to confirm the progress of the disease, while relying on electromyography (EMG) and muscle biopsy to objectively detect signs of LMN impairment [4]. Neuroimaging and laboratory tests allow to exclude ALS-mimicking diseases (ALS-mim); in particular, brain and/or spine MRI are usually performed to exclude tumors, ischemic or demyelinating lesions, spine compression, and other neurodegenerative diseases [5].

Recent neuroimaging research has been focused on the analysis of the iron deposition in the precentral cortex, which is thought to be a marker of upper motor neuron degeneration in ALS patients [6–10]. Since iron has high magnetic susceptibility, it appears as a low signal cortical rim on susceptibility-weighted image (SWI) sequence [11–13]. The diagnostic value of SWI-based visual assessment using qualitative rating scales of the hyperintense cortical rim of the precentral cortex for the diagnosis of UMN involvement has been investigated [3,

13–15]. The main limitation of SWI is that it does not provide quantitative measurements. This issue has been addressed with the development of a quantitative susceptibility mapping (QSM) algorithm that allows in vivo quantification of the magnetic susceptibility [16]. A fully automatic QSM measure assessing the iron-related alterations of the precentral cortex was used to predict the UMN impairment in a cohort of ALS subjects [8].

The aim of this study is to compare the SWI-based visual qualitative assessment and the QSM-based automatic quantitative measures and evaluate the diagnostic accuracy in suspected motor neuron disease and ALS phenotypes.

Materials and methods

Subject groups

We retrospectively enrolled 54 ALS patients who had consecutively undergone a comprehensive evaluation, including neurological history, neurophysiological assessment, and MRI the IRCCS Istituto Auxologico Italiano in Milan (Italy), between January 2016 and December 2017. Diagnosis of ALS was made according to the revised El Escorial criteria [4].

Neurological examination was performed by two neurologists with more than 15 years of experience in the assessment of neuromuscular disorders.

According to our clinical protocol, the patients underwent MRI within 1 day from the clinical assessment. In addition to the MR images, in all subjects we collected the following clinical data: predominance of UMN or LMN impairment signs, site of onset (spinal or bulbar), and disease duration. The severity of UMN impairment was quantified using the Penn UMN score [17], and the grade of disability was measured using the ALS Functional Rating Scale-Revised (ALSFRS-R) [18]. The exclusion criteria for patient selection were (a) concomitant psychiatric or neurological disorders; (b) MR imaging artifacts; or (c) brain lesions shown on MRI images involving the motor cortex and corticospinal tracts not related to ALS.

Patients were divided into three subgroups according to the predominance of UMN and LMN impairment: 12 UMN-predominant ALS (UMN-ALS), 16 LMN-predominant ALS (LMN-ALS), and 19 ALS patients with no clinically defined predominance (c-ALS).

We recruited 23 healthy controls (HC) including volunteers and non-blood relatives of the patients between January 2018 and September 2018. HC enrollment was performed according to the following criteria: (a) in the age range of the ALS group; (b) no history of psychiatric or neurological disorders; and (c) no reported substance abuse. The exclusion criteria were as follows: (a) image artifacts on the T1-weighted and/

or gradient-echo images, or (b) brain MRI showing abnormal findings, except for sporadic small gliotic lesions in the white matter. Study participants were selected from the cohort of previous studies on QSM assessment in ALS [7, 8, 19].

Additionally, we retrospectively collected images of 15 patients defined as mimics and chameleons (ALS-Mim), as defined by Turner and Talbot [5], who had undergone the same MRI protocol as ALS and HC patients, between January 2016 and September 2018. The definite diagnoses of ALS-Mim were extracted from clinical records as follows: two hereditary spastic paraparesis, one metabolic myelopathy with vitamin B12 deficiency, three corticobasal degeneration, two cervical myeloradiculopathy, five parkinsonism, and two frontotemporal dementia.

For statistical analysis purposes, we also considered the group of subjects with suspected motor neuron disease (SMD) composed of ALS and ALS-mim.

The research protocol was reviewed and approved by the local ethics committee, in accordance with the Declaration of Helsinki. All participants gave written, informed consent for the protocol.

