Cognitive phenotypes of sequential staging in amyotrophic lateral sclerosis

Research report

Cognitive phenotypes of sequential staging in amyotrophic lateral sclerosis

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Abstract

Sequential spread of TDP43 load in the brain may be a pathological characteristic of amyotrophic lateral sclerosis (ALS). Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) based marker of this pathological feature. Cognitive deficits known to be present in a subset of ALS patients might act as an additional in vivo clinical marker of disease spread.

N=139 patients with ALS were tested with the Edinburgh Cognitive and Behavioural ALS screen (ECAS) in addition to DTI brain measures of pathological spread. Executive function, memory and disinhibited behaviour were selected for Cognitive-Staging criteria, as these cognitive functions are attributed to cerebral areas analogous to the pattern of MRI markers of TDP43 pathology. ROC curve analyses were performed to define cut-off scores for cognitive stages 2 (executive function), stage 3 (disinhibited behaviour) and stage 4 (memory), and staging was performed according to the cognitive profile subsequently. Associations of Cognitive-Staging (stage 2-4) and MRI-Staging measures were determined.

In total, 77 patients (55%) performed below ROC cut-off scores in either executive function or memory or both and / or were reported to have disinhibited behaviour which permitted Cognitive-Staging. The cognitive profile of patients with discrete MRI stages 2 to 4 correlated significantly with DTI parameters. For those patients with cognitive impairment, there was a high congruency between MRI and Cognitive-Staging with high specificity and sensitivity of executive functions for MRI stage 2, disinhibited behaviour for MRI stage 3 and moderate of memory for MRI stage 4. Cognitive impairment follows specific patterns in ALS and these patterns can be used for Cognitive-Staging with a high specificity compared to MRI-Staging. For the individual, cognitive screening is a fast and easy to apply measurement of cerebral function giving valuable information in a clinical context.

Keywords: Amyotrophic Lateral Sclerosis, diffusion tensor imaging (DTI), cognition, staging, TDP43

Abbreviations: ALS = amyotrophic lateral sclerosis, ALS-FRS-R = ALS functional rating scale-revised, DTI = diffusion tensor imaging, FA = fractional anisotropy, FTD = fronto-temporal dementia

1. Introduction

There is clear evidence in anatomical post-mortem analyses that intraneuronal inclusions show a distinct pattern throughout the brains of patients with amyotrophic lateral sclerosis (ALS). These phosphorylated 43kDa TAR DNA-binding protein (pTDP-43) inclusions seem to follow a sequential pattern of cortical spread in at least four stages (Braak, Brettschneider, and Ludolph AC et al., 2013; Brettschneider, Del Tredici, and Toledo et al., 2013). This spread is closely linked to sequential propagation of oligodendroglia pathology (Fatima, Tan, and Halliday et al., 2015) and changes in the white matter connectome (Schmidt, de Reus, and Scholtens et al., 2015). Using fractional anisotropy (FA) mapping by diffusion tensor imaging (DTI), evidence for an in vivo marker of these pathological stages has been provided (Kassubek, Müller, and Del Tredici et al., 2014). About one fourth of ALS cases do not consecutively fulfill the DTI criteria of staging and therefore do not allow for conclusive in vivo MRI-Staging (Kassubek et al., 2014). Furthermore, given that MRI is not available in every clinic for every patient and can be difficult for ALS patients with respiratory insufficiency, there is a need to provide additional in vivo measures of spreading patterns. There is a missing link between microstructural changes and functional loss in ALS. Recently, evidence was provided that oculomotor dysfunction is a functional marker of ALS pathology (Gorges, Müller, Lulé et al., 2015). An additional functional parameter might be cognitive and behavioural changes which are the most common non-motor symptoms in ALS and occur in 30 to 50% of patients (Goldstein and Abrahams, 2013; Beeldman, Raaphorst, and Klein Twennaar et al., 2015). Behavioural abnormalities are present in up to 30% of patients and underlie the diagnosis of fronto-temporal dementia (FTD) in 5-10% of ALS patients (Strong, Grace, and Freedman et al., 2009). According to Bak's and Chandran's (2012) hypothesis, the decline in cognitive domains in ALS should be closely associated with disease spread in the motor system (Eisen, Turner, and Lemon et al., 2013). In fact, executive control, language (Taylor, Brown, and Tsermentseli et al., 2013) and verbal fluency are reported to be the most common domains affected in ALS (Goldstein and Abrahams, 2013). Dysfunction of the memory domain is less frequently described (Abrahams, Leigh, and Harvey et al., 2000; Abrahams, Newton, and Niven et al. 2014; Raaphorst, de Visser, and Linssen et al., 2010; Lulé, Burkhardt, and Abdulla et al., 2015; Wei, Chen, and Zheng et al., 2015). Since spread of TDP-43 pathology is associated with neuronal loss, it may be expected to

change the profile of cognitive performance mirroring functions of the affected brain areas. Therefore, we hypothesized that cognitive impairments in ALS show a distinct pattern and may serve as a clinical in vivo staging correlate for sequential spreading of DTI measures indicative TDP43 pathology (Kassubek et al., 2014). This study will determine 1.) whether there is a distinct pattern of cognitive and behaviour impairment which is directly associated with MRI staging, 2) whether this pattern may be useful for functional in vivo staging whether Cognitive-Staging is an accurate predictor of MRI-Staging. Cognitive-Staging is fast and easy to obtain, therefore, it may provide valuable information in clinical routine.

