Measurements of Cognitive Functioning in Amyotrophic Lateral Sclerosis Patients

A Longitudinal Study

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Abstract

This study aims to map cognition over time in patients Amyotrophic Lateral Sclerosis. The Edinburgh Cognitive Assessment Screen (ECAS) is used to measure multiple domains creating ALS-specific cognition and ALS non-specific cognition. Results show that there are learning effects regarding the ECAS in the control- and patient groups and that is probably why an improvement is found in cognition in this longitudinal study. Most learning effects are in executive and memory domains. The learning effects may mask the cognitive decline in patients with hexanucleotide *C9orf72* repeat expansion and patients with bulbar onset in who no significant increase in cognition is found. So, in the current study no decrease in cognition is found over time but, learning effects and heterogeneity of ALS patients in at least this sample might mask other results. In future studies a B-version of the ECAS should be used while correcting for disease progression to map the course of cognition in ALS patients.

Keywords: ALS, Cognition, Longitudinal, MND, ECAS

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a heterogenic progressive neurodegenerative disease, characterized by loss of motor neurons, in which a cognitive deficit exists in about 33-52% of patients (Lomen-Hoerth et al., 2003; Massman et al., 1996; Phukan et al., 2012; Ringholz et al., 2005). Approximately 5% of ALS patients develop frontotemporal dementia (FTD) (Strong, Grace, & Freedman, 2009; Neary, Snowden, & Mann, 2000). ALS and FTD can coexist and are regarded as two ends of the same spectrum (Montushi et al., 2015; Rosenfeld & Swash,

2006). Cognitive functioning in ALS is getting more attention in recent years, but little is known about possible changes in cognition in the course of the disease. The goal of this study is to measure cognitive functions in ALS patients on multiple points of time to see if there are changes in cognition.

It was thought that neuropathology in ALS was restricted to the frontal parts of the brain, which was reflected in cognitive deficits in executive function and changes in behavior/personality. Many new studies suggest that, next to the characterized frontal lobe impairment, more parts of the brain are affected in ALS, including the parietal lobe (Tsermentseli, Leigh, & Goldstein, 2012). Also, monitoring cortical thinning, mostly in frontal areas, is now appearing to be a superior method in detecting the degenerative effects of ALS (Agosta et al., 2012; Verstraete et al., 2012). Neuropathological changes in ALS-FTD patients are mostly found in both frontal and temporal areas (Nakano, 2000).

So, the degenerative effects of ALS can be monitored but longitudinal research on cognition in ALS is not abundant. Most longitudinal cognitive studies on ALS lack control groups or have small sample sizes, which is probably due to attrition considering illness progression. Control groups are important to investigate if any effects found are due to the disease or any other cause, whereas a small sample size can have negative effects on power. The longitudinal studies with small sample sizes seem to have consistent results though: cognitive decline in ALS might be a relatively slow process which occurs early in the disease (Abrahams et al., 2005; Kilani, Micallef, & Soubrouillard, 2004; Robinson et al., 2006; Schreiber et al., 2005). This decline appears to be slower than motor decline (Abrahams, Leigh, & Goldstein, 2005; Schreiber et al., 2005);. Longitudinal studies mostly find, if any deficit is found, early cognitive detoriation in verbal fluency, which measures executive dysfunction (Abrahams,

Leigh, & Goldstein, 2005; Gordon et al., 2010; Irwin & McMillan, 2013; Schreiber et al., 2005). The effect of verbal fluency is not found in letter fluency tasks with letters that tend to give higher word production. This indicates that these deficits come from a higher function like the supervisory attentional system or central executive component of working memory (Abrahams et al., 2000). Abrahams et al. (2005) showed that there is an association between working memory reduction and cognitive impairment in ALS patients. Some studies report impairment in other domains as well, these domains include memory (Gordon et al., 2010; Hanagasi et al., 2002; Schreiber et al., 2005), attention (Kilani, Micallef, & Soubrouillard. 2004) (which is also an executive function), and language (Abrahams, Leigh, & Goldstein, 2005; Gordon et al., 2010; Hanagasi et al., 2002; Roberts-South et al., 2012). The memory impairment might be originating from a failure to code information instead of poor memory (Mantovan et al., 2003). This reinforces evidence for frontal dysfunction, just like the statement that verbal fluency deficits might come from a higher order function.

Although ALS patients seem to perform significantly worse on some domains in comparison with controls in these studies, it is important to note that a significant decline over time is not always found in patients and that the evidence concerning a decline is mixed. A methodologically sound longitudinal study by Kasper et al. (2016) for example shows no general cognitive decline over time. It is also important to note that some studies show that patients with bulbar onset perform consistently poorer in many cognitive tests in comparison with patients with spinal onset (Kilani, Micallef, & Soubrouillard, 2004; Schreiber et al., 2005; Strong, Grace, Freedman, 2009). Cognitive impairment also appears more progressive over time in patients with bulbar onset, but absence of bulbar decline did not predict absence of cognitive decline (Strong, Grace, & Freedman, 2009).

Further on, it is found that patients who have a repeat expansion in the *C9orf72* gene, have an estimated shorter survival time, lower age of onset and more rapid cognitive decline compared to ALS patients without the genetic expansion (Byrne et al., 2012; Irwin et al., 2013; Murphy et al., 2016). Frontotemporal dementia develops more often in patients with this gene, but there are also patients with the genetic expansion who do not develop FTD (Byrne et al., 2012; Irwin et al., 2013; Murphy et al., 2016).

