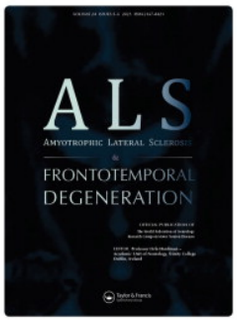

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RESEARCH ARTICLE

Italian reference values and brain correlates of verbal fluency index – *vs* standard verbal fluency test – to assess executive dysfunction in ALS

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Abstract

Objectives: In amyotrophic lateral sclerosis (ALS), verbal fluency index (V*fi*) is used to investigate fluency accounting for motor impairment. This study has three aims: (1) to provide V*fi* reference values from a cohort of Italian healthy subjects; (2) to assess the ability of V*fi* reference values (*vs* standard verbal fluency test [VFT]) in distinguishing ALS patients with and without executive dysfunction; and (3) to investigate the association between V*fi* and brain structural features of ALS patients. *Methods:* We included 180 healthy subjects and 157 ALS patients who underwent neuropsychological assessment, including VFT and V*fi*, and brain MRI. Healthy subjects were split into four subgroups according to sex and education. For each subgroup, we defined the 95th percentile of V*fi* as the cutoff. In ALS, the distributions of "abnormal" cases based on V*fi* and standard VFT cutoffs were compared using Fisher's exact test. Using quantile regressions in patients, we assessed the association between V*fi* and VFT scores, separately, with gray matter volumes and white matter (WM) tract integrity. *Results:* Applying V*fi* and VFT cutoffs, 9 and 13% of ALS cases, respectively, had abnormal scores ($p < 0.001$). In ALS, while higher V*fi* scores were associated with WM changes of callosal fibers linking supplementary motor area, lower VFT performances related to corticospinal tract alterations. *Discussion:* We provided Italian reference values for the spoken V*fi*. Compared to VFT, V*fi*s are critical to disentangle motor and cognitive deficits in ALS. In patients, abnormal V*fi*s were associated with damage to WM tracts specifically involved in ideational information processing.

Keywords: *Amyotrophic lateral sclerosis, cognitive classification, motor neuron disease, normative data, verbal fluency index*

Introduction

Amyotrophic lateral sclerosis (ALS), the most common form of motor neuron disease, is a progressive and fatal neurodegenerative disorder characterized by the degeneration of both the upper and lower motor neurons (1). In about 35–45% of ALS patients concomitant cognitive and/or behavioral deficits may occur, with 14% of these

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cases presenting with a comorbid behavioral variant frontotemporal dementia (bvFTD) (1,2). The most frequent cognitive changes are executive function and language deficits, while typical behavioral disturbances are apathy, loss of sympathy and empathy, stereotyped behaviors and dietary changes (2,3).

According to the revised consensus criteria (4), a diagnosis of ALS with cognitive impairment depends on the presence of executive and/or language dysfunction. The executive dysfunction is defined by the sole presence of deficits in verbal letter fluency or in two non-overlapping executive tests. Verbal letter fluency is assessed with the verbal fluency test (VFT), in which subjects are required to produce as many words as possible beginning with a certain letter within a limited amount of time (usually 60 s per letter). In ALS, VFT pathological scores are considered a sensitive early marker of executive function difficulties (5,6). However, a major concern is that VFT can be affected by patient speech motor disabilities resulting from bulbar involvement (7). In the original paper of Abrahams et al. (7), authors observed a performance decrement in the ALS patient group on the spoken VFT without the inclusion of the speech motor control condition. Being VFT determinant for the cognitive classification of ALS patients, this test is valid only if controlled for motor and/or speech impairment, otherwise would lead to an over estimation and misclassification of patients' cognitive deficits with implications in terms of prognosis, treatment, expectations about the adherence to treatment, prognosis and caregiver burden (8). To overcome such a limitation, the verbal fluency index (V*fi* = 60 s-seconds to read aloud words/correct words generated) was introduced, which allows the investigation of fluency accounting for motor impairment (7). V*fi* has been included in the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) (9).

