**Spectroscopic markers of neurodegeneration in the mesial prefrontal cortex predict survival in ALS**

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**Abstract**

**Background and objective:** N-acetylaspartate (NAA) and myo-inositol (mIns) are spectroscopic markers of neuronal integrity and astrogliosis, respectively. We performed a survival analysis to determine the prognostic value of the NAA/mIns metabolite ratio in ALS after a period of two and five years.

**Methods:** Twenty-four patients with ALS (two with ALS-FTD) were recruited to participate in a high-field MR spectroscopy study of the mesial prefrontal cortex. Univariate and multivariate Cox proportional hazards analyses were used to assess NAA/mIns as a predictor of survival alongside other demographic and clinical measures. Census dates were set at two and five years after the time of MR scan for each patient. Survival curves were calculated using the Kaplan-Meier method.

**Results:** After a five-year observation period, 19 patients had died and 5 were still alive. Median survival time from date of scan was 1.95 years. Univariate and multivariate Cox analysis showed NAA/mIns to be a significant independent predictor of survival at two years after scanning, but not at five years.

**Conclusions:** Cerebral degeneration in the mesial prefrontal cortex as detected by the NAA/mIns metabolite ratio is predictive of survival in ALS in a time-dependent manner.

**Introduction**

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by rapid progressive muscular weakness and a clinicopathological hallmark of degeneration and dysfunction of upper and lower motor neurons. Extra-motor involvement in ALS is now widely acknowledged with clinical, neuroimaging and pathological features of frontotemporal lobar degeneration (FTLD) resulting in a number of behavioural and cognitive deficits **[1-4].** Large-cohort studies suggest that as many as 50% of patients may suffer such a syndrome **[5,6]** and in 5-15% of ALS patients, the impairments reach the diagnostic threshold for frontotemporal dementia (FTD) **[7].**

The discovery of a hexanucleotide repeat expansion in *C9ORF72*, present in a significant proportion of familial ALS and FTD cases, confirmed previous hypotheses that some phenotypes of ALS and FTLD co-exist within the same pathological and molecular spectrum including abnormal cytoplasmic neuronal and glial aggregates of TAR DNA-binding protein 43 (TDP-43) **[8].**

Extra-motor changes have been demonstrated *in vivo* in ALS using advanced neuroimaging methods, including voxel based morphometry **[9],** diffusion tensor imaging **[10]**, resting state network analysis **[1]**,cortical thickness quantitation **[11]**, and magnetic resonance spectroscopy **[12,13]**. Such changes are demonstrated in patients even without clinical FTD.

In the absence of effective disease modifying treatment, median survival in ALS is between 2 and 5 years after symptom onset. However, there is considerable variation among patients; while some live for a little as 2 months following diagnosis, up to 30% live beyond 5 years **[14].**

Accordingly, there is a pressing need to identify prognostic indicators that can assist with patient counselling. The variability in progression rate and disease duration in ALS presumably has a biological basis. Stratification of patients based on survival with such a tool would thus assist in basic and translational research studies to improve our understanding of this biological variability. A survival predictor would play a key role in clinical trials in patient selection to improve the biological homogeneity of cohorts and minimizing enrolment of patients that, for example, are predicted to progress slowly. Recently, a survival model was developed using baseline data from 14 European countries, finding predictive variables to be site of onset, age, El Escorial diagnostic designation, time to diagnosis, forced vital capacity, progression rate, presence of frontotemporal dementia, and presence of a C9orf72 mutation **[15]**. These features have been found variably by other investigators to be predictive, with other predictors including the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) score **[16]**, body mass index **[17]**, and attendance at multidisciplinary ALS clinics **[18]**. Biomarkers will likely play an important role in refining survival models, with preliminary evidence present for fluid-based biomarkers such as cerebrospinal fluid neurofilament concentration **[19]**, and neuroimaging biomarkers derived from MRI **[20-22].**

Cerebral neurochemical changes quantified using MRS hold promise as a biomarker of neuro-degeneration in ALS. Studies have revealed evidence of decreased neuronal integrity in the motor **[23-28]** and prefrontal **[13]** cortices by the finding of reduced N-acetylaspartate (NAA) or ratios of NAA to choline (NAA/Cho), creatine (NAA/Cr) or myo-inositol (NAA/mIns). We recently demonstrated spectroscopic changes in the prefrontal cortex in ALS indicative of degeneration and that the NAA/mIns ratio is a more robust marker of such compared to changes in the individual metabolites alone **[13].** Furthermore, these changes were present in a cohort of subjects in which only a minority had clinical FTD. With attendant FTD in ALS being a poor prognostic sign and MRS possibly a more sensitive indicator of prefrontal degeneration, we hypothesized that MRS-derived neurochemical changes indicative of prefrontal degeneration are predictive of reduced survival.

Specifically, we sought to determine whether the NAA/mIns metabolite ratio is predictive of patient survival after observation periods of two and five years. These time limits encompass the range of the survival time in ALS after symptom onset, serving as a useful window for prognostic purposes.