2. Methods

2.1 Participants

In total, N=139 patients (55 female) with probable or definite diagnosis of amyotrophic lateral sclerosis (ALS) were included in the study. Patients underwent standardized clinical-neurological and routine laboratory examinations. They were all diagnosed with sporadic ALS by a board-certified neurologist according to the Airlie House criteria (Miller, Munsat, and Swash et al., 1999) and revised El Escorial criteria (Ludolph, Drory, and Hardiman et al., 2015). N=100 had a predominant spinal onset, N=34 a predominantly bulbar onset and N=5 had a mixed onset. Severity of physical symptoms were mild to moderate as measured with the revised ALS functional rating scale (ALS-FRS-R) (Cedarbaum, Stambler, and Malta et al., 1999) (for detailed sample characteristics see table 1).

	mean	SD	range
Age	*60.9*	*12.2*	*19-83*
Education [years]	*13.0*	*3.0*	*4-23*
Duration since onset [months]	*20.4*	*24.1*	*2-168*
ALS-FRS-R	*39.2*	*6.4*	*16-48*
Progression [48-ALS-FRS/duration in months]	*0.8*	*1.0*	*0.1-6.5*
Fluency	*13.3*	*5.9*	*0-22*
Language	*24.6*	*3.6*	*12-28*
Executive function	*35.5*	*6.8*	*8-45*
Memory	*15.2*	*4.3*	*0-23*
Visuospatial function	*11.4*	*1.3*	*7-19*
ALS specific score	*73.7*	*13.3*	*32-95*
ALS non-specific score	*26.6*	*4.8*	*8-35*
Total score	*100.3*	*16.5*	*41-125*

Table 1: Sample characteristics and performance in the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) of N=139 ALS patients.

None of the participants had signs of any neurological or psychiatric illness (other than ALS) or overt dementia as these were exclusion criteria for the study. They were all native German speakers. All patients eligible for MRI were consecutively recruited from the out- and inpatient clinics of the Department of Neurology at the Universitätsklinikum Ulm, Germany.
The study was approved by the Ethics Committee of the University of Ulm (No. 19/12) and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave written informed consent to the study according to institutional guidelines.

2.2 Study design

Patients were screened for cognitive dysfunction as a functional measure of ALS pathology by a board certified neuropsychologist within 3 days after MRI scanning. All patients received MRI scanning according to a standardized protocol (Kassubek et al., 2014). Staging (1-4) was then determined separately for each individual by a) cognitive and behaviour impairment (Cognitive-Staging) and b) MRI-DTI (MRI-Staging).

2.3 Cognitive and Behaviour assessment

Cognitive and behaviour profile was assessed with the German version of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS; Abrahams et al. 2014, Lulé et al. 2015; Loose, Burkhardt, and Aho-Oezhan et al., 2016), with subdomains of ALS specific cognitive functions including executive function, language and verbal fluency and non-ALS specific functions of memory and visuospatial perception. Maximum total ECAS score is 136 with decreasing score indicating lower cognitive performance (table 1). The test has been validated against extensive cognitive assessment with good sensitivity and specificity to cognitive dysfunction in ALS (Niven, Newton, and Foley et al., 2015; Lulé et al., 2014).

Primary caregivers (all 1st degree family members; N=89) reported behavioural changes according to criteria of the ECAS behavioural interview. In total, N=28 (31%) presented with changes in behaviour (in at least one domain) with N=9 with disinhibited behaviour, N=16 with apathy, N=6 with loss of empathy and social interest, N=6 with repetitive behaviour, and N=6 with hyperorality. In total, N=38 were

classified as ALSci and N=20 as ALSbi according to the revised Strong criteria (Strong, Abrahams, and Goldstein et al., 2017).
None of the patients presented with evidence of psychosis.

2.4 Theory of Cognitive-Staging

ECAS cognitive domains of interest were selected according to regional association of dysfunction and proposed ALS stages (Braak et al., 2013; figure 1; see also graphical abstract). In stage 1 where primary motor areas and the corticospinal tract of the cortex are affected, we did not expect impairments in cognition or behaviour. Those patients without any cognitive deficits were defined as cognitive stage 1. In stage 2, the pathological process in the motor cortex also expands into contiguous portions of the premotor and prefrontal regions (Braak et al., 2013; Brettschneider et al., 2013). The association between damage in prefrontal regions, in particular the dorsal part of the middle prefrontal cortex, and cognition is well established (Abrahams et al., 2014; Lillo, Savage, and Mioshi et al., 2012). Cognitive domains such as executive control (including verbal fluency as a measure of executive control) specifically attributed to structures involved in stage 2 including the dorsal part of the middle frontal cortex are associated with reduced cortical activity (Goldstein, Newsom-Davis, and Bryant et al., 2011; Witiuk, Fernandez-Ruiz, and McKee et al., 2014; Abrahams, Goldstein, and Simmons et al., 2004) and reduced white matter integrity in ALS (Pettit, Bastin, and Smith et al., 2013) in the respective area. Therefore, executive control may serve as marker for pathological stage 2.
During pathological ALS stage 3, involvement of prefrontal regions progresses to more anterior, ventromedial and orbital regions of the frontal cortex such as the gyrus rectus and orbitofrontal areas (Brettschneider et al., 2013). Among others, these areas are linked to inhibition of socially inappropriate behaviour also known to be involved in some ALS patients (Abrahams et al., 2014). Therefore, abnormal disinhibited behaviour may be attributed to cortical dysfunction in orbitofrontal cortex.
In pathological stage 4, hippocampal areas are involved and memory dysfunction in ALS has been attributed to structural alterations in these areas (Takeda, Uchihara, and Mochizuki et al., 2007; Abdullah, Machts, and Kaufmann et al., 2014; Stoppel, Vielhaber, and Eckart et al., 2014). Therefore, measures of memory may serve as marker of ALS pathology for stage 4.