In Italy, the need of *Vfi* reference values has been partially satisfied by the Italian ECAS, which provided a cutoff for the spoken letter fluency index of a single letter (10). However, the Italian most common VFT is that of Novelli et al. (11), which is still widely administered also to ALS patients. In this version of VFT, participants are required to produce as many words as possible beginning with the letters "F", "P", and "L" in three 1-min trials. Several findings suggested that the impact of executive abilities on fluency tests increases across the trials because patients need to remember earlier responses, suppress interference, and shift between proposed letters (12,13). Therefore, more trials should be more sensitive to executive dysfunctions than one trial alone, as in the case of ECAS. Italian normative values for V*fi* of this commonly used version of VFT (11) have not been provided yet.

With respect to this background, our study had three aims: (1) to provide *Vfi* reference values from a cohort of Italian healthy controls; (2) in a large cohort of ALS patients, to compare the ability of *Vfi* reference values *vs.* standard VFT cutoffs in distinguishing patients with and without executive dysfunction; and (3) to investigate the association between VFT and *Vfi* values, separately, with brain features of ALS patients. We hypothesized that a lower number of ALS patients would be classified as cognitively impaired based on the V*fi* reference values with respect to using the standard VFT cutoffs. We further expected that the relationship between patients' V*fi* performances and their brain integrity would include regions specifically involved in lexical and ideational information processing.

Materials and methods

Subjects

From a large sample of 311 healthy controls, we retrospectively selected 180 subjects, aged 29–85 years, who had an available neuropsychological assessment which included both VFT and V*fi*. Inclusion criteria were the following: normal neurological exam, Mini Mental State Examination (MMSE) score $\geq$27, and negative family history for neurodegenerative disorders. From a large sample of 235 patients with ALS, we retrospectively selected 157 cases, recruited in two expert centers in Milan (IRCCS Ospedale San Raffaele and IRCCS Istituto Auxologico Italiano), with a clinical diagnosis of probable or definite ALS (14), and with an available neuropsychological assessment which included both VFT and V*fi*. VFT and V*fi*. Disease severity in patients was assessed using the ALS Functional Rating Scale-revised (ALSFRS-R) (15). At study entry, all patients were receiving Riluzole and none had significant respiratory failure. A sub-sample of ALS patients ($N = 95$) also underwent a 3T MRI scan (see details below). Additional inclusion criteria for all participants were the following: native Italian-speaking, no significant medical illnesses or substance abuse that could interfere with cognitive functioning, no (other) major systemic, psychiatric, or neurological illnesses.

The study protocol was approved by local ethical standards committee on human experimentation and all participants provided written informed consent.

Cognitive and behavioral assessment

Neuropsychological assessments were performed by experienced neuropsychologists. Full details of the batteries administered to patients and controls are available on the supplementary material.

Among the executive functions, in patients and healthy controls, fluency was assessed with the spoken letter VFTs (11) and the relative *Vfis* (7). During the spoken letter VFT, each participant was asked to say aloud as many different words beginning with a given letter (P, F and L; excluding proper nouns and derived words) in a 60 s time period (11). In order to obtain *Vfi* values, we applied the following procedure: for each letter of the VFT, the participant was asked to read aloud the produced words as fast as possible and the reading time (seconds) was recorded; a *Vfi* for each letter was calculated with the following formula: 60 s-seconds to read aloud words/correct words generated (7); the three obtained V*fi*s (one for each letter) were finally averaged, thus obtaining one *Vfi* for each participant.

MRI acquisition

Using a 3.0 T scanner (Intera, Philips Medical Systems, Best, the Netherlands), the following brain MRI sequences were obtained from 95 ALS patients at the same center: T2-weighted spin echo; fluid-attenuated inversion recovery; 3D T1-weighted fast field echo (FFE); and pulsed-gradient SE echo planar. Full details on MRI acquisition are available on the supplementary material.

MRI analysis

MRI analysis has been previously described (16). Full details on volumetry, diffusion map reconstruction and fiber tracking are available on the supplementary material.

Grey matter volumes. To obtain quantitative measures of regional gray matter (GM) volumes, GM maps of patients were parcellated into 90 Automated Anatomical Labeling (AAL) regions of interest.

White matter tractography. DT MRI analysis was performed using the FMRIB Diffusion Toolbox in FSL and the JIM6 software. Maps of mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity (axD) and radial diffusivity (radD) were obtained. Seeds for tractography of the corticospinal tract (CST), corpus callosum (CC), cingulum, superior longitudinal (SLF), inferior longitudinal and uncinate fasciculi were defined, as previously described (17,18). In addition, using a "seed to target" approach, the CC was segmented into three portions to identify the callosal fibers linking the primary motor cortices, lateral premotor cortices and supplementary motor areas (CC-SMA) (17). Fiber tracking was performed using a probabilistic tractography algorithm in FSL (probtrackx, Bedpostx) (19).