As noted, most longitudinal studies on cognition in ALS do not have many participants and sometimes lack control groups. A longitudinal research that is methodologically stronger, is that of Elamin, Bede & Byrne (2013), because of their use of control groups and a large sample. In their study, 98 ALS patients were retested with a neuropsychological test battery with home visits in a 6-month interval. The study used an age, education and sex matched healthy control that is recruited using a volunteer network. The aim of Elamin, Bede & Byrne (2013) was to investigate whether cognitive functioning in ALS patients at first testing was predictive for cognitive and motor decline. Here, multiple groups were distinguished in the course of cognition in ALS patients. The group without any cognitive deficits at first testing (baseline) had better prognosis. The group with patients who had deficits in executive functioning or had ALS-FTD diagnosis at first measurement, showed a more rapid decline in motor strength. Eighty percent of patients with executive dysfunctions at baseline developed non-executive dysfunctions in the course of the disease. Patients who showed cognitive deficits at baseline, showed cognitive deficits on multiple domains of cognitive testing in follow-up. Twenty percent of patients without executive dysfunction at baseline did appear to have executive dysfunction later on in follow-up. ALS patients performed cognitively worse than a matched healthy control group. Executive decline is a negative predictor of prognosis in the study by Elamin, Bede & Byrne

(2013). A disadvantage of this research is the fact that it did not include any *C9orf72*-related patients; these patients seem to have more rapid cognitive decline than other ALS patient (Irwin et al., 2013). Including *C9orf72*-related patients in a longitudinal study gives opportunity to compare cognition in familiar and sporadic patients. Furthermore, the test battery used in this study only has one neuropsychological test per cognitive domain in language and visuo-constructive skill, which means that subtle changes in cognition can hardly be detected, this could mean that all patients might experience a form of cognitive decline.

Drawing scientific conclusions on studies from which only one seems methodologically sound is always a risk and therefore more evidence is needed. Furthermore, the study by Kasper et al. (2016) replicated the previous study without successfully showing that there is any decline among patients who have executive dysfunctions at baseline. The current study will focus on cognition in the course of ALS. A longitudinal study will be conducted including ALS patients and controls. This project will also address the methodological weak points in the study by Elamin, Bede & Byrne (2013) by including *C9orf72*-related patients and using multiple tests per domain. The following are will be pursued:

Aim 1: to explore longitudinal cognitive functions in ALS.

Aim 2: to investigate if there are any learning effects found in the test battery used.

Aim 3: to investigate if *C9orf72* hexanucleotide repeat expansion and onset influence cognitive functions.

For aim 1, the following research question will be answered: is there a difference in cognitive functioning on ECAS scores between measurements? The hypothesis is as followed:

H0: there is no statistical difference in cognitive functioning in ALS patients on ECAS scores between baseline and follow-up measurements.

Since it is not sure if there are any learning effects within this particular test battery, a control group will be used to check whether controls will improve or not on the specific tests. So for aim 2 the research question is: are there any learning effects found in this study? The hypothesis is:

H0: there are no learning effects found in this particular test battery.

Not investigated by Elamin, Bede & Byrne (2013) are some specific factors like the influence of repeat expansion in the *C9orf72* gene and site of onset. The research question is: are *C9orf72* and site of onset of influence in cognition of ALS patients? The following hypothesis will be tested:

H0: *C9orf72* repeat expansion and site of onset have no influence on the course of cognition in ALS patients.

In line with the study of Elamin, Bede & Byrne (2013), cognitive functions are expected to decline when executive functions are impaired and cognitive functions are expected to be spared over time when they are not impaired at baseline. So a subhypothesis will be tested regarding this study.

H0: patients with executive impairment at baseline, are not more impaired in cognition on follow-up than patients without this impairment.

It is expected that no learning effects will be found because of a minimum 3 month time interval. If any learning effects are found, these are likely to occur in the memory subtests in which a story needs to be remembered, because it might be easy to recognize for controls.

C9orf72 repeat expansion will have effect on cognitive functions over time. It is expected that *C9orf72* repeat expansion will cause a faster rate of decline in cognition which is in line with studies from Irwin et al. (2013), Byrne et al. (2012) and Murphy et al. (2016). In multiple studies is found that patients with bulbar onset, show more rapid decline in cognition than patients with other types of onset (Kilani, Micallef, & Soubrouillard, 2004; Schreiber et al., 2005; Strong, Grace, Freedman, 2009). Therefore, it is expected that patients with bulbar onset show more cognitive decline in this sample.

Identifying cognitive deficits in ALS is important, because any cognitive deficit found can be taken into account in treatment and contact with patients. Thereby, it is shown that caregivers can experience a great burden when helping ALS patients who experience cognitive deficits (Pagnini et al., 2010). The goal of this project is to map cognition in the course of ALS. This will be done by examining a test battery on multiple points in time with ALS patients.

Methods

Participants: Using the database of ALS patients from UMC Utrecht, and testing/retesting patients has provided approximately 100 patients and 50 healthy controls. Inclusion criteria for patients were: being diagnosed with ALS, not participation in another study that used neuropsychological testing, declared to be willing to be approached for scientific research, completed the ECAS once with a 3 month or more interval or completed the ECAS once and willing to be retested. Exclusion criteria for patients were: having a comorbid psychiatric disease (e.g. depression) or a condition that possibly affects cognition , incomplete ECAS on first measurement, change of diagnosis over time or having declared not wanting to be approached for research. The inclusion criteria for controls were: completed the ECAS twice with a 3 month or more interval and having a complete ECAS at baseline. An exclusion criterion for controls

were: not having a comorbid disease and not willing to be retested.

