**A computational PET/CT approach to spinal cord metabolism in**

**spinal onset amyotrophic lateral sclerosis: methodology and prognostic value**

Cecilia Marini1, Angelina Cistaro2, Cristina Campi3, Andrea Calvo4, Caponnetto5, Silvia Morbelli6, Piercarlo Fania7, Mauro Beltrametti3, Cristina Moglia4,4, Genova neuro5, Ambra Buschiazzo6, Torino IRMET2, Annalisa Perasso3, Antonio Canosa4, Genova neuro5, Elena Pomposelli6, Torino IRMET2, Genova Matematica3, Stefania Cammarosano4, Flavio Mariano Nobili5, Paolo Bruzzi8, Gianmario Sambuceti6, Gianluigi Mancardi5, Michele Piana3, Adriano Chio’4

1 CNR Institute of Bioimages and Molecular Physiology, Milan, Section of Genoa, Italy

2 Positron Emission Tomography Centre IRMET, Affidea, Turin, Italy

3 Department of Mathematics (DIMA), University of Genoa, Genoa, Italy

7 Institute of Cognitive Sciences and Technologies, CNR, Rome, Italy

4 ALS Center, ‘Rita Levi Montalcini’ Department of Neuroscience, University of Turin,Turin, and AUO Città della Salute e della Scienza, Turin, Italy

5 Department of Neuroscience, IRCCS San Martino IST, Italy

6 Nuclear Medicine, IRCCS San Martino IST, and Depth of Health Science, University of Genoa, Genoa, Italy

7 Statistics and Epidemiology Unit, IRCCS AOU San Martino-IST, Genoa, Italy

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*Address for correspondence:*

Cecilia Marini, MD,

CNR Institute of Bioimages and Molecular Physiology, Section of Genoa

C/o Nuclear Medicine

IRCCS AOU San Martino-IST,

160143- Genoa,

Italy

Email: Cecilia.Marini@unige.it

**Abstract**

**Background** In amyotrophic lateral sclerosis, functional alterations have been intensively assessed within the brain, while the progression of lower motor neurons damage has been scarcely defined. The present study aims to develop a computational method to systematically evaluate spinal cord metabolism as a tool to monitor disease mechanisms in amyotrophic lateral sclerosis.

**Methods***.* 30 patients with spinal onset amyotrophic lateral sclerosis and 30 controls were submitted to a new computational three-dimensional method to extract spinal cord uptake of 18F-fluorodeoxyglucose combining functional and structural maps of co-registered PET/CT images. The algorithm identified the skeleton on the CT images by using an extension of the Hough Transform, and then extracted the spinal canal and the spinal cord. In these districts 18F-fluoroedoxyglucose standardized uptake values were measured to estimate metabolic activity of spinal canal and cord. Measurements were performed in cervical and dorsal spine districts and normalized for the corresponding value in the liver.

**Findings** Uptake of 18F-fluoroedoxyglucose was significantly higher in spinal cord of patients as compared with controls (p<0•05). By contrast, no significant differences were observed in spinal cord and spinal canal volumes between the two groups. 18F-fluorodeoxyglucose uptake was completely independent from age, gender, degree of functional impairment and disease duration or riluzole treatment. Kaplan-Mayer analysis documented a significantly higher mortality rate in patients with standardized uptake value >5th decile in three years follow-up (log-rank test, p<0•01). The independent value of this information was confirmed by multivariate Cox analysis.

**Interpretation**Our computational three-dimensional method permitted to evaluate spinal cord metabolism and volume and might represent a potential new window on the pathophysiology of amyotrophic lateral sclerosis.