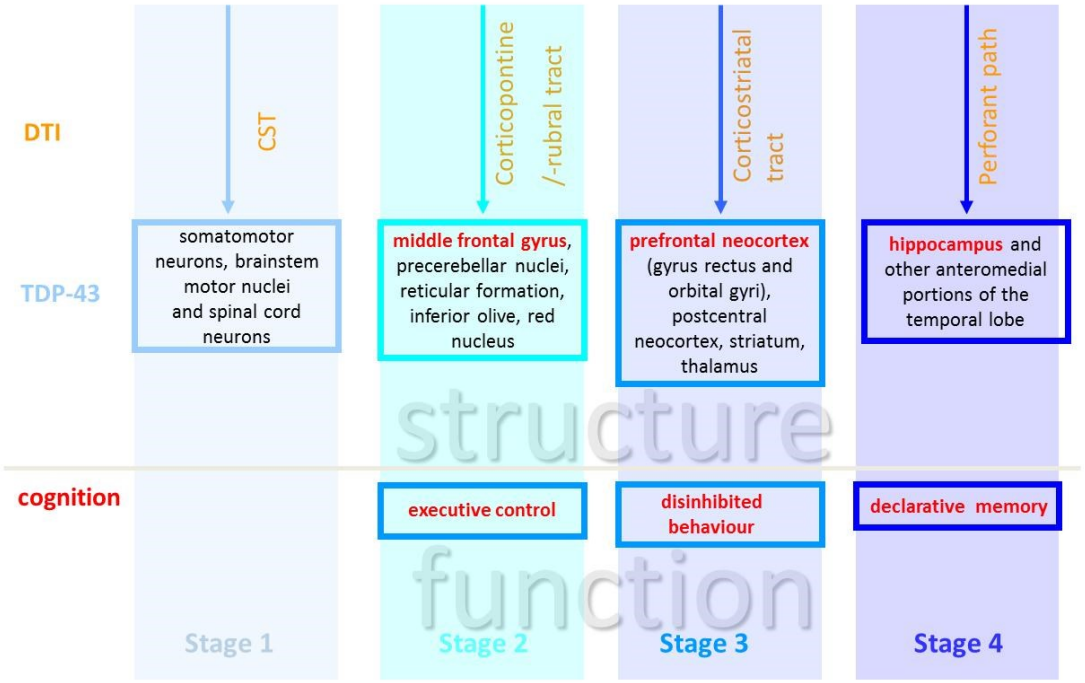

Figure 1: Scheme of pathological involvement of either structure (upper part: measures of DTI and measures of TDP43 pathology) and function (lower part: measures of cognition). All measures may serve as markers of the four pathological stages in ALS according to Braak and colleagues (2013).

2.5 Factors associated with cognitive performance

To see how genotypes were associated with Cognitive-Staging, blood samples were derived and genotyped for N=70 patients for which N=4 were positive for C9orf72 mutations and N=1 was positive for SOD mutations (for detailed protocol see Diekstra, Van Deerlin, and van Swieten et al. 2014).

2.6 MRI

Patients received staging through in vivo diffusion tensor imaging (DTI) markers of ALS pathology (Kassubek et al., 2014) according to the post-mortem staging theory of ALS by Brettschneider and Braak (Brettschneider et al., 2013).

For N=99 patients, MRI data were acquired on a 1.5 T clinical scanner (Magnetom Symphony, Siemens Medical), the other 40 patients were investigated on a 3.0 T head scanner (Allegra, Siemens Medical).

The scanning protocol for 1.5T was as follows: 2 x 31 gradient directions, including two b = 0 gradient directions, 64 slices, 64 x 64 pixels, slice thickness 3.0 mm, in-plane pixel size 3.3 mm x 3.3 mm, echo time 28 ms, repetition time 3080 ms, b = 1000 s/mm2. The scanning protocol for 3T was as follows: 49 gradient directions, one b = 0 gradient direction, 52 slices, 96 x 128 pixels, slice thickness 2.2 mm, in-plane pixel size 2.2 mm x 2.2 mm, echo time 85 ms, repetition time 7600 ms, b = 1000 s/mm2. For further details see the work of Kassubek and colleagues (2014).

2.7 MRI - Staging

The DTI analysis software Tensor Imaging and Fiber Tracking (TIFT; Müller, Unrath, and Ludolph *et al*., 2007) was used for post processing and statistical analysis according to Kassubek and colleagues (2014). Fractional anisotropy (FA) values for reference areas were calculated: for DTI stage 1, FA values of corticospinal tract (CST) were determined. For DTI stage 2, FA values in pontine and rubral tract were used as reference and for DTI stage 3 striatal paths. For reference, FA values of tractus opticus were defined. Overall, 75% of the patients included in the study consecutively fulfilled the DTI criteria of staging (i.e. conclusive stage 2 only if criteria for stage 1 *and* stage 2 are fulfilled), whereas the remaining 25% did not allow for conclusive in vivo MRI-Staging (called “undefined”; Kassubek et al., 2014).

2.8 Statistics

All analyses were performed using IBM® SPSS version 21.0 except for definition of cut-off points in ROC analyses which were defined with R Statistical Computing software version 3.1.3. A priori, data were analyzed for normal distribution using the Kolmogorov-Smirnov test. Following, non-parametric tests and Z transformation were performed.

ROC-Analyses were performed for definition of cut-off points for abnormality in executive function (stage 2) and memory (stage 4). Disinhibition was indicated as present or not.