Tasks: A neuropsychological test-battery taking approximately 40 minutes was administered at both baseline and follow-up. This battery includes:

- **Edinburgh Cognitive Assessment Screen** (ECAS) (Abrahams et al., 2014): This is a test used at UMC Utrecht to discriminate between cognitive deficits that are common in ALS patients and cognitive deficits that do not fit an ALS profile. The ECAS determines the presence, severity and type of cognitive and behavioral changes. The domains in this test include: verbal fluency, language, memory, executive functioning, and visuospatial ability. Multiple tests measure each domain. There are domain-specific, ALS-specific, -non-specific and total cut-off scores to discriminate between cognitive deficits. The ECAS also includes a part where a caregiver is asked about behavioral changes and psychotic symptoms that might occur in patients.
- **Frontal Assessment Battery** (FAB) (Dubois, Slachevsky, Litvan, & Pillon, 2000): a screening used to measure deficits in frontal regions of the brain. This battery uses 6 individual smaller tests measuring conceptualization, mental flexibility, programming, sensitivity to interference, inhibitory control, and environmental autonomy. On every test a maximum of 3 points can be scored which adds up to a maximum total of 18 points. A cut off score of 12 on the FAB has a sensitivity of 77% and specificity of 87% in differentiating between frontal dysexecutive type dementias and other types of dementia.
- **Hospital Anxiety and Depression Scale** (HADS) (Zigmond & Snaith, 1983): a questionnaire with 14 questions giving an indication about anxious and depressive symptoms. There are 7 questions measuring anxiety symptoms and 7 that measure depressive symptoms. Al questions have 4 multiple choice options giving 0 to 3 points

for a question. A higher score means more symptoms. For anxiety and depressive symptoms the maximum score is 21. The cutoff lies at 8-10 for a possible indication of anxiety/depressive symptoms and 11 or more for an indication of these symptoms.

- **ALS Frontotemporal Dementia Questionnaire** (ALS-FTD-Q) (Raaphorst et al., 2012): this is a questionnaire that must be filled in by a caregiver of the patient to see if there are any signs of frontotemporal dementia. This questionnaire consists of 25 multiple choice questions with 4 answering options. This makes the maximum score 100, a higher score means a higher indication of frontal dysfunction. The cutoff is >21 points for mild behavioral disturbances and >28 for severe behavioral disturbances.

Procedure: Baseline testing was mostly done at UMC Utrecht on the day patients receive diagnosis. Here patients undergo multiple tests including the neuropsychological test battery to see if there are signs of ALS-specific cognitive deficits. During neuropsychological testing the psychologist informs the patient on the testing. At the same time the neuropsychologist tells a caregiver (mostly a partner, sibling or child) that they will be asked a few questions in private later on. The caregiver then leaves the room and patients will be tested. There is an alternative version of the ECAS developed for when patients are not able to speak/write. Retesting procedure was at the homes of the patients, because some patients were unable to come to the hospital because of disease progression. The same tests were completed at retesting. The neuropsychological testing took up about 45 minutes with 30 minutes of testing and 15 minutes of seeing a caregiver/loved one. All tests were administered in the same order at baseline and retesting.

Analysis: In order to test cognitive function declines over time, a statistical significance between neuropsychological test scores on 2 points in time must be found. It was checked if

there were changes in individual domains to see if certain domains are more or less impaired than others. This is tested with ECAS total scores for significance using a paired-sample t-test or a non-parametric equivalent when assumptions were not met. For testing individual domains mean scores were calculated for each domain and were tested for significance over time with a related sample t-test or a non-parametric equivalent.

To clarify whether having the *C9orf72* mutation will affect the course of cognition, delta scores were calculated between T1 and T2 for patients with and without this particular specific factor. This was tested for significance with a within sample t-test or a non-parametric equivalent. As outlined in the 2nd paragraph, *C9orf72*-related ALS gene ALS patients were expected to influence the cognition in ALS patients over time. The same procedure was followed regarding bulbar and spinal onset. As discussed, patients with bulbar onset are more likely to experience cognitive decline.

Results

Sample characteristics: originally 116 patients underwent both baseline and follow-up testing with the ECAS. There are 9 patients with missing variables and other exclusion criteria made up for loss of 3 patients from whom 2 are diagnosed with COPD and 1 with a psychiatric disorder. The exacerbation of COPD is strongly correlated with cognitive decline (Zhang et al., 2016). This takes the number of subjects to 104. From 9 patients there is no data regarding the site of onset. All tests have occurred between 2014 and the start of 2016. From the 57 controls who underwent testing at baseline and follow-up, none have missing variables. All sample characteristics are shown in table 1.

We must know if ECAS scores are normally distributed so the right statistical test can be applied. A Kolmogorov-Smirnov test of normality shows us that baseline and/or follow-up

distributions for all tests are significantly different from a normal distribution ($p<.01$). There is one exception, the *C9orf72* sample have normally distributed scores on both baseline and follow-up. This means all statistical analysis has to be non-parametric except for the *C9orf72* sample. The lowest Kolmogorov-Smirnov statistic is shown for every test (table 2-6).

Table 1

Sample characteristics

	Patients	Healthy controls
# subjects	104	57
Male (percentage)	70 (67%)	22 (38.6%)
Thoracic onset	1	-
Bulbar onset	20	-
Spinal onset	72	-
Generalized onset	2	-
C9orf72 (percentage)	14 (13.4%)	-

The influence of disease progression on cognition: Mean total ECAS scores are at baseline 105.462 (SD=15.263) and in follow-up 108.882 (SD=14.664). For measuring statistical difference a Wilcoxon Signed Ranks test was used which demonstrated in statistically significant results between baseline and follow-up total scores ($p<.05$). ECAS total scores seem to not decrease over time. Statistics are shown in table 2, the corresponding scatter dots can be found in figure 1.

But, this might not prove all that much, because the ECAS includes both ALS specific and ALS non-specific domains, therefore the same analysis will be executed for ALS specific

(executive functioning, language, fluency and social cognition) and non-specific scores (memory, visuospatial function). The average ALS specific scores is 77.5 (SD=12.383) at baseline and 79.365 (SD=11.887) in follow-up. For ALS non-specific scores this is 27.961 (SD=4.435) at baseline and 29.3558 (SD=4.043) in follow-up. This analysis resulted in a statistical significance in the non-specific domains with $p < .01$, with more positive ranks than negative ranks which means that most patients scores higher in follow-up than baseline as shown in figure 1. A statistical difference in ALS specific scores is demonstrated ($p<.05$). Just like non-specific scores, most patients tend to improve in ALS specific scores in follow-up in comparison with baseline testing.