**Methods**

*Participants:* Patient characteristics, imaging methods and spectroscopic findings are detailed in our original report **[13].**

Patients were recruited from the ALS Clinic at the University of Alberta Hospital with a revised El Escorial classification of possible, probable, or definite ALS. At the time of imaging, the median symptom duration was 14 months (range 5 - 75 months). Participants gave informed consent, and the study was approved by the Health Research Ethics Board.

Twenty-four patients were included in this analysis. Age, site of onset, and symptom duration were recorded at the time of scanning. Forced vital capacity (FVC), ALSFRS-R score, and letter F verbal fluency score were collected within seven days of the MRS scan (patient demographics in Table 1).

The clinical and MR spectroscopic results from this cohort have been previously reported **[13].** We continued to follow up with patients post-study for up to five years after the time of their MRS scan and updated their survival status as necessary.

*MR Imaging and Spectroscopy:* MR scanning was performed between February 2006 and October 2010 at 3T with single voxel spectroscopy and metabolite-specific sequences optimized for the detection of NAA and mIns from a voxel placed midline in the prefrontal lobe anterior to the genu of the corpus callosum **[13,29].** Data were acquired by using single-voxel localized proton MRS. The voxel measured 25x25x30 mm and was centered in the mesial prefrontal cortex, anterior to the genu of the corpus callosum.

*Statistics:* A univariate and multivariate Cox analysis was used to compare the significance of variables as predictors of survival. The Kaplan-Meier method was used to calculate survival curves, which were compared using the log-rank test. Due to the limitations of the sample size, an automated backward stepwise regression was adopted for the multivariate Cox proportional hazards model, with a retention threshold of p < 0.15, though an exploratory complete Cox model with all covariates was also performed. Survival time was calculated as time to death or to permanent assisted ventilation from the date of the MR scan. Analyses were performed for two follow-up windows, 2 and 5 years. Patients were censored if lost to follow-up or were still alive without permanent assisted ventilation or at the end of each of these respective observation periods. Statistical significance was accepted at p<0.05.

Age at time of scan, site of onset, symptom duration, ALSFRS-R score, FVC, and letter F verbal fluency were included in the analysis due to their status as well-established prognostic factors in ALS **[14, 28, 30, 31].**

**Results**

In the medial prefrontal region selected for this study, the segmentation of the volume of tissue from which the MRS data is acquired typically composed of 67% GM, 20% WM and 13% CSF.’

*Two years after scan:* Two years after the MR scan, a univariate Cox analysis found age, ALSFRS-R score, FVC, and NAA/mIns to be significant independent predictors of survival. Multivariate Cox regression with all covariates returned only NAA/mIns as a significant predictor of survival. Using a backward Cox regression with all covariates included in the initial step, baseline NAA/mIns was retained as a significant predictor of survival (HR 0.01, p < 0.01) along with symptom duration (HR 0.52, p = 0.07) and ALSFRS-R score (HR 0.83, p < 0.01). All other covariates were eliminated by the retention threshold of p > 0.15 (Table 2).

Patients divided into two groups at the median NAA/mIns were compared using a univariate Kaplan-Meier analysis (Figure 1). Mean survival time differed significantly between the groups, with 1.24 years (95% CI: 0.96 – 1.52) in patients below the median compared with 1.86 years (95% CI: 1.86 – 1.87) for those above the median (χ21 = 7.07, p < 0.01).

*Five years after scan:* Five years after the MR scan, the univariate Cox analysis found age, FVC, and verbal fluency to be significant independent predictors of survival. A trend (p=0.06) was present with ALSFRS-R, but NAA/mIns was not a significant predictor of survival five years after the time of scan. A multivariate Cox regression with all covariates included returned age (HR 1.05), as a significant predictors of survival. A backward Cox regression with all covariates included in the initial step retained age (HR 1.06, p < 0.01), and FVC (HR 0.97, p = 0.04) (Table 3).

**Discussion**

We have found that the ratio of NAA/mIns in the mesial PFC of ALS patients is predictive of survival over an observation period of two years. Univariate and multivariate Cox analyses identified higher levels of NAA/mIns as a positive indicator of prognosis two years after the MR scan. However, this result was not replicated when the prognostic window was extended to five years, suggesting that the significance of this spectroscopic marker as a predictor of survival is limited to the upper range of median ALS survival. Our finding that age, ALSFRS-R score, and FVC were significant predictors of survival are consistent with previous studies establishing these as prognostic factors in ALS **[14, 31, 32].** However, a stepwise multivariate analysis showed that their predictive abilities was also time dependent. At 2 years, reduced ALSFRS-R accompanied reduced NAAmIns in being predictive of shorter survival. Whereas higher age and lower FVC at time of MRI were predictive of reduced survival.