Statistical analysis

Sociodemographic, cognitive, and behavioral data. Sociodemographic, cognitive, and behavioral features were compared between ALS and healthy controls using Mann–Whitney's test and Fisher's exact test for continuous and categorical variables, respectively. The statistical analyses were performed with using SPSS software (version 24.0; IBM Corp., Armonk, NY, USA). In order to verify the homogeneity of the ALS samples, these analyses were repeated between ALS patients who underwent ($N = 95$) or not ($N = 62$) the MRI scan.

Vfi reference values. In healthy controls, the relationship between age ($\leq$60 vs >60 years), sex and education ($\leq$13 vs >13 years) with *Vfi* scores was assessed using multiple quantile regression. As in the Italian version of ECAS (10), which includes V*fi* cutoffs for the letter S, we used 13 years of education for separating low and high educated participants. *Vfi* scores were found be significantly associated with education ($p = 0.001$), but not with sex ($p = 0.051$), and amply not with age which was removed by backward variable selection. Thus, for the definition of the reference cutoffs, healthy controls were split into four subgroups based on sex and education: $N = 37$ females, education >13; $N = 76$ females, education $\leq$13; $N = 27$ males, education >13; $N = 40$ males, education $\leq$13 (see Supplementary Table 1 for sociodemographic, cognitive and behavioral features of each of the four subgroups).

In each healthy control subgroup, the distribution of the *Vfi* scores was examined. Since the distributions were not normal, we identified the cutoff differently from Abrahams et al. (9). For each healthy control subgroup, we firstly estimated the 95th percentile of *Vfi* and its 95% confident interval (CI). In order to be highly conservative in the definition of normal scores (and thus reducing false positives cases), we defined the upper bound of the 95% CI as the cutoff to assess abnormal scores (Supplementary Figure 1).

As for the healthy control group, the ALS patient group was divided in four subgroups according to sex and education. *Vfi* reference cutoffs obtained in healthy controls were then applied to determine the possible presence of abnormal *Vfi* scores in patients. ALS *Vfi*s were considered altered if they were greater than the reference cutoff. In the ALS cohort, the distribution of "abnormal" cases defined with the cutoffs of the *Vfi* and of the standard VFT (11) was compared using Fisher's exact test.

Finally, in order to verify the correlation between V*fi* and other executive domain tests, in healthy controls we computed the Spearman's correlation coefficient between VFT and *Vfi* scores,

separately, with the performances at the Digit Span backward, Trail Making Test B-A, and Attentive Matrices.

The analyses were performed with R 3.5.0 (http://www.R-project.org/) and SPSS software (version 24.0; IBM Corp., Armonk, NY, USA). The quantreg R package was used for the estimation of the quantile regression and the quantileCI R package for the calculation of the 95% CI of the percentiles.

MRI analysis. In the group of ALS patients who also underwent MRI ($N = 95$), we assessed the association between VFT and *Vfi* values, separately, with GM volumes and WM tract integrity measures, by using quantile regression adjusted for age, sex and education (age and education considered as continuous variables). Bonferroni-correction for multiple comparisons was applied to the resulting p values considering GM and WM, separately. The quantreg package of R 3.5.0 (http://www.R-project.org/) was used for estimating the quantile regression.

Results

Sociodemographic, cognitive, and behavioral data

Sociodemographic and clinical features of healthy controls and ALS patients are presented in Table 1, while details on their neuropsychological features are shown in Table 2. ALS patients and healthy controls differed in age, sex and education (Table 1). The neuropsychological assessment revealed significant differences between groups in almost all the tests administered, including the VFT and the *Vfi* (Table 2).

When classifying our ALS patient sample according to consensus criteria (4), 13% presented with cognitive impairment, 10% had behavioral impairments, 6% presented both cognitive and behavioral difficulties, and 3% were ALS-FTD.

The comparison between ALS cases who underwent or not the MRI revealed that patients who performed the MRI ($N = 95$) were older. The two groups were similar for the remaining sociodemographic, clinical, and cognitive variables (Supplementary Table 2).