Image acquisition and processing

All subjects underwent a brain MRI on a 3T SIGNA General Electric (GE Healthcare Medical Systems) unit at the Istituto Auxologico Italiano, IRCCS, Milan (Italy). The protocol included the following: whole-brain sagittal three-dimensional FSPGR BRAVO T1-weighted sequence (TR = 8.7 ms, TE = 3.2 ms, inversion time = 450 ms, voxel size = $1 \times 1 \times 1$ mm^3, flip angle = 12°, acquisition matrix 256×256); sagittal 3D fluid-attenuated inversion recovery (FLAIR) (TR = 6000 ms, TE = 108 ms, inversion time = 1824 ms, voxel size = $1 \times 1 \times 1.4$ mm^3, flip angle = 90°, acquisition matrix 224×224); axial T2-weighted fast spin-echo (FSE) (TR= 3000 ms, TE = 82 ms, pixel size = 0.234×0.234 mm^2, slice thickness = 2 mm, slice gap = 0.2 mm, flip angle = 111°, acquisition matrix 320×320); three-dimensional spoiled gradient-echo multi- echo (GRE) pulse sequences (TR = 39 ms, TE = 24 ms, delta TE = 3.3 ms, number of echoes = 7, voxel size = $0.468 \times 0.468 \times 1.4$ mm^3, flip angle = 20°, acquisition matrix 416×320). The images collected from the different echoes of the spoiled GRE were averaged and the phase images were high-pass filtered by the scanner.

The brain MRIs were assessed by two neuroradiologists (with 4 and 30 years of experience) to define motion artifacts in the T1 or GRE sequences, arriving at a consensus. The brain MRI assessments identified severe motion artifacts in seven ALS patients, who were excluded from further analysis.

Visual qualitative assessment

The Motor Cortex Susceptibility (MCS) score described in detail in [13] was used for the visual qualitative assessment of the motor cortex susceptibility. Briefly, for each of the six subregions of the primary motor cortex (upper limb, lower limb, and bulbar musculature in each hemisphere) 0 to 2 points on a discrete scale were allotted, according to the level of hypointensity of the primary motor cortex in a curvilinear multi-planar reconstruction of the cortical surface in magnitude images of the SWI (Fig. 1A).

Automatic quantitative assessment

The primary motor cortex QSM was performed as described in Contarino et al [8]. Briefly, the whole-brain QSM was calculated from the 3D spoiled gradient-echo pulse sequence by using STI Suite tool (https://people.eecs.berkeley.edu/~chunlei.liu/software.html) and coregistered to the 3D T1-weighted image. The 3D T1- weighted images were segmented by using FreeSurfer [20, 21] and the Desikan-Killiany atlas [22] (Fig. 1B). Then, the susceptibility mean (SuscMean), susceptibility median (SuscMedian), susceptibility standard deviation (SuscSD), susceptibility skewness (SuscSkew), and susceptibility kurtosis (SuscKurt) neuroimaging metrics were calculated from the susceptibility values distribution in the automatically segmented precentral cortex.

Statistical analysis

The statistical analysis was performed using R 4.1.2 [R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing. URL https://www.R-project.org/]. A p-value smaller than 0.05 was considered significant.

Shapiro-Wilk tests were performed to test the distribution normality of the continuous variables.

The Mann-Whitney/Kruskal-Wallis tests were used to compare the continuous and ordinal variables between/among groups, and a chi-square test was used to compare nominal variables. Dunn-Holm's tests were carried out for post hoc pairwise comparisons. In light of the distribution of the values of the qualitative and quantitative variables into the groups, we defined the following group-comparison and accuracy assessment: (1) SMD vs HC and (2) UMN-ALS vs ALS-mim.

Spearman's test was used to assess the correlation between SuscSkew and MCS.

The area under the curve (AUC) value of the receiver operating characteristic (ROC) curve was used to evaluate the accuracy of MCS and QSM metrics in differentiating SMD and HC, and UMN-ALS and ALS-Mim. Sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were then calculated for the MCS, SuscSD, SuscSkew, using the value that maximizes SP and PPV as a cut-off. Delong's test was used to compare AUCs.