Raw data of cognitive function was Z transformed with Fisher transformation. Kendall-Tau correlation analyses were performed for association of Z transformed cognitive raw-scores of all ECAS subdomains, subtests and FA DTI values and between MRI-Staging and Cognitive-Staging. Differences in cognitive parameters between DTI stages and cognitive deficits and between those with Cognitive-Staging

vs. those without and demographics (age, education) / clinical phenotypes (ALS-FRS, region of onset, disease duration, progression) were determined with Mann-Whitney U-Test. Chi² analysis was performed for Cognitive-Staging and MRI-Stages, and Cognitive-Staging and presence of genetic mutation (C9orf72, SOD). Sensitivity and specificity of Cognitive-Staging for MRI stages 2 to 4 were determined. All analyses were two-sided and the significance level was adjusted at p=0.05.

3. Results

3.1 Association of cognitive scores with DTI FA values for MRI-Staging

Cognitive scores on executive function correlated significantly with the FA values in structures associated with MRI stage 1 to 4. Memory correlated significantly with the FA values in structures indicating MRI stage 1, 2 and 4 but not with stage 3. Other cognitive functions known to be involved in ALS such as verbal fluency (correlation with DTI FA measures 2 to 4), language (no correlation with any DTI FA measure) or visuospatial function (correlation with DTI FA measures 2 only) similar to subtests in the ECAS (supplement table) did not provide any additional information for Cognitive-Staging.

Neither cognitive dysfunction correlated with the tractus opticus as reference structure (table 2).

DTI stages	tract for DTI	executive function	memory	fluency	visuospatial	Language
stage 1	*corticospinal tract*	*0.005*	*0.05*	*n.s.*	*n.s.*	*n.s.*
stage 2	*pontine tract*	*0.003*	*n.s.*	*0.008*	*0.05*	*n.s.*
	rubral tract	*0.002*	*0.008*	*0.029*	*0.014*	*n.s.*
stage 3	*striatal path*	*0.02*	*n.s.*	*0.005*	*n.s.*	*n.s.*
stage 4	*perforant path*	*0.04*	*0.01*	*0.007*	*n.s.*	*n.s.*
reference	*tractus opticus*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Table 2: Association of FA values in structures used for MRI-Staging and Z-transformed scores of performance in cognitive ECAS subdomains including domains used for Cognitive-Staging (highlighted in green; Kendall-Tau correlation).

Mean Z scores of executive function (r=-0.15 p=0.5) and memory (Kendall-Tau correlation r=-0.16 p=0.04) were significantly associated with MRI stages (Figure 2). A between group comparison between those in MRI stage 1 to those in MRI stage 2 or higher revealed significant differences in executive function (Mann-Whitney-U test p=0.004) and memory score (Mann-Whitney-U test p=0.03).

Memory was different between groups with MRI stage 4 compared to MRI stage 3 or lower (Mann-Whitney-U test p=0.08), but this difference did not reach significance.

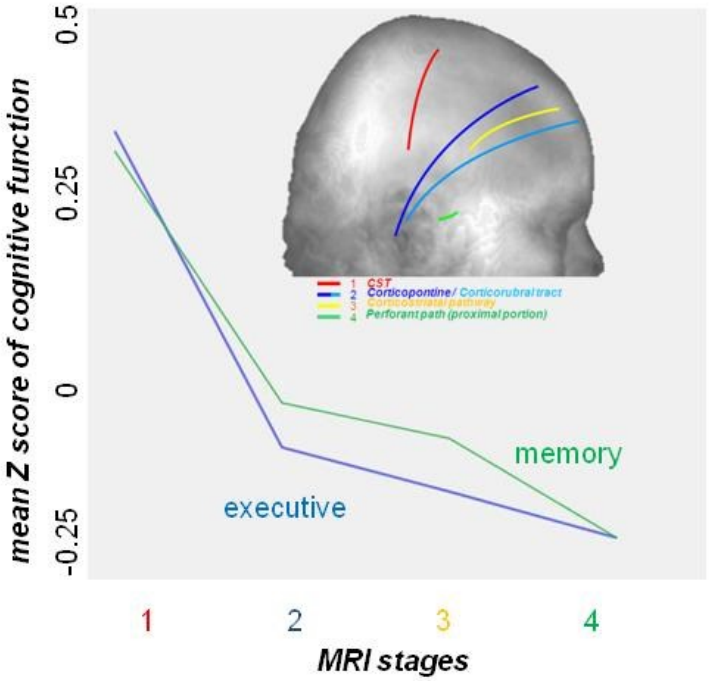

Figure 2: Association of Z transformed raw scores in cognitive subdomains (executive, blue, and memory function, green, in ECAS) and MRI stages 1 to 4 according to DTI parameters (Kassubek et al., 2014); Kendall-Tau correlation.

3.2 Cognitive-Staging

Cut-offs in cognitive performance in ECAS subdomains for executive function and memory were defined according to ROC-analysis as following. For cognitive ROC analysis separating MRI stage 2 from the other stages, executive function was a significant predictor with p=0.04 and a cut-off point of ≤ 35 was defined. For cognitive ROC analysis separating MRI stage 3 from the other stages, memory was a significant predictor with p=0.03 and a cut-off point of ≤ 14 (ROC curves see supplement) was determined. To separate MRI stage 3 from other stages with regards to cognitive performance, disinhibited behaviour was regarded as either present or not and cut-off point was defined as presence of disinhibited behaviour.

In total, cognitive and behavioural scores indicated Cognitive-Staging ≥ 2 in N=77 patients (55%) whereas N=62 had no cognitive and / or behavioural impairment and

provided evidence for cognitive stage 1. . N=75 (53%) had impairment in cognitive functions or combined with behavioural impairment and N=2 had changes in behaviour only. N=53 were impaired in the executive domain, N=53 in the memory domain and N=31 in both domains. Of those N=9 with disinhibited behavior, N=7 also had deficits in executive and/or memory domains and N=2 patients were reported to have disinhibited behavior only, one of them with involuntary emotion expression disorder (IEED). According to Cognitive-Staging criteria, all patients below cognitive ROC cut-off points provided evidence for Cognitive stage 2 or higher, with executive scores below cut-off score indicating Cognitive stage 2, disinhibited behavior indicating Cognitive stage 3 and reduced memory scores indicating Cognitive stage 4 according to a priori hypothesis mentioned above and ROC analysis.