Vfi *reference values*

Table 3 shows the resulting normative data of the *Vfi* for each healthy control subgroup. Applying *Vfi* cutoffs, 9% of ALS patients had an abnormal V*fi* score. On the other hand, applying standard VFT cutoffs, 13% of patients with ALS showed a pathological performance. The distribution of the abnormal performances classified with the VFT ($n = 20/157$) and with the *Vfi* ($n = 14/157$) differed significantly ($p < 0.001$; see Figure 1).

The 8 cases classified as pathological by VFT (but not by *Vfi*) showed moderate disease severity (mean ALSFRS-R = 37.17; mean disease progression rate = 0.68). In the diagnostic classification according to Strong's criteria (4), 4/8 cases were classified as cognitively impaired by VFT only; the remaining four cases were classified in any case as ALS-FTD ($N = 1$), behaviorally impaired ($N = 1$), and cognitive impaired ($N = 2$) due to the presence of executive dysfunctions in other tests than fluency.

The 2 cases classified pathological by V*fi* (but not by VFT) had bordeline performances based on standard VFT cutoffs (equivalent score = 1, between the 13.6th and the 5th percentile) and mild disease severity (mean ALSFRS-R = 44.00; mean disease progression rate = 0.25). According to the cognitive/behavioral classification (4), these two cases were classified as ALS-FTD and cognitively and behaviorally impaired.

*Relationship between VFT/*Vfi *and another executive test in healthy controls*

In healthy controls, significant correlations were found between VFT and *Vfi* performances, separately, with executive functions scores, assessed with the Digit Span backward (VFT: rho = 0.237, $p = 0.01$; *Vfi*: rho = −0.199, $p = 0.02$), Trail Making Test B-A (VFT: rho = −0.282, $p = 0.002$; *Vfi*: rho = 0.297, $p = 0.001$), and Attentive Matrices (VFT: rho = 0.301, $p = 0.001$; *Vfi*: rho = −0.300, $p = 0.001$).

Table 1. Sociodemographic and clinical features of the sample.

	HC	ALS	*p* Values
N	180	157	
Age [years]	63.44 ± 10.49 (29–85)	59.71 ± 10.68 (24–83)	0.001
Sex, women	114 (63.33%)	73 (46.50%)	0.001
Education [years]	12.88 ± 4.08 (5–23)	11.14 ± 4.32 (4–28)	<0.001
ALSFRS-R, 0–48	–	38.00 ± 6.11 (22–48)	–
Disease duration [months]	–	20.96 ± 17.68 (4–94)	–
Disease progression rate	–	0.70 ± 0.63 (0 – 2.67)	–

Notes: Values denote mean ± standard deviation (percentages or minimum-maximum values). *p* Values refer to Mann–Whitney's test and Fisher's exact test for continuous and categorical variables, respectively. Abbreviations: ALS: Amyotrophic Lateral Sclerosis; ALSFRS-R: ALS Functional Rating Scale revised; HC: Healthy Controls. Disease Progression Rate has been obtained as following: (48–ALSFRS-R score)/time between symptom onset and first visit.

Table 2. Neuropsychological assessment of the sample.