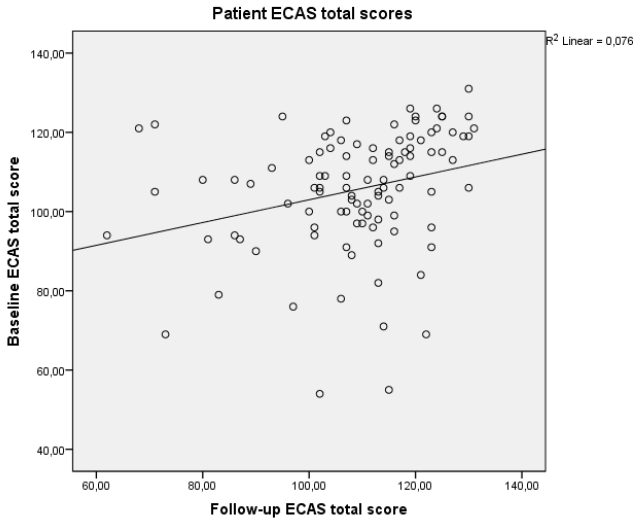
So patients are more likely to improve on the ECAS scores in general and in specific/non-specific domains separately, but individual domains could explain the unexpected average improvement in follow-up. To test this, a mean difference score is calculated for each domain over time. Wilcoxin signed ranks are used to see if scores in domains differ significantly. There are no significant differences in fluency (p=.235), visuospatial ability (p=.808) and language (p=.930). As hypothised a significant average increase in scores is found in memory (p=.000). This change is mainly due to the 'recall' task where patients need to remember a story and try to reproduce it (p=.001). Recognition (p=.048) and retention (p=.053) were less influential. For executive function a significance is found when using an alpha of .05 (p=.043).

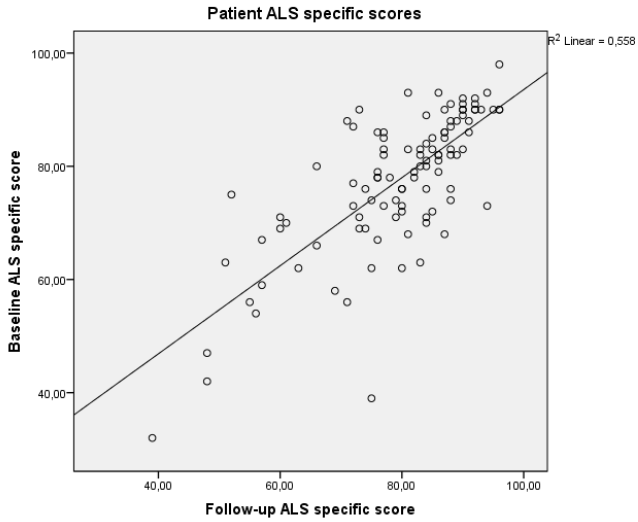
Table 2

Sample Wilcoxin signed ranks tests for ECAS scores in ALS patients.

	Z	K-S***	*P*	Neg.	Pos.	Ties	Total
Patients							
Total	-2.235	.001**	.025*	37	61	4	102
Specific	-2.480	.000**	.013*	32	63	9	104
Non-specific	-3.870	.000**	.000*	26	64	14	104
Fluency	-1.186	.000**	.235	31	38	35	104
Visuospatial	-.243	.000**	.808	23	20	61	104
Language	-.088	.000**	.903	29	33	42	104
Executive	-1.994	.000**	.000**	36	60	8	104
Memory	-4.293	.000**	.043*	26	62	16	104

Note: *p < .05. **p < .01. ***K-S stands for lowest p-value regarding baseline and follow-up Kolmorov-Smirnov testing. (A positive ranks is a subject who scored higher on follow-up then on baseline). 'Neg.'= number of negative ranks, 'Pos.'= number of positive ranks.





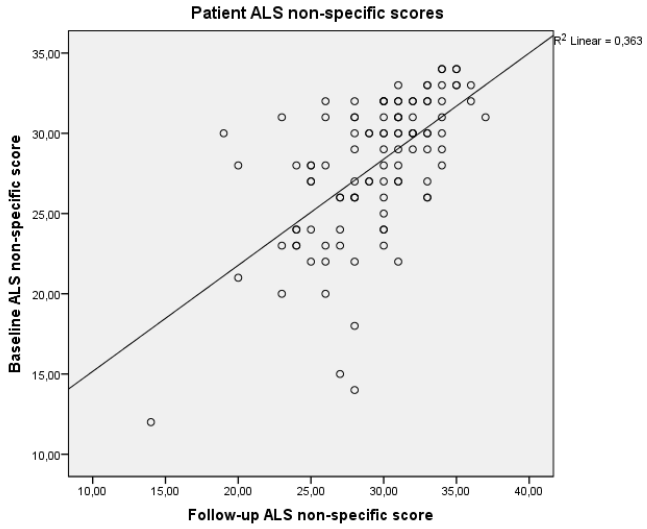


Figure 1. Scatterplots of ECAS total/specific/nonspecific scores at baseline and follow-up.

Reproducing the study of Elamin, Bede & Byrne (2013), it is tested if executive dysfunction (scoring under executive cutoff in ECAS) predicts a more rapid rate of decline. An independent sample non-parametric test is used with ECAS total scores in follow up as dependent variable and executive function (above/below cutoff) as grouping variable. In this sample 39 out of 102 subjects had an executive dysfunction at baseline. To see if there is a decline over time a Wilcoxin signed ranks test is used to check if patients with executive impairments on baseline show significant differences in follow-up. For ECAS total scores in follow-up a significant difference ($p<.05$) is found toward an increase in scores for patients with an executive impairment at baseline (see table 3 and figure 2). Also for ALS specific domains, a significance is found towards an increase in scores ($p<.01$).