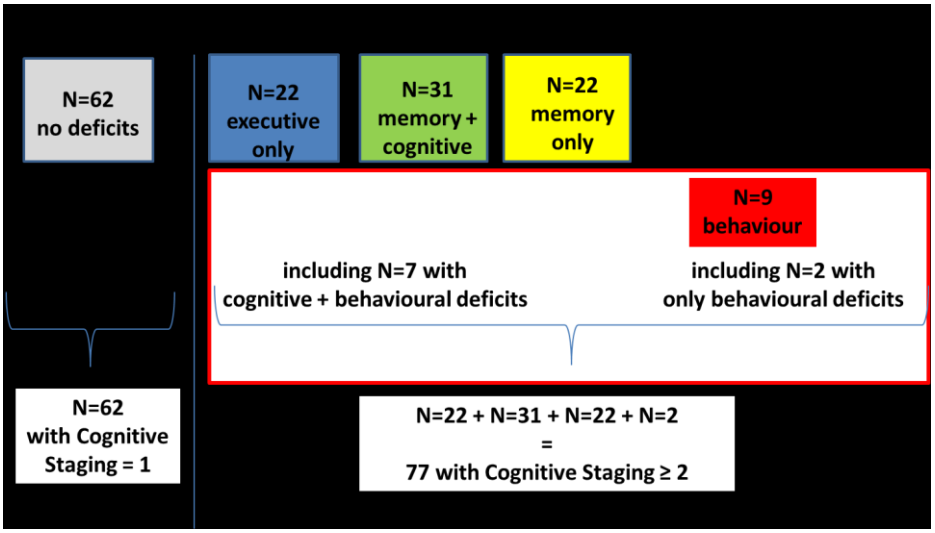

Figure 3: Scheme of cognitive and behavioural impairments critical for Cognitive Staging in the study cohort of N=139 patients.

3.3 Association between Cognitive- and MRI-Staging

All N=77 with cognitive stage 2 or higher also had an MRI stage of 2 or higher except the patient with IEED who had MRI stage 1. Those with MRI stage 1 had no cognitive impairment and received Cognitive-Stage 1, whereas those with MRI stage 2 or higher were more likely to have cognitive impairment in the executive (Chi^2=9.59 df=3 p=0.022) or memory domain (Chi^2=11.75 df=3 p=0.008).

For those patients with cognitive deficits, Cognitive-Staging was very specific for MRI-Staging: all patients with cognitive dysfunction in executive domain had an MRI

stage of at least 2 (100% specificity and sensitivity for stage 2). All patients with disinhibited behavior (except the one patient with IEED) had an MRI stage of 3 or higher (95% specificity and 100% sensitivity for stage 3). Patients with additional memory deficits were more likely to have MRI stage 4 (52% specificity and 55% sensitivity for stage 4).

Of those N=35 who had no conclusive MRI-Staging (25%), N=18 patients received Cognitive-Staging ≥2 and N=17 (12%) received cognitive staging 1.

3.4 Association of clinical phenotypes and cognitive impairment

Compared to those with cognitive-Staging ≥2, those with no cognitive impairment and therefore with Cognitive-Stage 1 were younger (mean age 57.1 vs. 66.1 years; Mann-Whitney-U-Test $p<0.001$), better educated (mean education 13.7 vs. 11.9 years; Mann-Whitney-U-Test $p<0.001$), and they were less physically impaired (mean ALS-FRS 40.3 vs. 37.5; Mann-Whitney-U-Test $p=0.04$) but there was no difference for gender ($Chi^2=1.3$ $df=2$ $p=0.5$), region of onset, disease duration nor progression of the disease (Mann-Whitney-U-Test all $p=0.05$).

Those N=4 patients with C9orf72 mutation were either Cognitive-Stage 3 (N=1) or 4 (N=4) but never Cognitive-Stage ≤ 2 compared to those tested negative for C9orf72 (N=65; $Chi^2=21.1$ $df=6$ $p=0.002$). The patient with SOD mutation presented with no cognitive deficits.

4. Discussion

The study provides evidence for a distinct pattern of cognitive and behavioural alterations in ALS patients consistent with patterns of pathological cerebral involvement in ALS (Brettschneider et al., 2013). Cognitive and behavioural deficits might be similarly used as in vivo functional markers of ALS pathology, allowing Cognitive-Staging for stages 1-4. These cognitive measures were highly associated

with DTI parameters used for MRI-based in vivo staging, providing additional evidence for a functional measure of pathological patterns in ALS such as it has been provided for oculomotor changes (Gorges et al., 2015). For Cognitive-Staging, impairment in executive function attributed to prefrontal cortex was highly specific for MRI stage 2, disinhibited behavior indicating possible orbitofrontal involvement was highly specific for MRI stage 3 and memory dysfunction attributed to parahippocampal areas was specific for MRI stage 4 although only in a poor range. Other cognitive functions such as verbal fluency or language functions known to be involved in ALS (Hervieu-Bègue et al., 2016; Grossman, 2008; Davies et al., 2005) did not provide any additional information for Cognitive-Staging. Verbal fluency is an executive function providing similar Cognitive-Staging information as the executive function score. Language is closely related to motor function and possibly degrades much earlier and more closely with bulbar involvement ("What fires together dies together"; Bak and Chandran, 2012) which is not fully represented by the DTI FA Staging system. Cognitive-Staging provides highly specific clues for in vivo staging in ALS complementary to MRI in those patients with cognitive deficits. Major advantage is that Cognitive-Staging can be acquired in clinic within 20-25 minutes with low strain for the patient and in ALS patients with breathing difficulties unable to perform an MRI scan.