	HC	ALS	*p* Values
N	180	157	
Global cognition			
MMSE	29.32 ± 0.87 (27–30)	28.37 ± 1.84 (20–30)	<0.001
ECAS language	–	24.21 ± 4.23 (12–28)	–
ECAS fluency	–	16.76 ± 6.62 (0–22)	–
ECAS executive functions	–	35.91 ± 7.47 (10–46)	–
ECAS ALS-specific functions	–	77.00 ± 16.31 (38–96)	–
ECAS memory	–	16.26 ± 4.94 (1–23)	–
ECAS visuospatial	–	11.44 ± 1.19 (6–12)	–
ECAS ALS nonspecific functions	–	27.71 ± 5.14 (12–35)	–
ECAS total score	–	103.30 ± 20.82 (45–129)	–
Memory			
RAVLT, delayed recall	9.74 ± 2.89 (3–15)	8.62 ± 3.36 (0–15)	0.01
Digit span forward	6.01 ± 0.98 (4–9)	5.47 ± 1.08 (3–8)	<0.001
Spatial span	5.18 ± 1.08 (2–7)	4.77 ± 0.82 (3–6)	0.11
Executive functions			
WCST, perseverations	8.82 ± 5.46 (4–25)	21.97 ± 22.90 (3–101)	0.02
MCST, perseverations	4.39 ± 5.28 (0–39)	4.75 ± 5.90 (0–25)	0.61
Digit span, backward	4.69 ± 1.11 (2–8)	4.12 ± 1.20 (0–6)	0.001
Raven's matrices	31.29 ± 3.59 (17–36)	28.74 ± 5.31 (14–36)	<0.001
Cognitive estimation task	–	14.32 ± 3.94 (6–26)	–
Stroop test, errors	–	4.02 ± 9.57 (0–50)	–
Weigl's sorting test	–	11.53 ± 3.10 (1–15)	–
Trail making test B–A	64.92 ± 35.95 (0–265.12)	–	–
Attentive matrices	52.29 ± 6.24 (27–60)	–	–
Fluency			
Phonemic verbal fluency test (VFT)	39.06 ± 10.64 (12–81)	30.92 ± 12.28 (4–59)	<0.001
Phonemic fluency index (*Vfi*)	4.68 ± 1.97 (1.7–17.82)	7.61 ± 7.70 (1.81–68.79)	<0.001
Semantic fluency test	47.71 ± 10.12 (25–72)	39.01 ± 11.06 (4–66)	<0.001
Semantic fluency index	3.96 ± 1.72 (0–12.56)	5.41 ± 4.92 (2–44.49)	<0.001
Language			
BADA, nouns	29.59 ± 1.12 (25–30)	28.73 ± 2.16 (13–30)	0.002
BADA, actions	27.34 ± 1.40 (21–28)	26.39 ± 2.51 (17–30)	0.03
Token test	33.83 ± 1.99 (27–36)	–	–
Visuospatial abilities			
Rey figure, recall	16.16 ± 5.90 (4.5–29)	–	–
Benson figure, recall	11.25 ± 2.97 (4–17)	–	–
Social cognition			
SET global score	–	14.85 ± 3.17 (7–18)	–
SET intention attribution	–	4.88 ± 1.32 (2–6)	–
SET causal inference	–	5.06 ± 1.03 (2–6)	–
SET emotion attribution	–	4.85 ± 1.37 (1–6)	–
Psychopathology			
HDRS	–	6.23 ± 4.91 (0–24)	–
BDI	6.73 ± 5.07 (0–29)	9.59 ± 7.84 (0–37)	0.03
ALS-FTD-Q	–	11.79 ± 12.57 (0–52)	–
NPI	–	5.31 ± 6.38 (0–28)	–

Notes: Values denote mean ± standard deviation (minimum–maximum values). *p* Values refer to Mann–Whitney's test. Abbreviations: ALS: Amyotrophic Lateral Sclerosis; ALS-FTD-Q: ALS-Frontotemporal lobar degeneration Questionnaire; BADA: Italian battery for the assessment of aphasic disorders; BDI: Beck Depression Inventory; ECAS: Edinburgh Cognitive and Behavioral ALS Screen; HC : Healthy Controls; HDRS: Hamilton Depression Rating Scale; MCST: Modified Card Sorting Test; MMSE: Mini Mental State Examination; NPI: Neuropsychiatric Inventory; RAVLT: Rey Auditory Verbal Learning Test; SET: Story-Based Empathy Task; WCST: Wisconsin Card Sorting Test.

VFT and Vfi *performances vs MRI features in ALS*

In ALS, adjusted quantile regression between VFT and *Vfi* values, separately, with GM volumes and WM tract integrity measures showed that poor VFT scores were associated with WM alterations of the entire CC, CC-SMA, bilateral SLF, and right CST. Higher *Vfi* scores (reflecting a poor performance) were associated with WM alterations of the CC-SMA. Details of the DTI measures which were significantly associated with VFT scores and *Vfi*s are reported in Table 4 and Figure 2. In ALS cases, no associations were found between any fluency score and GM volumes.

Discussion

In the present study, we provided Italian *Vfi* reference values for the adjustment for ALS motor

Table 3. Reference data and cutoffs for the verbal fluency index (*Vfi*) in each subgroup of healthy controls.

	Males				Females			
Education (years)	<=13		>13		<=13		>13	
No. of subjects	(N = 40)		(N = 27)		(N = 76)		(N = 37)	
Spoken V*fi*	Mean ± SD (median; range IQR)	Cutoff[a]	Mean ± SD (median; range IQR)	Cutoff[a]	Mean ± SD (median; range IQR)	Cutoff[a]	Mean ± SD (median; range IQR)	Cutoff[a]
	5.26 ± 1.84 (5.06; 1.95)	16.05	4.37 ± 1.49 (3.90; 1.42)	12.41	4.92 ± 2.31 (4.33; 2.07)	16.89	3.79 ± 1.18 (3.74; 1.50)	6.88

[a]The cutoff refers to the upper limit of the 95th percentile confidence interval (see distributions of healthy control performances in the Supplementary Figure 1).