Due to the high variability and heterogeneity intrinsic to ALS, a sensitive and reliable biomarker is needed to guide patient counselling, clinical research, and prognostication. MR techniques such as spectroscopy permit the identification of specific biomarkers and are advantageous because they are readily accessible on clinical scanners and importantly non-invasive **[33].** Few studies, however, have investigated the prognostic value of biomarkers derived from MRI. Previously, we determined the spectroscopic index NAA/Cho measured in the motor cortex to be predictive of survival in ALS **[20]**; in a multivariate analysis reduced NAA/Cho had a greater correlation with reduced survival (HR 0.24, p=0.01) than other clinical variables such as age, shorter symptom duration, ALSFRS, and FVC Others have applied DTI in 24 patients with ALS showing decreased FA of the corticospinal tract (HR 0.94, p=0.06) and ALSFRS deterioration rate to be retained in the multivariate survival model **[21]**. In another study, survival prediction at 18 months was studied in 60 patients with ALS using DTI and cortical thickness. The prediction accuracy was higher for MRI data alone (77.08%) than clinical data alone (66.67%), with a slight improvement in prediction accuracy when the two were combined (79.17%). **[34]**. Recently, whole brain 3D texture analysis of T1-weighted images acquired in 83 patients with ALS showed changes in the image texture *autocorrelation* (autoc) in both gray and white matter, including the motor cortex, corticospinal tract, basal ganglia, hippocampus and frontal lobe. When the cohort was divided at the median survival, those in the longer surviving group had autoc changes relatively restricted to the motor cortex and corticospinal tract, whereas those with a shorter survival had more widespread cerebral degeneration including the frontal subcortical white matter, basal ganglia, insula and hippocampus **[35].** The differing patient cohorts and survival analysis methods between studies do not permit a comparison of the survival predictability of the imaging techniques.

The TDP-43 proteinopathy of ALS is centred on the motor cortex and spreads to surrounding regions as the disease becomes more advanced **[36]**. In this 4-stage classification system, anterior cingulate pathology is present only in the latter two stages (stages 3 and 4) **[37]**. The appearance of neurochemical abnormalities in the mesial PFC of these patients was demonstrated in our previous study **[13]** confirms in vivo the presence of pathology in this region.

NAA/mIns was a significant predictor of survival in the univariate and multivariate Cox models and was retained in the stepwise regression at two years after scan, whereas verbal fluency was not. Impaired verbal fluency is a common sign of executive dysfunction in ALS **[1, 2, 32].** There does not appear to be a correlation between mesial PFC NAA/mIns and verbal fluency **[13],** though other studies have associated poor verbal fluency with abnormalities in the dorsolateral PFC and mid-frontal cortex **[4, 38].** However, another study suggests is in line with our finding that letter-guided fluency is not a predictor of survival in ALS **[39].** Our results suggest that, in a prognostic window of two years, mesial prefrontal NAA/mIns is a more sensitive prognosticator in ALS than a clinical measure such as verbal fluency.

It is unclear why there is a lack of prognostic significance for NAA/mIns at five years. It is possible that the prognostic significance of spectroscopic markers of NAA/mIns holds only in the short term because such imaging reflects only a snapshot of the extent of neurochemical abnormalities, without elucidating its trajectory.

A limitation of the study is that the scanning technique we utilized precluded the quantification of other metabolites, including choline, whose prognostic value has been explored previously **[20].** The status of a C9ORF72 mutation is unknown for these patients, as the study was conducted before the discovery of this cause of ALS. As the C9ORF72 hexanucleotide expansion is accompanied by differing CNS changes as determined by MRI **[22]**, future studies could examine the neuroanatomical basis of reduced survival in this group compared to those without the expansion **[38, 40].** A further limitation is the small sample size. This study is being replicated in the Canadian ALS Neuroimaging Consortium (CALSNIC), wherein the baseline MRS characteristics have recently been reported in 65 patients with ALS **[41]**. The patients will be followed prospectively to determine the prognostic utility of MRS (among other imaging measures) on survival. The CALSNIC study will also be able to determine the contribution of other potentially important clinical factors that were not available in our cohort of patients, such as diagnostic delay and the rate of ALSFRS-R decline.

In conclusion, the data we have presented suggest that the NAA/mIns ratio as a biomarker of survival is indeed predictive at the 2-years mark post scan, and provides potentially useful clinical information on an individual basis in the move towards precision medicine.

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**Table Captions**

Table 1: Demographic Data.

Table 2: Survival Analysis Results two years after the MR scan show the hazard ratio (HR) with 95% confidence interval (CI), standard error (SE) and p-value statistic. Significant changes are indicated in bold where the p-value is less than 0.05 with one asterisk, and 0.01 or lower with 2 asterisks.

Table 3: Survival Analysis Results five years after the MR scan show the hazard ratio (HR) with 95% confidence interval (CI), standard error (SE) and p-value statistic. Significant changes are indicated in bold where the p-value is less than 0.05 with one asterisk, and 0.01 or lower with 2 asterisks.

**Figure Captions**

Figure 1: Plot of survival time in years versus the percent probability of survival for ALS patients who were dichotomized at the median NAA / mIns ratio of 1.55, as measured at their MR scan session. Those patients greater than the median are illustrated with the dashed line, and those lower with the solid line. The separation of these two survival curves at the 2 and 5-year times post MRS exam are highlighted.