4.1 Cerebral involvement in ALS

Clinical phenotypes of cognitive functions in ALS are diverse (Strong, Grace, and Freedman et al., 2009; Bak, 2010) which is further supported by the current data. In the literature, there are inconclusive results with regards to the progressive nature of cognitive deficits in ALS (Schreiber, Gaigalat, Wiedemuth-Catrinescu et al., 2005), possibly explained by heterogeneity of the cognitive pattern within cross-sectional comparisons. In a subset of ALS patients, cognitive deficits become more prominent over time (Robinson, Lacey, and Grugan et al., 2006) and patients with early onset of cognitive impairment show a high probability of progressive nature of cognitive dysfunction (Elamin, Bede, and Byrne et al., 2013). Analogous to the spreading nature of structural changes of TDP43 and DTI alterations (Braak et al., 2013; Kassubek et al., 2014) frequency of functional changes might also increase in the course of ALS. The current data suggest that cognitive performance correlates with structural disease pathology, providing an indirect link between clinical alterations

and structural changes in DTI. The increased involvement of different cognitive domains in the course of ALS extends our understanding of an increased impairment in one cognitive domain in the course of ALS pathology (Robinson et al., 2006).

4.2 Association of cognition and clinical features

The current study provides evidence for the association of anatomical (Braak et al., 2013) and clinical stages in ALS (Balendra, Jones, and Jivraj et al., 2014), further supporting previous findings on the association of clinical phenotypes and pathological TDP43 spreading (Tan, Kril, and Fatima et al., 2015). Incidence of cognitive deficits has been regarded in the context of clinical parameters such as survival and respiratory function (Beeldman, Raaphorst, and Klein Twennaar et al., 2015). In the current study, physical function was more advanced in patients with cognitive deficits compared to those without, providing possible evidence for progressive nature of cognition; however, in a cross sectional approach only. Furthermore, we provide additional evidence that age and education might be a protective factor for cognitive decline as it was previously shown for dementia (Terrera, Minett, and Brayne et al., 2014). Finally, C9orf72 mutations were associated with Cognitive-Staging providing further support for a pathological link of this mutation and cognition in ALS and FTD patients (Montuschi, Iazzolino, and Calvo et al., 2015).

Providing that all demographic and affective parameters are carefully controlled for, cognitive and behavioural measures are highly specific for MRI stages in ALS, indicating TDP pathology in prefrontal, orbitofrontal and parahippocampal areas but in the subset of patients with cognitive alterations only. Memory was only poorly specific for parahippocampal involvement. For the memory task there is not only temporal but also prefrontal cortex involvement supported by the fact that raw scores of memory correlated with DTI parameters in pontine and rubral tract indicative of MRI stage 2. For those patients with a possible FTD phenotype, temporal involvement attributed to memory function may be more specific for Cognitive-Stage 2 (Brettschneider et al., 2014). Taken together, the majority of 88% received either MRI- or Cognitive-Staging or both. Those 12% with no in vivo-staging were rather young and therefore, structural (i.e. TDP pathology) and functional changes (possibly due to compensatory processes in young age; Terrera et al., 2014) might show an ALS-untypical pattern of propagation.

4.3 Limitations

In total, 45% of all patients present with no cognitive deficits (Phukan, Elamin, and Bede et al., 2012; Beeldman et al., 2010) and evidence for Cognitive-Stage 1 can be given only. This lack of cognitive impairment might be either explained by cognitive reserve and compensatory processes in some cases (Perquin, Diederich, and Pastore et al., 2015) with e.g. high education as protective factor or it might be attributed to a non-cognitive clinical phenotype of ALS, emphasizing the heterogeneity in ALS. Furthermore, staging 3 using behavioural alterations showed a rather poor performance for Cognitive-Staging and needs further validation in future studies.

A major limitation is that the current cross-sectional study does not provide proof of progressive nature of Cognitive-Staging within an individual so that future longitudinal studies are needed.

Furthermore, for the MRI data collection different systems were used but Mueller and colleagues have provided evidence that different MRI data collection systems provide similar results with regard to DTI measures for ALS Staging (Kassubek et al., 2014).

Finally, DTI and cognitive screening are two techniques which measure completely different parameters. DTI is a measure of structural alterations in axons of white matter while cognitive screening is a functional measure of cognitive performance known to be dependent on correct functioning within specific cortical networks. Both measures have a very high congruency. However, DTI and cognitive measures at the very best describe different aspects of the same pathological mechanism in ALS.

4.4 Conclusion

The current study provides evidence that frequency and pattern of functional measures of cortical performance such as cognition and behaviour show a distinct pattern in ALS which might be useful for in vivo Cognitive-Staging in ALS. Cognitive measures were closely linked to structural alterations of DTI MRI signals indicative of TDP43 pathology in ALS. Consistency of MRI staging and cognitive screening parameters provides suggestive evidence that both methods might add complementary in vivo information for pathological staging according to Braak et al. (2013) in the subset of cognitively impaired patients but longitudinal studies are

warranted to highlight the progressive nature of the hereby described pathology pattern.

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References

Abdulla S, Machts J, Kaufmann J, Patrick K, Kollewe K, Dengler R, *et al*. Hippocampal degeneration in patients with amyotrophic lateral sclerosis. Neurobiol Aging. 2014; 35: 2639-45.

Abrahams S, Leigh PN, Harvey A, Vythelingum GN, Grisé D, Goldstein LH. Verbal fluency and executive dysfunction in amyotrophic lateral sclerosis (ALS). Neuropsychologia. 2000; 38: 734-47.