Fig. 1 A SWI-based MCS score: hypointensities in the primary motor cortex are visually evaluated in the subregions of lower limbs (in blue), upper limbs (in red), and bulbar musculature (in yellow) on both hemispheres as described in [13]; **B** QSM-based metrics: QSM values in the precentral gyrus (in red) are automatically extracted by image segmentation and coregistration to compute SuscMean, SuscMedian, SuscSD, SuscSkew, and SuscKurt as described in Contarino et al [8]
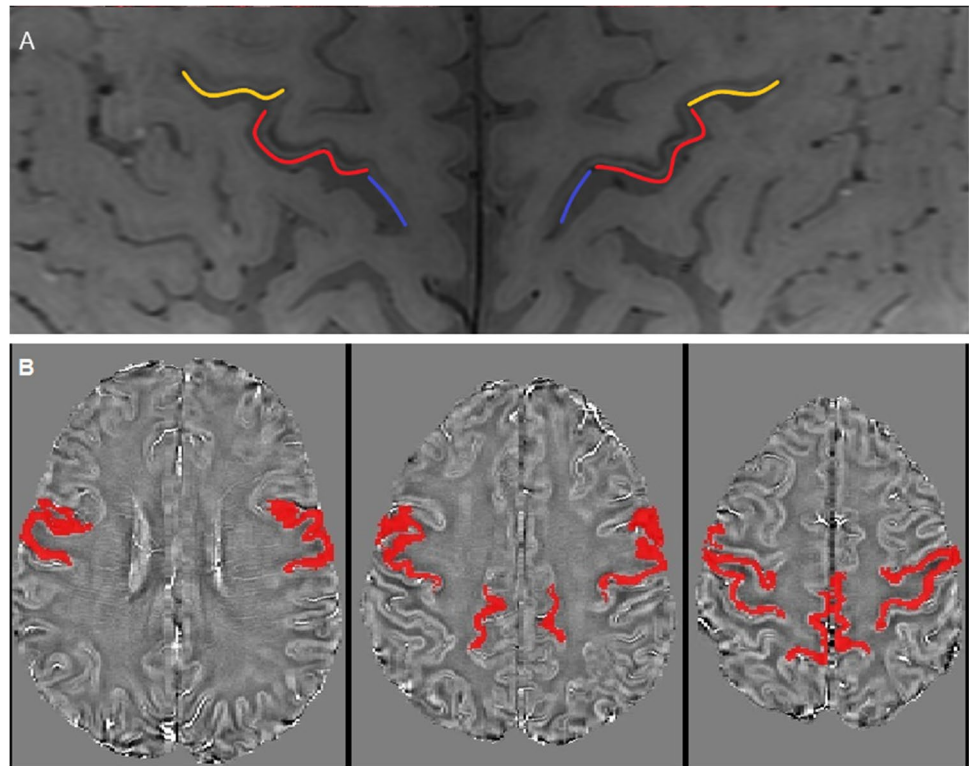
Results

Group-comparison in demographic, clinical, and neuroimaging data

The cohort of ALS subjects (male: 20/47) had a median age of 62 years (IQR: 54.5–68.5 years) and a median duration of illness of 18 months (IQR 11.5–25.5 months). The ALS-mim subjects (male: 7/15) had a median age of 70 years (IQR: 57–74.5 years) and the HC (male: 8/23) had a median age of 57 years (IQR: 53–62.5 years). Table 1 summarizes the demographic, clinical, and neuroimaging data in the subgroups.

No statistically significant difference was found among c-ALS, UMN-ALS, LMN-ALS, ALS-mim, and HC in terms of gender ($\chi^2(4) = 1.24$, $p = 1$) and age (H(4) = 10.6, $p = 0.371$). c-ALS, UMN-ALS, and LMN-ALS did not statistically differ in terms of ALSFRS-R (H(2) = 0.00617, $p = 1$), and disease duration (H(2) = 2.72, $p = 1$). UMN-ALS showed significantly higher Penn UMN scores than LMN-ALS and c-ALS (H(2) = 34.5, $p < 0.0001$).