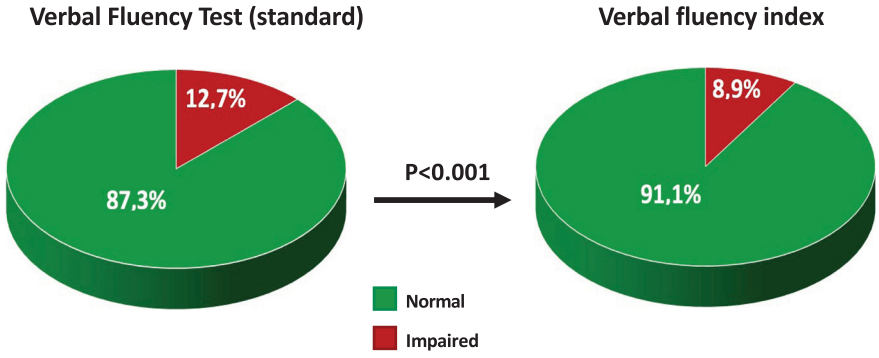

Figure 1. Application of VFT and *Vfi* cutoffs. Graphs display the percentages of ALS patients with an impaired and normal performance applying VFT and *Vfi* cutoffs. *p* Value refers to Fisher's exact test.

and/or speech impairment during fluency performances. Applying the standard and the identified V*fi* reference cutoffs in a large cohort of ALS patients, we demonstrated a significant difference in the classification of patients with abnormal fluency. As hypothesized, using the normative *Vfi* cutoffs defined in this study, the percentage of patients who had abnormal fluency indices (and thus potentially classified as ALS with cognitive impairment) was significantly lower compared to standard VFT. The eight patients classified as pathological by VFT (but not by V*fi*) showed moderate to severe disease severity (in terms of ALSFRS-R and progression rate), reflecting the possibility of a misclassification of patients' executive dysfunction due to motor impairment. On the other hand, one can hypothesize that these cases classified "normal" by V*fi* are false negatives. In fact, among these patients, one was classified as ALS-FTD according to Strong's criteria (4), one had behavioral impairment and two had executive impairments on tests other than verbal fluency. The V*fi* cutoffs that we proposed allow to define or support the diagnosis for the fluency domain only, neither for other executive dysfunctions nor for behavioral impairment. Although we cannot totally exclude the presence of false negatives when using V*fi*, we believe that those subjects (including the case of ALS-FTD) who were classified "normal" by our V*fi* cutoffs did not have a clear fluency impairment.

Although the Italian version of ECAS is an accurate measure for screening the presence of fluency dysfunction in patients with ALS accounting for motor disability (10), the V*fi* values obtained using Novelli's VFT (11) can be useful for assessing cognitive decline in those patients who present with high level of physical fatigue and are unable to perform the entire ECAS battery, or in those centers with a limited number of neuropsychologists.

Furthermore, the V*fi* values obtained using Novelli's VFT, have the potential to increase the accuracy of ECAS in detecting a possible impairment. This is due to the robustness of the test presenting more than one trial (11), which guarantees a reliable assessment of executive abilities, such as inhibition and switching, beyond the verbal (e.g. lexical/vocabulary knowledge) function (12,13). In this regard, we observed an association between V*fi* values and higher order executive functions (as indexed by the digit span backward, Trail Making Test B-A, and Attentive Matrices performances) in healthy subjects.

In this study, we obtained V*fi* reference values from a large and well-defined sample of healthy adults. In defining the cohort of controls, we took care to enroll participants with a normal score on MMSE (≥27) and negative family history for neurodegenerative disorders.

As in a previous study on normative data in a Dutch population (20), we did not find age-related differences on V*fi* performance. While the effect of age on semantic fluency has been consistently demonstrated (21), the total number of produced words in letter fluency seems to be stable across

Table 4. Significant relationships between patients' verbal fluency test and verbal fluency index performances and their white matter tract integrity.