Table 3

Wilcoxin Signed Ranks test for patients with executive dysfunction at baseline.

	Z	K-S***	P	Neg.	Pos.	Ties	Total
ECAS							
Total	-3.997	.001*	.025*	6	31	2	39
Specific	-3.352	.000**	.001**	9	29	2	39

Note: *p < .05. **p < .01. ***K-S stands for lowest p-value regarding baseline and follow-up Kolmorov-Smirnov testing. 'Neg.'= number of negative ranks, 'Pos.'= number of positive ranks.

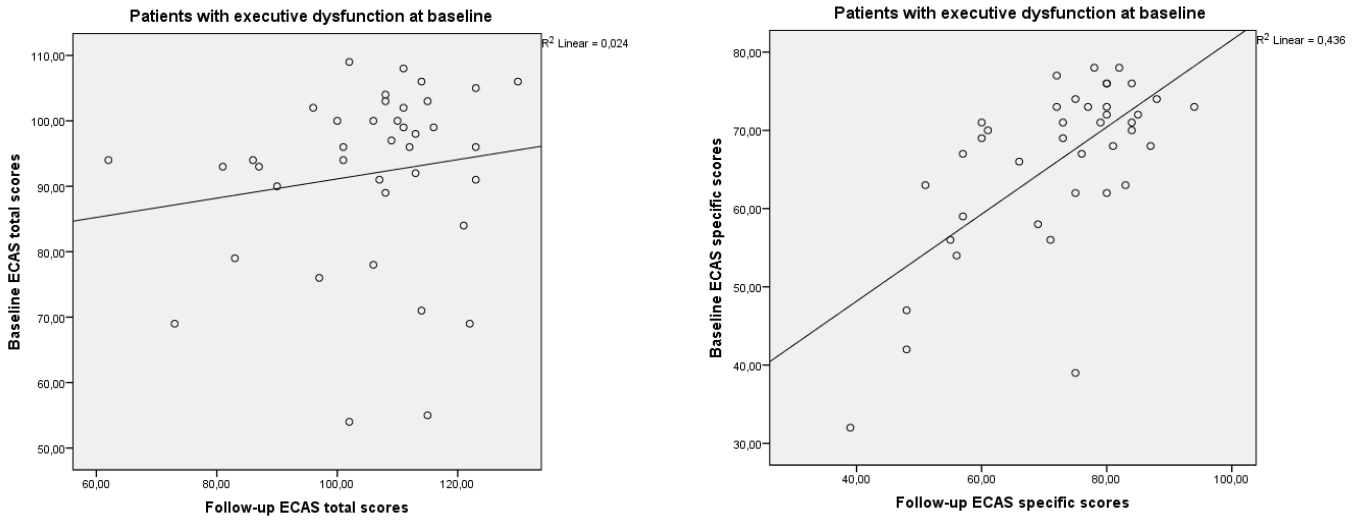


Figure 2. Scatterplots of ECAS total scores (left) and ECAS specific scores (right) on baseline and follow-up in patients with executive dysfunction on baseline.

The influence of genetics and site of onset: thus far, there is no cognitive decline found in this sample for ECAS total scores, ALS-specific scores or separate domains. The heterogeneity of the disease might make it hard to find significant differences. Therefore, specific important predictors of cognitive decline in ALS were selected to see if these make for

early/rapid decline in ALS patients. The same analysis as earlier will be ran again but now with multiple groups splitting patients with *C9orf72* hexanucleotide repeat expansion and patients who do not have this expansion, and bulbar versus spinal onset. Generalized and thoracic onset are taken out of this analysis because there are not enough patients with these types of onset in this sample.

The file is split into a group with bulbar onset and a group with spinal onset. Using a Wilcoxin signed ranks test it is tested if there is a significant decline in cognition between baseline and follow-up. Mean ECAS scores for bulbar onset on baseline and follow-up are 101.05 (Sd=20.278) and 108.667 (Sd=16.471) respectively. For spinal onset these are 106.444 (sd=13.884) and 109.167 (sd=13.925). For bulbar onset (p>.05) no significant difference is found in cognition over time, suggesting no changes in cognition. For spinal onset (p<.05) a difference is found, which suggests an increase in overall cognition (as seen in table 4 and figure 3).

Table 4

Sample Wilcoxin Signed Ranks test on onset in ECAS total scores.

	Z	K-S	P.	Pos.	Neg.	Ties	Total
Onset							
Bulbar	-.758	.000**	.449	8	9	1	18
Spinal	-2.222	.000**	.026*	24	45	3	72

Note: *p < .05. **p < .01. ***K-S stands for lowest p-value regarding baseline and follow-up Kolmorov-Smirnov testing.

'Neg.'= number of negative ranks, 'Pos.'= number of positive ranks.