MSC score of 1 and 2 was more frequent in UMN-ALS than LMN-ALS, c-ALS, ALS-mim and HC (H(4) = 33.4, $p = 0.00114$, Table 1 and Supplementary Table 1).

SuscSD was significantly higher in all ALS phenotype groups than in HC (H(4) = 20.1, $p = 0.00563$) (Table 1 and Supplementary Table 1).

SuscSkew was significantly higher in UMN-ALS compared to other c-ALS, LMN-ALS, ALS-mim and HC (H(4) = 23.0, $p = 0.00154$, Table 1 and Supplementary Table 1).

Figure 2 shows the distribution of the neuroimaging metrics in the subjects' groups.

For SMD subjects, no statistically significant differences were found in terms of gender from HC ($\chi^2(1) = 0.232$, $p = 1$) and age (U = 502, $p = 0.23$).

MCS score of 1 and 2 was more frequent in SMD than in HC (SMD MCS 0 = 45; 1 = 4; 2 = 13; HC MCS score 0 = 23; 1 = 0; 2 = 0; U = 518; $p = 0.048$). SuscSD was significantly higher in SMD than HC (respectively median = 21.8 ppb, IQR 20.3–24.3, and median = 19.9, IQR = 18.8–20.7; U = 264, $p = 0.0001$). No other susceptibility metrics significantly differ between groups.

Correlation between qualitative and quantitative assessment and stratification according to MCS score

The MCS score showed a significant correlation with Susc-Mean (Rho = 0.27, $p = 0.01$), SuscSD (Rho = 0.45, $p < 0.001$), and SuscSkew (Rho = 0.55, $p < 0.001$) (Fig. 3). The correlations are shown in Supplementary Table 2.

Subjects grouped according to the MCS score showed statistically significant differences in QSM metrics (Table 2). In particular, subjects with MCS score of 0 had lower Susc-Skew values (H(2) = 25, $p < 0.0001$) and lower SuscSD

Table 1 Demographics, clinical and neuroimaging data in subjects' groups. Categorical variables are reported as absolute frequency. Continuous variables are reported as median (interquartile range). Chi-square and Crammers' V were used to assess differences in distribution and effects sizes for categorical variables. For continuous and ordinal variables, Kruskal-Wallis test and Eta2 were used. *p*-values were corrected using Bonferroni's method

	c-ALS	UMN-ALS	LMN-ALS	ALS-Mim	HC	*p* values	Effect size
Male (frequency/total)	7/19	5/12	8/16	7/15	8/23	1	0.1207
Age (years)	58 (51.5–64.5)	59 (54.2–68.2)	66.5 (58.8–70.8)	70 (57–74.5)	57 (53–62.5)	0.371	0.0820
Illness duration (months)	16 (7–24)	20.5 (18.8–33)	17 (12–23.2)	-	-	1	0.00864
ALSFRSR	39 (31–42)	38.5 (32.8–42.5)	38 (34–41)	-	-	1	0.04
Penn UMN	**10 (7–12.5)**	**21 (16.8–23.2)**	**3.5 (0.75–6)**	**-**	**-**	**< 0.0001*****	**0.739*****
MCS (0/1/2)	**13/1/5**	**3/2/7**	**15/0/1**	**14/1/0**	**23/0/0**	**0.00144****	**0.301*****
SuscMean (ppb)	13.7 (11.2–15.9)	13.0 (11.1–14.9)	12.5 (10.6–14.1)	13.7 (11.7–14.9)	12.1 (11.0–13.7)	1	0.004
SuscMedian (ppb)	13 (11.8–15.7)	12 (10.7–13.9)	12.5 (10.5–14.2)	14 (11–15)	12 (11.2–14)	1	0.0285
SuscSD (ppb)	**20.9 (20.2–23.9)**	**22.2 (20.2–24.7)**	**21.4 (20.5–23.9)**	**23.2 (21.3–24.0)**	**19.9 (18.8–20.7)**	**0.00563****	**0.199*****
SuscSkew	**−0.098 (−0.232 to 0.137)**	**0.189 (0.077–0.422)**	**−0.185 (−0.284 to −0.045)**	**−0.11 (−0.172 to −0.036)**	**−0.186 (−0.212 to −0.099)**	**0.00154****	**0.234*****
SuscKurt	3.48 (3.30–5.26)	3.64 (3.3–4.2)	3.77 (3.44–4.19)	3.28 (3.20–3.72)	3.43 (3.21–3.68)	1	0.0119