	Verbal fluency test (VFT)	Verbal fluency index (V*fi*)
CC, MD	C: −40.11; $p = 0.04$	
CC, radD	C: −42.68; $p = 0.01$	
CC-SMA, FA	C: 97.27; $p = 0.002$	
CC-SMA, MD	C: −68.30; $p = 0.01$	C: 24.46; $p = 0.01$
CC-SMA, radD	C: −64.35; $p = 0.002$	C: 18.42; $p = 0.02$
SLF R, FA	C: 71.33; $p = 0.02$	
SLF R, MD	C: −48.88; $p < 0.001$	
SLF R, radD	C: −49.18; $p < 0.001$	
SLF L, radD	C: −59.76; $p = 0.02$	
CST R, MD	C: −23.96; $p < 0.001$	
CST R, axD	C: −22.18; $p = 0.04$	

Notes: Significant results of adjusted quantile regression between verbal fluency test and verbal fluency index scores and white matter tract integrity, accounting for age at MRI, sex, and education. Abbreviations: axD: axial diffusivity; C: regression coefficient; CC: corpus callosum; FA: fractional anisotropy; L: left; MD: mean diffusivity; R: right; radD: radial diffusivity; SLF: superior longitudinal fasciculus; SMA: supplementary motor area.

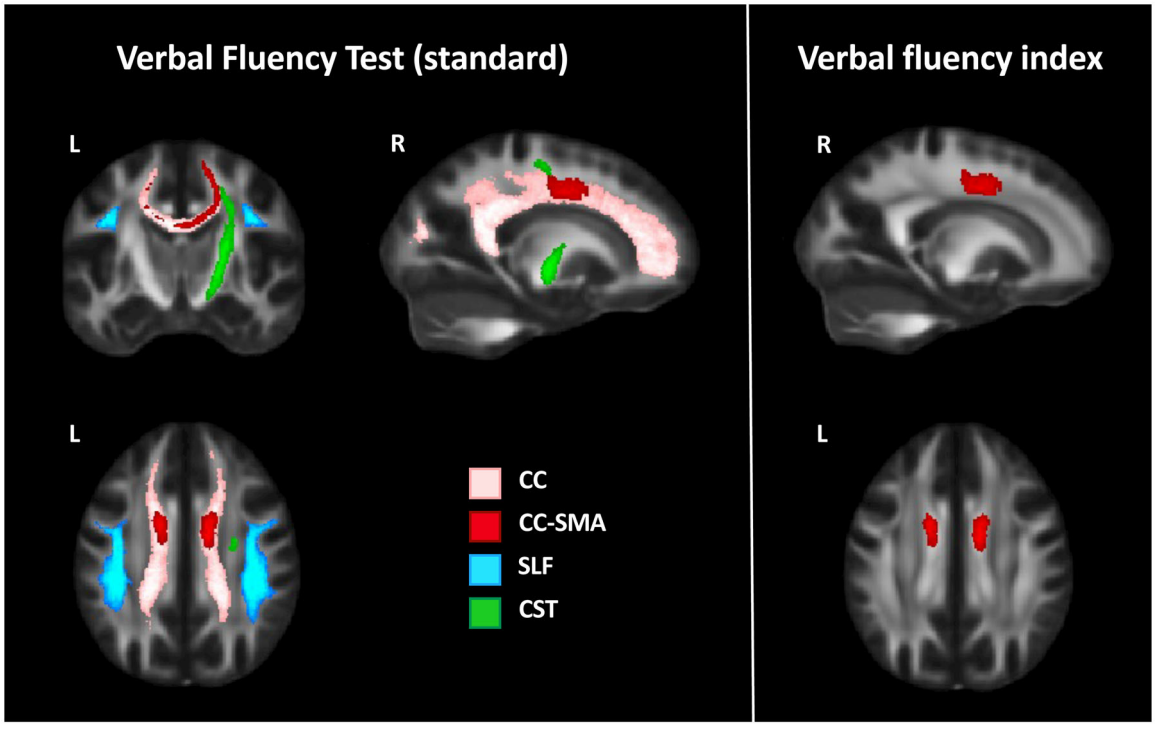

Figure 2. Significant relationships between patients' verbal fluency test and verbal fluency index performances with their white matter tract integrity. Abbreviations: CC: corpus callosum; CC-SMA: callosal fibers linking the supplementary motor area; CST: corticospinal tract; L: left; R: right; SLF: superior longitudinal fasciculus.

the tested age-range (22,23). In fact, letter fluency performance on conventional tests increases until 39 years of age with only a mild decline after the age of 70 (24). Most of the normal subjects in our cohort were between 40 and 70 years of age, which might explain the absence of an age effect in this sample. On the other hand, V*fi* values were associated with years of education and sex, with higher performances in highly educated subjects and females, which is consistent with other studies on V*fi* and standard VFT (20,25).