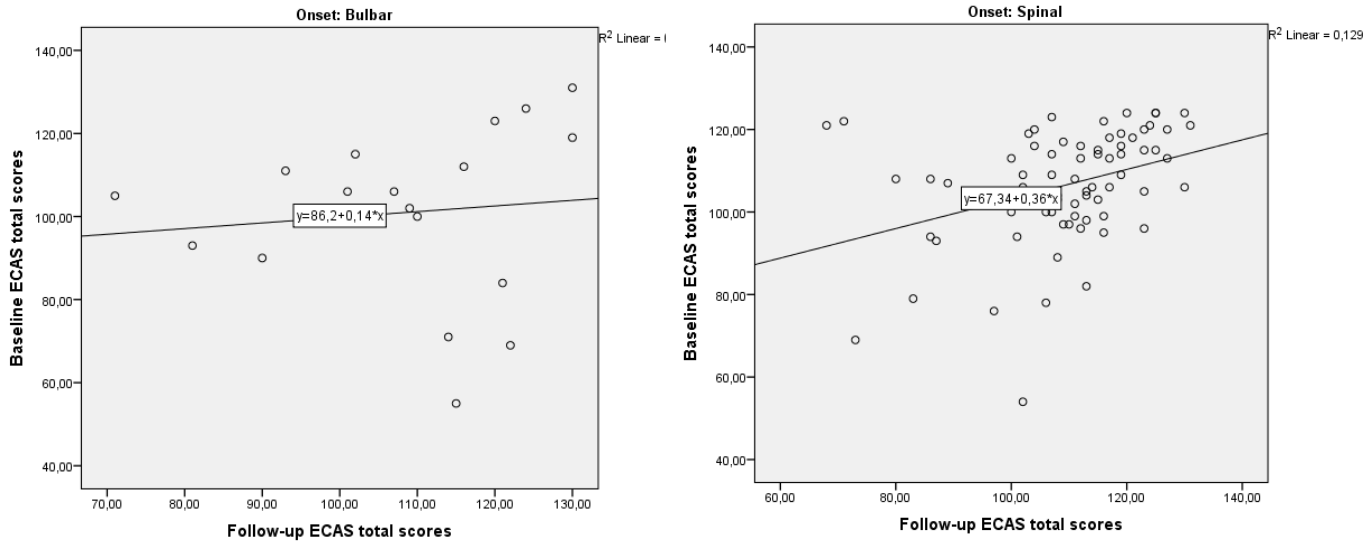


Figure 3. Scatterplots of ECAS total scores on baseline and follow-up in patients with spinal onset (left) and bulbar onset (right)

When analyzing *C9orf72*-related patients, the file is split into patients with expressed expansion and patients who do not have similar genetic expression. A Wilcoxin signed ranks test is used to analyze if patients without the expansion show differences in total ECAS scores over time. Mean ECAS total scores for non-genetic patients are 105.5 at baseline and 109.4 for follow-up, this difference is significant ($p<.05$) which shows an increase in cognition. To see if the same applies for people with the repeated genetic expansion a within sample t-test is used because total ECAS scores on baseline and follow-up are normally distributed (see table 5). For patients with expressed repeated expansion of the *C9orf72* gene the mean total ECAS score on baseline is 105.167 and 105 at follow-up testing. Patients with the expressed gene show no improvement or decrease in cognition ($p>.05$).

Table 5

Sample Wilcoxin Signed Rank and Within sample t-test on ECAS total scores for patients with and without C9orf72 repeat expansion

	Z	K-S	P	Pos.	Neg.	Ties	Total
C9orf72							
Yes	-.089	.196	.969	-	-	-	-
No	-2.356	.001**	.018*	31	56	3	90

Note: *p < .05. **p < .01. ***K-S stands for lowest p-value regarding baseline and follow-up Kolmorov-Smirnov. 'Neg.'= number of negative ranks, 'Pos.'= number of positive ranks. testing

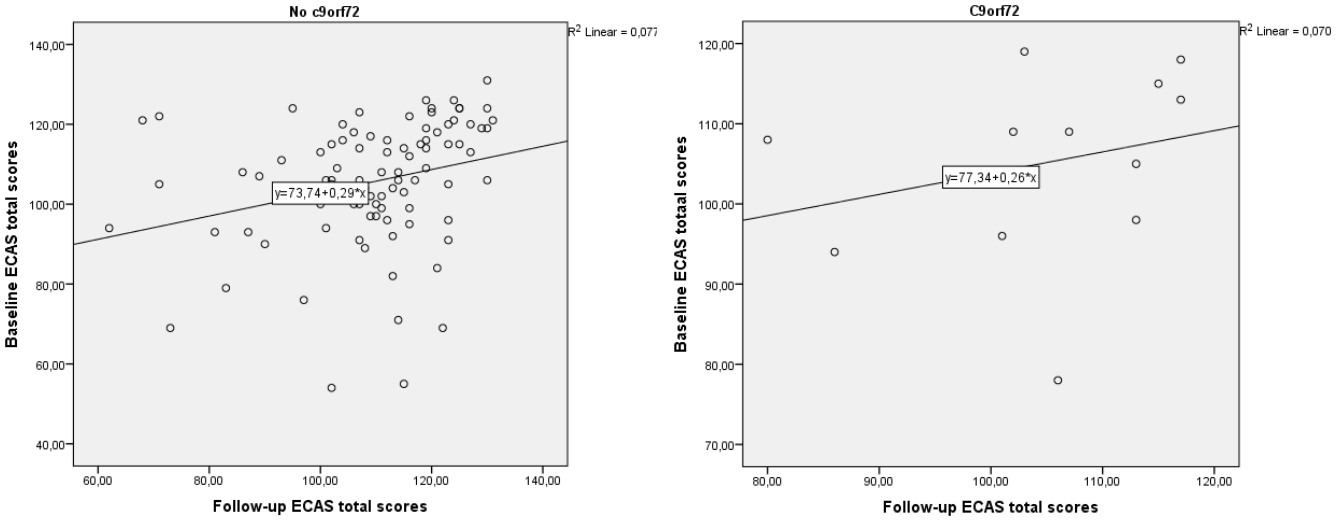


Figure 4. Scatterplots of ECAS total scores on baseline and follow-up in patients without *C9orf72* repeat expansion (left) and with the expansion (right)

Learning effects in controls: For all controls, ECAS scores at baseline are not differing from a normal distribution according to a Kolmogorov-Smirnov test (p>.05), ECAS total scores in follow-up are significantly different from a normal distribution (p<.01) so a non-parametric test is used to see if there are learning effects. Averaged ECAS total scores for all controls add up to 114 (sd=9.214) at baseline testing and 117.228 (sd=8.135) in follow-up. This improvement

in total cognition is significant using a Wilcoxin signed ranks test (p<.01).

For ALS specific scores the same analysis is used. Only ALS specific scores at baseline resemble a normal distribution, so a Wilcoxin Signed Ranks test is used again. A significant improvement is seen in both ALS specific scores ($p < .01$) and ALS non-specific scores ($p < .05$). There is no difference in non-specific scores using an alpha of .01. Z-values and ranks are found in table 6, whereas the scatterdots are found in figure 5.