ALS amyotrophic lateral sclerosis, *c-ALS* classic ALS phenotype, *UMN-ALS* upper motor neuron predominant ALS, *LMN-ALS* lower motor neuron predominant ALS, *ALS-Mim* mimics of ALS phenotype, *HC* healthy control, *SuscMean* mean of distribution susceptibility values of the motor cortex, *SuscMedian* median of susceptibility values of the motor cortex, *SuscSD* standard deviation of distribution of susceptibility values of the motor cortex, *SuscSkew* skewness of distribution of susceptibility values of the motor cortex, *SuscKurt* kurtosis of distribution of susceptibility values of the motor cortex, *MCS* Motor Cortex Susceptibility score, *ALSFRSR* ALS Functional Rating Scale
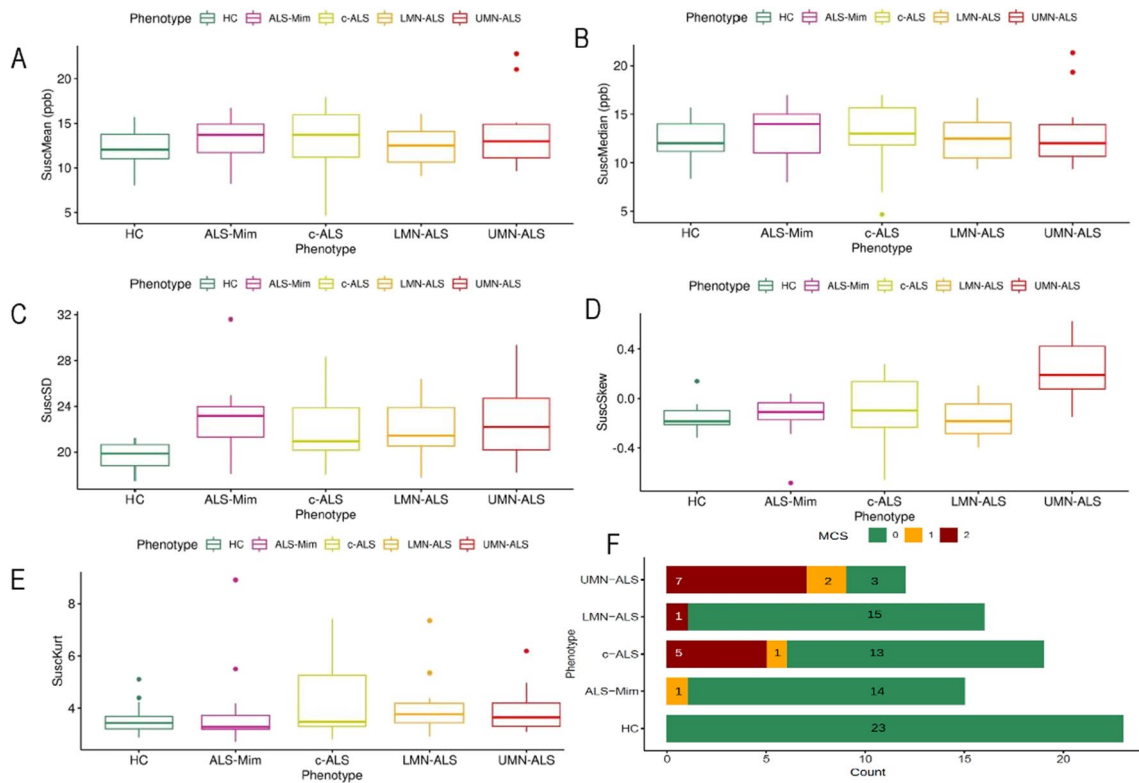