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**Introduction**

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder of adult life characterized by a progressive loss of upper and lower motor neurons1. Survival usually averages 3–4 years from symptom onset2, however, lifespan can prolong up to more than 10 years in a small number of patients3. Due to this heterogeneous nature, a large research effort has been put forward to develop biomarkers able to objectively define disease aggressiveness and to complement the clinical evaluation4. In this line, several studies suggested a great potential for imaging techniques due to their capability to explore both motor cortex and spinal cord (SC) as disease targets documented at pathology5. More recently, brain imaging of 18F-fluorodeoxyglucose (FDG) uptake documented the relevance of inflammatory signals related to microglial activation or lymphocytes/macrophage infiltration6–10 as possible correlates of disease phenotype11. However, while presence and degree of functional alterations have been intensively assessed within the brain, a large uncertainty still exists about their extension in lower motor neurons and thus in the SC anterior horns.

To expand the functional evaluation to this district, we recently developed a computational approach able to automatically recognize the spinal canal and the SC using FDG-positron emission tomography (PET) co-registered with computed tomography (CT). In this study, we analysed the accuracy of this method and its feasibility in evaluating SC metabolism.

**Subjects and Methods**The study included 30 patients with spinal onset ALS (20 men, mean age 66±10 years, range 34-82) who signed the informed consent to enter the study that was approved by the Ethical Committees of IRCCS AOU San Martino-IST in Genova and of AUO Città della Salute e della Scienza in Torino, Italy. ALS diagnosis was defined according to the revised El-Escorial Criteria12. The Revised ALS Functional Rating 0-48 Scale (ALSFRS-R)13 was used to evaluate overall patients’ functional status at the study time.

Data obtained in patients were compared with 30 healthy controls without any history of neurodegenerative disease, randomly selected from a previously published normalcy database14 according to a case-control criterion considering, age, sex and used scanner.

*PET/CT acquisition*

All subjects were studied in the early morning after 12 hours fasting. Serum glycaemia was assessed as to ensure glucose level ≤2 g/l. A bolus injection of FDG was performed (4•8-5•2 MBq/kilogram of body weight) with patient lying in supine position in a quiet room and instructed not to move or talk. A 3D whole body scan (arms down position) started 60-75 minutes after tracer administration using an integrated PET/CT scanner (Hirez, Siemens Medical Solutions or Discovery GE Healthcare).

PET raw data were reconstructed by means of ordered subset expectation maximization (OSEM) and attenuation correction was performed using CT data. The entire CT dataset was co-registered with the 3-dimensional PET images using commercially available software interfaces. For each patient, ideal body weight (IBW) was calculated according to the conventional Robinson formulation15.

*Image analysis*The different spinal canal and SC districts were defined on anatomical basis considering cervical segment of the region between skull basis and the plane adjacent to the caudal face of C7 vertebral body. Dorsal segment was defined as the district included between this plane and the one adjacent to the caudal face of D12. Sacral and lumbar canal districts were *a priori* considered free from SC and thus excluded from the analysis.

Image analysis was performed according to a previously validated method16,17 based on a generalization of the Hough transform technique for pattern recognition18. According to the original definition, given a point $P=(x\_{P},y\_{P})$ in the image plane satisfying the equation of a straight line

$y=ax+b$ (1)

the Hough transform of *P,* with respect to the class of straight lines, is the straight line of equation

$y\_{P}=ax\_{P}+b$ (2)

into the parameter space where the two independent real parameters *a*, *b* vary19. This definition implies that all points on the straight line (1) in the image space correspond to straight lines in the parameter space that all intersect in the point (*a, b*) uniquely identifying the original straight line. This correspondence between the image and the parameter spaces holds not only for straight lines, but also for several classes of algebraic curves. This simple fact inspires the following pattern recognition algorithm for the identification of curves in a digital image:

1. Apply a traditional edge detection algorithm to extract discontinuities;
2. Compute the Hough transforms, with respect to the selected family of curves, of all points in the image plane highlighted by the edge detection process;
3. Discretize the parameter space into cells of appropriate dimension;
4. Construct an accumulator function defined on the discretized parameter space such that, for each cell, the value of the accumulator function is equal to the number of Hough transforms passing through that cell;
5. Search for the parameter values identifying the cell where the accumulator function reaches its maximum.