Abrahams S, Newton J, Niven E, Foley J, Bak TH. Screening for cognition and behaviour changes in ALS. Amyotroph Lateral Scler Frontotemporal Degener. 2014; 15: 9-14.

Abrahams S, Goldstein LH, Leigh PN. Cognitive change in amyotrophic lateral sclerosis: a prospective study. Neurology 2005; 64: 1222-1226

Abrahams S, Goldstein LH, Simmons A, Brammer MJ, Williams SCR, Giampietro V, *et al*. Word retrieval in amyotrophic lateral sclersosis: a functional magnetic resonance imaging study. Brain 2004; 127: 1507-1517.

Bak TH, Chandran S. What wires together dies together: verbs, actions and neurodegeneration in motor neuron disease. Cortex. 2012; 48: 936-44.

Bak TH. Motor neuron disease and frontotemporal dementia: One, two, or three diseases? Ann Indian Acad Neurol. 2010; 13: 81-8.

Balendra R, Jones A, Jivraj N, Knights C, Ellis CM, Burman R, *et al*. Estimating clinical stage of amyotrophic lateral sclerosis from the ALS Functional Rating Scale. Amyotroph Lateral Scler Frontotemporal Degener. 2014; 15: 279-84.

Beeldman E, Raaphorst J, Klein Twennaar M, de Visser M, Schmand BA, de Haan RJ. The cognitive profile of ALS: a systematic review and meta-analysis update. J Neurol Neurosurg Psychiatry. 2015 Aug 17. 10.1136/jnnp-2015-310734.

Braak H, Brettschneider J, Ludolph AC, Lee VM, Trojanowski JQ, Del Tredici K. Amyotrophic lateral sclerosis--a model of corticofugal axonal spread. Nat Rev Neurol. 2013; 9: 708-14.

Brettschneider J, Del Tredici K, Toledo JB, Robinson JL, Irwin DJ, Grossman M, *et al*. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann Neurol. 2013; 74: 20-38.

Brettschneider J, Del Tredici K, Irwin DJ, Grossman M, Robinson JL, Toledo JB, *et al*. Sequential distribution of pTDP-43 pathology in behavioral variant frontotemporal dementia (bvFTD). Acta Neuropathol. 2014; 127: 423-39.

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, *et al*. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999; 169: 13-21.

Davies RR, Hodges JR, Kril JJ, Patterson K, Halliday GM, Xuereb JH. The pathological basis of semantic dementia. Brain. 2005;128(9):1984-95.

Diekstra FP, Van Deerlin VM, van Swieten JC, Al-Chalabi A, Ludolph AC, Weishaupt JH, *et al*. C9orf72 and UNC13A are shared risk loci for amyotrophic lateral sclerosis and frontotemporal dementia: a genome-wide meta-analysis. Ann Neurol. 2014; 76: 120-33.

Eisen A, Turner MR, Lemon R. Tools and talk: an evolutionary perspective on the functional deficits associated with amyotrophic lateral sclerosis. Muscle Nerve. 2014; 49: 469-77.

Elamin M, Bede P, Byrne S, Jordan N, Gallagher L, Wynne B, *et al*. Cognitive changes predict functional decline in ALS: a population-based longitudinal study. Neurology. 2013; 80: 1590-1597.

Fatima M, Tan R, Halliday GM, Kril JJ. Spread of pathology in amyotrophic lateral sclerosis: assessment of phosphorylated TDP-43 along axonal pathways. Acta Neuropathol Commun. 2015; 3: 47.

Goldstein LH, Abrahams S. Changes in cognition and behaviour in amyotrophic lateral sclerosis: nature of impairment and implications for assessment. Lancet Neurol. 2013; 12: 368-80.

Goldstein LH, Newsom-Davis IC, Bryant V, Brammer M, Leigh PN, Simmons A. Altered patterns of cortical activation in ALS patients during attention and cognitive response inhibition tasks. J Neurol. 2011; 258: 2186-98.

Gorges M, Müller HP, Lulé D, Del Tredici K, Brettschneider J, Keller J, P *et al*. Eye Movement Deficits Are Consistent with a Staging Model of pTDP-43 Pathology in Amyotrophic Lateral Sclerosis. PLoS One. 2015; 10: e0142546.

Grossman M, Anderson C, Khan A, Avants B, Elman L, McCluskey L. Impaired action knowledge in amyotrophic lateral sclerosis. Neurology. 2008;71(18):1396-401.

Hervieu-Bègue M, Rouaud O, Graule Petot A, Catteau A, Giroud M. Semantic memory assessment in 15 patients with amyotrophic lateral sclerosis. Rev Neurol (Paris). 2016;172(4-5):307-12.

Kassubek J, Müller HP, Del Tredici K, Brettschneider J, Pinkhardt EH, Lulé D, *et al*. Diffusion tensor imaging analysis of sequential spreading of disease in amyotrophic lateral sclerosis confirms patterns of TDP-43 pathology. Brain. 2014; 137: 1733-40.

Lillo P, Savage S, Mioshi E, Kiernan MC, Hodges JR. Amyotrophic lateral sclerosis and frontotemporal dementia: A behavioural and cognitive continuum. Amyotroph Lateral Scler. 2012; 13(1): 102-9.

Loose M, Burkhardt C, Aho-Oezhan H, Keller J, Abdulla S, Böhm S, *et al*. Age and education-matched cut-off-scores for the revised German/Swiss-German version of ECAS. Amyotroph Lateral Scler Frontotemporal Degener. 2016 Mar 30:1-3. [Epub ahead of print]

Ludolph A, Drory V, Hardiman O, Nakano I, Ravits J, Robberecht W, *et al*. A revision of the El Escorial criteria - 2015. Amyotroph Lateral Scler Frontotemporal Degener. 2015; 16: 291-2.