Fig. 2 Distribution of the neuroimaging metrics in the subject's groups (**A**) SuscMean, (**B**) SuscMedian, (**C**) SuscSD, (**D**) SuscSkew, (**E**) SuscKurt, and (**F**) MCS
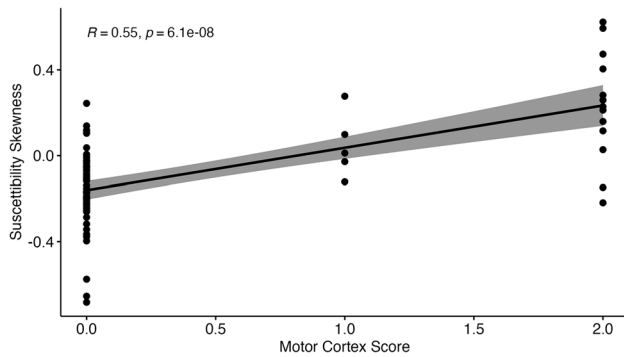
Fig. 3 Spearman's correlation analysis for MCS score and SuscSkew distribution metrics with 95% confidence intervals shaded in gray

(H(2) = 18, p = 0.000122) compared to subjects with MCS score 2, and lower SuscSkew (H(2) = 25.1, p < 0.0001) than subjects with MCS score 1 as shown in supplementary Table 3. No statistical differences were found in SuscMean, SuscMedian, and SuscKurt.

Diagnostic accuracy of qualitative and quantitative measures

The ROC analysis demonstrated that SuscSD (AUC = 0.809, p < 0.0001) and MCS (AUC = 0.64, p = 0.003) (Fig. 4A) are able to differentiate SMD from HC with specificity and a positive predictive value of 100% considering a cut-off of 21.26 ppb and a cut-off of 1 for SuscSD and MCS, respectively. SuscSD was a significantly better discriminative metric than MCS (Delong's test Z = −3.384, p-value = 0.00071).

The ROC analysis demonstrated that SuscSkew (AUC: 0.936, p < 0.0001) and MCS (AUC: 0.861, p < 0.0001) are able to differentiate UMN-ALS versus ALS-Mim (Fig. 4B). SuscSkew did not have a significantly higher AUC than MCS (Delong's test Z = 0.81; p = 0.21). SuscSkew showed the highest specificity (92.3%) and positive predictive value (91.7%) considering a cut-off of −0.017.

Discussion

Our study shows a good concordance between the SWI-based visual qualitative and QSM-based automatic quantitative measures in assessing the magnetic susceptibility alteration in the motor cortex. We demonstrated sufficient and very good diagnostic accuracy to differentiate subjects with SMD from HC respectively for MCS and SuscSD and very good and excellent accuracy to differentiate ALS-UMN from ALS-Mim respectively for MCS and SuscSkew.

Indeed, the concordance between visual qualitative and automatic quantitative assessment was highlighted by significant positive correlations between MCS and QSM metrics and the strongest correlations were demonstrated for SuscSD and SuscSkew. Since the two quantitative and qualitative measures that are based on different images and different imaging methods provide similar results, that reinforces their role in representing the alterations in the motor cortex.

SMD showed altered MCS and SuscSD values compared to HC. In the ROC analysis, MCS and SuscSD showed high accuracy, SP and PPV in differentiating the two groups, suggesting two promising methods to detect non-specific cortical iron-related alterations. Moreover, the automatic quantitative SuscSD showed better performances than the qualitative visual MSC with the advantage being unbiased by the reader.