Table 6

Sample Wilcoxin Signed Ranked table for ECAS scores in controls

	Z	K-S***	P	Negative	Positieve	Ties	Total
Total	-3.456	.000**	.001**	15	35	5	57
Specific	-3.044	.000**	.002*	15	34	8	57
Nonspecific	-2.574	.000**	.010*	20	34	3	57

Note: *p < .05. **p < .01. ***K-S stands for lowest p-value regarding baseline and follow-up Kolmorov-Smirnov testing

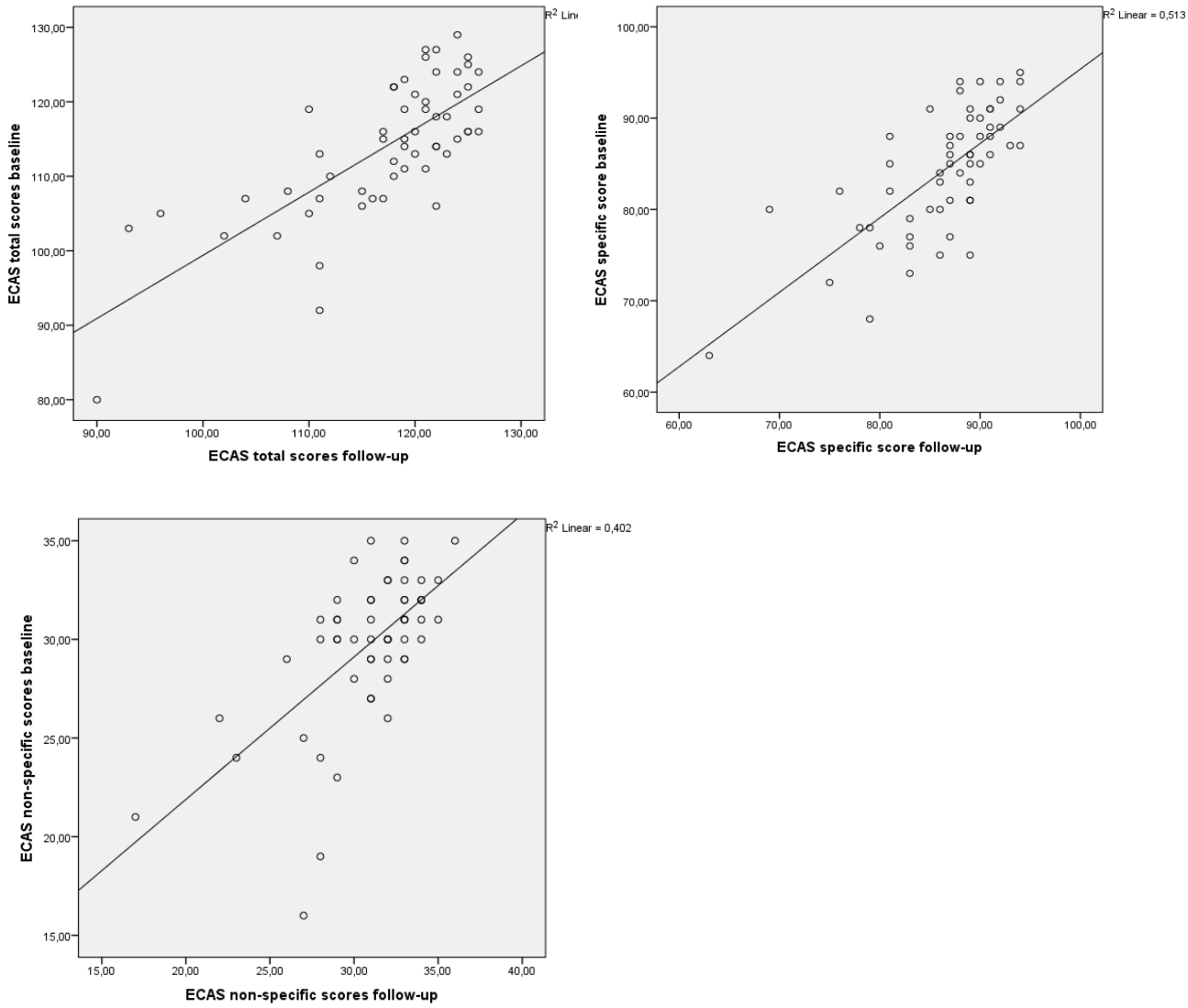


Figure. 5: Scatterdots of ECAS total (top left), ALS specific (right) and ALS non-specific (bottom left) scores at baseline and follow-up for controls.

Just like the patient group, controls are likely to improve in cognition in general and in specific/non-specific domains separately (see figure 5). To test if these differences come from a specific domain, the same analysis was used for all domains. There is no significant difference between measurements in language (p >.05) , visuospatial ability (p >.05) and fluency (p >.05). When α=.05 a significant improvement is found in both memory (p =.019) and executive

function (p =.013) suggesting a learning effect for controls in these domains. For all domains there were more positive ranks (improvement) then negative ranks (decline) within the Wilcoxin signed ranks test.

Discussion and Conclusion

The aims of this longitudinal study on cognition in Amyotrophic Lateral Sclerosis were 1) to explore cognitive functions in ALS over time, 2) to investigate if there are any learning effects found in the test battery used using a control group and testing this group twice, 3) to investigate if *C9orf72* repeat expansion and site of onset influence cognitive functions in ALS patients. These aims were realized by mapping cognition in 104 patients at diagnosis and a follow-up moment with the Edinburgh Cognitive Assessment Screen (ECAS).

It was expected that at least ALS specific functions would decrease over time. Also it was expected that executive dysfunction at baseline predicts a more decline in follow-up just like spinal onset and *C9orf72*-related patients.