In patients with ALS, we observed that lower VFT scores were associated with a widespread pattern of WM tract alterations, including both motor (CST) and extra-motor (bilateral SLF, CC) pathways, reflecting the influence of ALS motor impairment on the spoken fluency performed in a standard modality. On the other hand, abnormal V*fi*s were associated with a focal damage to callosal fibers linking to SMA. SMA is significantly involved in speech production (26), working memory, sequence processing and cognitive control (27,28). The link of this structure with fluency and executive performances has been corroborated by functional-MRI overt verbal fluency paradigms in healthy controls (29–32) and by findings on

patients affected by the "SMA syndrome" (33,34). These latter cases, who underwent a neurosurgical resection of the dorsal part of the superior frontal cortex (35), showed a poor letter (more than category) fluency and decreased performances on dual tasks and cognitive control.

Interestingly, although it is known that letter fluency involves different GM areas, especially the left inferior frontal gyrus (36,37) and the subcortical brain regions (38), we did not find association between fluency performances and GM volume integrity in our patient sample. Other GM metrics, such as cortical thickness, could be more informative on the associations with fluency in these patients.

Some caveats need to be considered when interpreting our findings. In this study we did not solve the potential inability of V*fi* in detecting those cases with severe motor impairment and concomitant executive dysfunction. Furthermore, although we recruited a large population of healthy subjects, their distribution was not normal and, differently from Abrahams et al. (9), we were compelled to identify the cutoffs based on percentile confidence intervals. Finally, due to the cross-sectional nature of the study, the evolving trajectory of the proposed V*fi*s (in parallel with the progression of motor impairment) as well as their ability to predict clinical worsening should be further investigated.

In conclusion, the present study provides Italian normative values of the spoken V*fi*, which can be applied for detecting cognitive impairment in ALS accounting for patients' motor impairment. Moreover, it demonstrates that, compared to standard VFT, V*fi* is critical to disentangle motor and cognitive deficits in ALS. Finally, the correlation with MRI in ALS patients shows the association of abnormal V*fi*s with damage to WM tracts specifically involved in lexical and ideational information processing, such as callosal fibers linking to SMA.

Declaration of interest

V. Castelnovo, P.M.V. Rancoita, M. Leocadi, A. Lamanuzzi, E.G. Spinelli, S. Basaia, N. Riva, B. Poletti, F. Solca, S. Abrahams report no conflict of interest. E. Canu has received research supports from the Italian Ministry of Health. F. Verde is review editor for Frontiers in Aging Neuroscience. N. Ticozzi received compensation from consulting services from Amylyx Pharmaceuticals and Zambon Biotech SA and lecture fees from Italfarmaco. He received research funding from the Italian Ministry of Health and AriSLA. He is associate editor for Frontiers in Aging Neuroscience. V. Silani received compensation for consulting services and/or speaking activities from AveXis, Cytokinetics, Italfarmaco, and Zambon; and receives research supports from the Italian Ministry of Health (Grant RF-2013-02355764), Fondazione Regione per la Ricerca Biomedica Regione Lombardia (Project nr.2015-0023), and E-RARE JTC 2018 (Project Repetomics). M. Filippi is Editor-in-Chief of the *Journal of Neurology*; received compensation for consulting services and/or speaking activities from Alexion, Almirall, Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Janssen, Merck, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi Genzyme, Takeda, and Teva Pharmaceutical Industries; and receives research support from Biogen, Merck, Novartis, Roche, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA). F. Agosta is Section Editor of *NeuroImage: Clinical*; has received speaker honoraria from Biogen Idec, Roche and Zambon; and receives or has received research supports from the Italian Ministry of Health, AriSLA (Fondazione Italiana di Ricerca per la SLA), the European Research Council and the Foundation Research on Alzheimer Disease.

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Data availability statement

The dataset used during the study will be made available by the corresponding author upon request to qualified researchers (i.e. affiliated with a university or research institution/hospital).

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