Iron-related alteration in the cortex is one of the etiopathogenetic mechanisms suggested for pathologies other than ALS that involve neurodegeneration and can mimic the clinical pictures of motor neuron disease [5, 10, 23]. Thus, alteration of iron homeostasis in the cortex measured by QSM could be of help in the discrimination of subjects with a SMD from HC, ruling in pathological subjects with involvement of the motor cortex. We might speculate that the SuscSD might also be linked to a less clearly defined boundary between gray matter and white matter due to the degeneration of the motor cortex and the

Table 2 Subjects' susceptibility distribution variable assessed by MCS score. Variables are reported as median (interquartile range). Differences in variable distribution are assessed with Kruskal-Wallis test. Eta2 was used for effect size

	MCS 0	MCS 1	MCS 2	p	Effect size
SuscMean (ppb)	12.4 (11.0–14.4)	12.5 (9.91–13.7)	14.8 (13.0–16.9)	0.0735	0.0776*
SuscMedian (ppb)	12.7 (11–14.7)	12 (10–13)	13.7 (12–15.7)	0.795	0.0202
SuscSD (ppb)	**20.7 (19.3–21.7)**	**23.2 (20.1–25.0)**	**24.3 (23.3–25.4)**	**0.00061****	**0.193*****
SuscSkew	**−0.169 (−0.23 to −0.058)**	**0.012 (−0.028 to 0.098)**	**0.228 (0.115–0.404)**	**< 0.0001*****	**0.278*****
SuscKurt	3.47 (3.21–4.16)	13.5 (9.5–18.8)	3.70 (3.40–4.31)	1	0.00146

SuscMean mean of distribution susceptibility values of the motor cortex, *SuscMedian* mean of susceptibility values of the motor cortex, *SuscSD* standard deviation of distribution of susceptibility values of the motor cortex, *SuscSkew* skewness of distribution of susceptibility values of the motor cortex, *SuscKurt* kurtosis of distribution of susceptibility values of the motor cortex, *MCS* Motor Cortex Susceptibility score

Fig. 4 ROC curves analysis comparing the performance of (**A**) SuscSD and MCS in distinguishing SMD from HC; **B** SuscSkew and MCS in distinguishing UMN-ALS and ALS-Mim
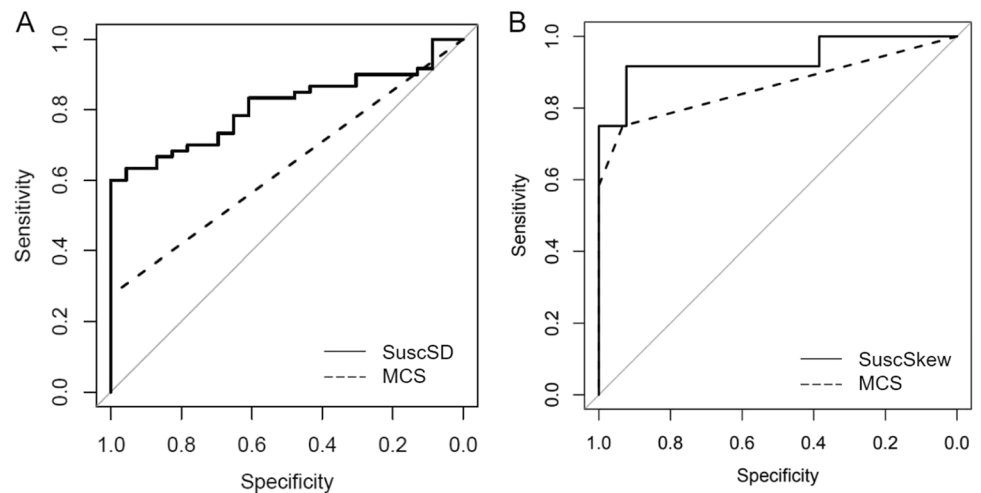
white matter, a process that has been recognized for ALS and ALS mimics subjects [10, 24–26].

UMN-ALS demonstrated altered MCS and SusckSkew values compared to other ALS phenotypes, HC and, more interestingly, to ALS-Mim. In literature, iron-related hypointensity in the motor cortex can be influenced by disease phenotype, in particular by the degree of UMN impairment [27]. Studies combining imaging with anatomopathological assessment showed that MRI signal changes of the motor cortex correspond to intracellular ferritin inclusion in microglial cells [10, 11]. We support the thesis that iron accumulation may be one of the mechanisms of degeneration in UMN-ALS subjects, while in LMN-ALS subjects the pathological mechanism is likely to be TDP-43 proteinopathy such as aggregates of cleaved and hyperphosphorylated TDP-43 and FUS-positive basophilic inclusion bodies [28, 29]. TDP-43 proteinopathy that starts in the LMNs and eventually spreads from the spinal cord and brainstem to the primary motor cortex was demonstrated in the LMN-ALS subgroup of progressive muscular atrophy [29].