Almost all analyses were non-parametric because baseline and follow-up scores differ significantly from a normal distribution, most distributions are left-skewed, suggesting a ceiling effect in the tests or sample. A difference in global cognition is found between baseline and follow-up showing that global cognition increases significantly. Both ALS specific cognition and ALS non-specific cognitive functioning are significantly increased in follow-up. It is also demonstrated that the individual domains of memory and executive function increase over time and the other domains (visuospatial ability, verbal fluency, and language) did not show an increase or decrease over time. The findings of Elamin, Bede & Bryne (2013) could not be reproduced because an executive dysfunction at baseline did not show a decrease in cognition in follow-up, but an increase in cognition was demonstrated in global cognition and ALS specific

cognition. A significant improvement in cognition over time is found in the patients with spinal onset. Patients with bulbar onset did not increase or decrease significantly over time in cognition. Patients with the *C9orf72* repeat expansion show no increase or decrease in global cognition while an improvement is found in patients without this expansion. The control group showed learning effects in global-, ALS specific- and ALS non-specific cognition. Just like the patient group, memory and executive domains show an increase over time in controls. Taken together, these are not the results which were to be expected, but how come?

First of all, a learning effect is found in the control group. This means that presumably, the memory and executive function tests are sensitive to learning effects which maintain over time. The increase in memory and executive function is shown in both the patient sample as the control group. In both groups all other domains remained statistically stable. Although only in memory and executive function a significant improvement is found, all domains show a higher mean score in follow-up, which might explain the increase in global cognition found in this study. Also, the tests that are administered at baseline are also used in follow-up which gives more chance of learning effects. There are also some situational differences between baseline and follow-up testing. Baseline testing was in the hospital on the day a formal diagnosis is mostly formed, this could provide stress to the patients which in turn can make them perform better or worse which depends on how they react on this pressure. In follow-up this possible stress is less of a deal because the diagnosis is already formed and patients will not be informed on how they performed on the follow-up testing. Furthermore, the conditions between baseline and follow-up testing also differ. The testing procedure is more controlled at baseline because this takes place in the same quiet testing room for every patient. Most follow-up testing takes place at the homes of the patients which is another environment in which patients might feel

more comfortable or have more distractions e.g. a pet or partner who is in the direct environment. So, learning effects can explain the improvement in cognition over time.

Second, this sample is sensitive to a bias towards the less affected patients; most patients were recruited through a study containing a MRI scan, which means that patients in this sample were able to go alone inside a MRI scan for several minutes by themselves. These patients are able to come to the hospital alone so disease is mostly not in a far stage of the disease. So there probably is a bias towards the cognitively 'less affected' patients which might be responsible for the ceiling effects and non-normal distributions found in this study.

A third reason why the results are not as expected, is that results/patients are not controlled for by disease progression. Baseline testing is at diagnosis, but at the time of diagnosis some patients are in a further stage than others. This means that the sample consists of an even more heterogeneous group when not controlling for disease progression/stages. The heterogeneous ALS population might explain earlier contradicting evidence found in other studies. Furthermore, respiratory weakness may cause or exaggerate cognitive deficits (Newsom-Davis et al., 2001). So learning effects, together with a probable sample bias within an already heterogeneous group, might explain the results that are found.

Fourth, the differences in the ECAS scores between baseline and follow-up testing are mostly smaller than the standard deviations so there is a fair chance of error, demonstrating a low power. Also, the heterogeneity of the sample is forcing to use non-parametric testing whereby no means are tested for significance but ranks (is the first scores higher, lower or tied to the follow-up score). Non-parametric testing is less powerful which lowers the chance of correctly rejecting the null-hypothesis.

Although the sample was large, it was split when testing multiple hypothesis. It is necessary to mention that the groups that emerged from this were not always large enough. The *C9orf72* patient sample only counts 12 patients. Although this is a small sample size, the test was run to see if there are any observable differences in groups. The results show that this small group shows no cognitive decline or improvement and average baseline cognition is not differing from the non-*C9orf72* group. The non-*C9orf72* group showed an improvement in cognition. This difference might be explained by the found learning effects. It is possible that *C9orf72* repeat expansion in patients is a marker for decline in this group but learning effects are keeping this from happening so the non-genetic group improves and the genetic group may be balanced by cognitive decline over time versus learning effects. The group with bulbar onset is, with 18 patients, also small. This group did not show any cognitive decline or improvement whereas the spinal onset group did show an improvement in cognition. Maybe, this can also be explained by learning effects which mask the cognitive decline in the bulbar-onset group.

In future studies it is important that the ECAS is used because of its good sensitivity, but a B-version must be administered at follow-up to diminish the learning effects found in this study. This B-version already exists and is being translated and validated in Dutch research. Furthermore, in future studies there must be controlled for disease progression as Trosji et al. (2016) did in their study, where patients were classified according to King's staging, to find out if patients tend to decrease in cognition throughout the disease. This lessens the heterogeneity bias found in the sample because cognition is linked to staged motor decline (Elamin, Bede, Byrne, 2013) and when there are several groups with patients who are in the same stage, statistical analysis will be parametric because those groups will be homogenous. Future studies should also address the quick cognitive decline at onset of the disease, this might predict further

decline just like Elamin, Bede & Byrne (2013) mentioned considering executive decline. We know not only executive function can decline so in future studies baseline cognitive dysfunctions should be taken into account. Future studies should address the cognitive decline in patients with bulbar onset and *C9orf72* repeat expansion and look at individual domains within these samples to see at what exactly takes in a specific place in time/disease progression.

To conclude, a learning effect is found in this study and the sample seems biased in an already heterogeneous population. On the other hand there are reasons to suggest that a cognitive decline in ALS patients, especially with a repeat expansion and/or bulbar onset is evident. More research with the Edinburgh Cognitive Assessment Screen should be done to complete the puzzle of cognitive functioning in patients with Amyotrophic Lateral Sclerosis.

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