Lulé D, Burkhardt C, Abdulla S, Böhm S, Kollewe K, Uttner I, *et al*. The Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen: a cross-sectional comparison of established screening tools in a German-Swiss population. Amyotroph Lateral Scler Frontotemporal Degener. 2015; 16: 16-23.

Miller RG, Munsat TL, Swash M, Brooks BR. Consensus guidelines for the design and implementation of clinical trials in ALS. World Federation of Neurology committee on Research. J Neurol Sci. 1999; 169: 2-12.

Montuschi A, Iazzolino B, Calvo A, Moglia C, Lopiano L, Restagno G, *et al*. Cognitive correlates in amyotrophic lateral sclerosis: a population-based study in Italy. J Neurol Neurosurg Psychiatry. 2015; 86: 168-73.

Müller HP, Unrath A, Ludolph AC, Kassubek J. Preservation of diffusion tensor properties during spatial normalization by use of tensor imaging and fibre tracking on a normal brain database. Phys Med Biol. 2007; 52: 99-109.

Niven E, Newton J, Foley J, Colville S, Swingler R, Chandran S, *et al*. Validation of the Edinburgh Cognitive and Behavioural ALS Screen: A cognitive tool for motor disorders. Amyotroph Lateral Scler Frontotemporal Degener. 2015; 16: 172-9.

Perquin M, Diederich N, Pastore J, Lair ML, Stranges S, Vaillant M, *et al*. Prevalence of Dementia and Cognitive Complaints in the Context of High Cognitive Reserve: A Population-Based Study. PLoS One. 2015;10:e0138818.

Pettit LD, Bastin ME, Smith C, Bak TH, Gillingwater TH, Abrahams S. Executive deficits, not processing speed relates to abnormalities in distinct prefrontal tracts in amyotrophic lateral sclerosis. Brain. 2013; 136: 3290-304.

Phukan J, Elamin M, Bede P, Jordan N, Gallagher L, Byrne S, *et al*. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. J Neurol Neurosurg Psychiatry. 2012; 83: 102-8.

Raaphorst J, de Visser M, Linssen WH, de Haan RJ, Schmand B. The cognitive profile of amyotrophic lateral sclerosis: A meta-analysis. Amyotroph Lateral Scler. 2010; 11: 27-37.

Robinson KM, Lacey SC, Grugan P, Glosser G, Grossman M, McCluskey LF. Cognitive functioning in sporadic amyotrophic lateral sclerosis: a six month longitudinal study. J Neurol Neurosurg Psychiatry. 2006; 77: 668-70.

Schmidt R, de Reus MA, Scholtens LH, van den Berg LH, van den Heuvel MP. Simulating disease propagation across white matter connectome reveals anatomical substrate for neuropathology staging in amyotrophic lateral sclerosis. Neuroimage. 2015; 124: 762-769.

Schreiber H, Gaigalat T, Wiedemuth-Catrinescu U, Graf M, Uttner I, Muche R, *et al*. Cognitive function in bulbar- and spinal-onset amyotrophic lateral sclerosis. A longitudinal study in 52 patients. J Neurol. 2005; 252: 772-81.

Stoppel CM, Vielhaber S, Eckart C, Machts J, Kaufmann J, Heinze HJ, *et al*. Structural and functional hallmarks of amyotrophic lateral sclerosis progression in motor- and memory-related brain regions. Neuroimage Clin. 2014; 5: 277-90.

Strong MJ, Grace GM, Freedman M, Lomen-Hoerth C, Woolley S, Goldstein LH, *et al*. Consensus criteria for the diagnosis of frontotemporal cognitive and behavioural syndromes in amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2009; 10: 131-46.

Strong MJ, Abrahams S, Goldstein LH, Woolley S, Mclaughlin P, Snowden J, Mioshi E, Roberts-South A, Benatar M, HortobáGyi T, Rosenfeld J, Silani V, Ince PG, Turner MR. Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. Amyotroph Lateral Scler Frontotemporal Degener. 2017 Jan 5:1-22. doi: 10.1080/21678421.2016.1267768. [Epub ahead of print]

Takeda T, Uchihara T, Mochizuki Y, Mizutani T, Iwata M. Memory deficits in amyotrophic lateral sclerosis patients with dementia and degeneration of the perforant pathway. A clinicopathological study. J Neurol Sci. 2007; 260: 225-30.

Tan RH, Kril JJ, Fatima M, McGeachie A, McCann H, Shepherd C, *et al*. TDP-43 proteinopathies: pathological identification of brain regions differentiating clinical phenotypes. Brain. 2015; 138: 3110-22.

Taylor LJ, Brown RG, Tsermentseli S, Al-Chalabi A, Shaw CE, Ellis CM, *et al*. Is language impairment more common than executive dysfunction in amyotrophic lateral sclerosis? J Neurol Neurosurg Psychiatry. 2013; 84: 494-8.

Terrera GM, Minett T, Brayne C, Matthews FE. Education associated with a delayed onset of terminal decline. Age Ageing. 2014; 43: 26-31.

Wei Q, Chen X, Zheng Z, Huang R, Guo X, Cao B, *et al*. Screening for cognitive impairment in a Chinese ALS population. Amyotroph Lateral Scler Frontotemporal Degener. 2015; 16: 40-5.

Witiuk K, Fernandez-Ruiz J, McKee R, Alahyane N, Coe BC, Melanson M, *et al*. Cognitive deterioration and functional compensation in ALS measured with fMRI using an inhibitory task. J Neurosci. 2014; 34: 14260-71.