A high SusckSkew means that the distribution of the QSM values is asymmetrical due to a larger right tail representing high susceptibility values. Interesting, previously published anatomopathological analysis of the precentral cortex of subjects affected by ALS demonstrated sparsely and intensely Perls-stained for ferric iron astrocytes and macrophages [10, 30]. A more recent study by [10] demonstrated how the microglia of the middle and deep layer of the motor cortex of subjects affected by ALS are selectively involved and the pattern of involvement is different from other pathologies that can affect the motor cortex. Indeed, the motor cortex of subjects affected by Alzheimer's and Parkinson's disease, is more diffusely involved and iron accumulation is also present in the superficial white matter of the gyrus. Thus, we postulate that the increase in the

SuscSD and SuscSkew could reflect the non-uniform and selective increase in ferric iron of the microglia of the precentral cortex of subjects affected by UMN-ALS. In our cohort, UMN-ALS were discriminated from ALS-Mim with high accuracy by using MSC, and SuscSkew. A previous publication [13] demonstrated how visual assessment of susceptibility of the motor cortex can be helpful in differentiating the UMN-ALS subjects from HC and ALS-Mim, being able to rule in ALS in subjects without LMN impairment using a MCS score ≥ 2 as a cut-off. Another study, using manually drawn ROIs [9], demonstrated how the relative mean and relative maximum QSM value of the cortex of the hand knob can differentiate ALS subjects from ALS-Mim with a specificity of 100%. In our study, SuscSkew is the most accurate metric to differentiate UMN-ALS from ALS-Mim, with sensibility, specificity, negative predictive value, and positive predictive value above 90% considering a cutoff of −0.017. The accuracy of SuscSkew is greater than MCS score, though not significantly. We can conclude that both SucSkew and MCS are reliable metrics to rule in subjects affected by ALS with prevalence of upper motor neuron signs. We provided a highly specific user independent cut-off that might ease the diagnosis of UMN-ALS and the enrollment in future clinical trials that would benefit from quantification of iron content alteration before and after treatment, such as the iron-chelation trials.

However, some limitations of our study need to be acknowledged. First, the sample size of our cohort is small and needs to be enlarged to validate the promising results of the present study.

Tissue susceptibility measured by QSM in the precentral cortex is determined by concentration of paramagnetic (iron) and diamagnetic (cortical myelin) species in a voxel. Combined MRI-histological studies or advanced processing and modelling of QSM data (source separation) could be useful to discriminate the contribution of each

component to the susceptibility properties of the tissue [31].

Further prospects might be the validation of the SWI-based qualitative visual and the QSM-based quantitative automatic measures in multicentric studies and the assessment of their accuracy in the early stages of the disease and their predicting roles in subjects at risk.

In conclusion, the SWI-based visual qualitative and the QSM-based automatic quantitative measures were concordant in assessing iron-related alterations in the primary motor cortex and might be evaluated in larger multicentric studies to validate their role in the identification of UMN-ALS among patients with suspected motor neuron disease.

Declarations

Guarantor The scientific guarantor of this publication is Claudia Morelli.

Conflict of interest Fabio Maria Triulzi is a member of the *European Radiology* Scientific Editorial Board. The remaining authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry The statistics were defined by Conte and Lo Russo

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Study subjects or cohorts overlap There exists a partial overlap with:

- Contarino VE, Conte G, Morelli C, et al (2020): Development of the QSM method, no SWI assessments were performed
- Conte G, Contarino VE, Casale S, et al (2021): Evaluation of the QSM measures accross the ALS clinical phenotypes, no SWI assessments were performed
- Conte G, Sbaraini S, Morelli C, et al (2021): Development of the SWI method, no QSM assessments were

Methodology

- Retrospective
- observational
- performed at one institution

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