We applied this scheme to the recognition of both the spinal canal and the SC districts in whole body CT images of control subjects and ALS patients. Specifically, the family of curves with 3 convexities, represented by the equation

$C\_{a,b}: (x^{2}+y^{2})^{3}=(a(x^{2}+y^{2})-b(x^{3}-3xy^{2}))^{2},$ (3)

was particularly appropriate to optimally detect the spinal canal (Figure 1).

By contrast, the 4-parameter family of ellipses expressed in the form

$E\_{a,b,c,d}: b^{2}(x-c)^{2}+ a^{2}(y-d)^{2}-a^{2}b^{2}=0$ (4)

was the best candidate to identify the SC district.

For each CT slice, the two curves identifying the spinal canal and SC were used to create two sets of binary masks with zero outside and one inside each curve, respectively. These masks were multiplied against the co-registered PET slice in order to digitally extract the metabolic information represented as Standardized Uptake Value (SUV) of local FDG radioactivity20. Finally, average SUVs of both spinal canal and SC were divided by the corresponding average SUV value in liver, in order to account for possible differences in scanner sensitivity, thus obtaining the normalized SUVs (NSUVs). NSUVs of the whole SC were computed according to the formula

$SC\\_NSUV= \frac{\left(C\\_NSUV × cervical SC volume\right)+(D\\_NSUV × dorsal SC volume)}{cervical SC volume+dorsal SC volume}$ , (5)

where C\_NSUV and D\_NSUV indicate the averaged NSUV of cervical and dorsal SC segments, respectively.

*Statistical analysis*All data are reported as mean ± SD. Unpaired or paired t-tests were used, as appropriate. Linear regression analysis was performed using the least squares method. P values <0•05 were considered significant.

The 30 ASL patients were divided in two groups using the 5th decile NSUV (0•67). The survival experience of these 2 groups was described with the Kaplan-Meier method and compared with the log-rank test. To assess the prognostic relevance of SC\_NSUV, a set of univariate and multivariate Cox proportional hazard models were fitted to the data: in univariate analyses, death incidence was modelled as a function of each of the following variables: age, sex, time from ALS diagnosis to PET/CT scan, riluzole therapy, ALS functional score and average SC\_NSUV (below and above the 5th decile). Then, all 6 variables were tentatively included in a multivariate Cox’s model by means of a step-down (backward) procedure, based on the likelihood ratio test: variables with a p value <0•1 were removed from the model. Proportionality assumptions were assessed as previously described21.

**Results**

*Clinical characteristics of patient population*

Main clinical findings of ALS patients and of control subjects are reported in Table 1. According to the case-control selection criterion, age, sex, body weight and ideal body weight were similar in the two groups. ALSFRS-R score, updated for each patient at imaging date, ranged from 20 to 46/48.

The time elapsed from ALS onset and PET/CT scanning was 18±15 months (range 2-69) with a median value of 16 months. Subsequent follow-up lasted 1-36 months after imaging (median 14 months). During this period, 13 patients died from respiratory complications (Table 1).

*Shape and volume of spinal canal and spinal cord*

Spinal canal profile significantly changed across the whole stack of CT images; however, the Hough transform method allowed the curve with 3 convexities to automatically change its parameters and to optimally adapt itself to the profile. Differently from spinal canal, the shape of SC was characterized by a relative stability and was adequately fitted throughout cervical and dorsal segments by the ellipse equation (4). This formulation was not changed throughout spine districts and segmentation of the cord was obtained by adjusting numerical parameters.

As shown in Figure 2, the disease did not affect the anatomical features of spinal canal whose volume was similar in ALS patients and in control subjects both in cervical (32•08±7•12 mL vs 31•36±5•99 mL, respectively, p=ns) and in dorsal districts (65•60±10•09 mL vs 68•84±12•61 mL, respectively, p=ns) (Figure 2A-E).

A similar consideration also applied to the extracted SC volume: it was analogous in patients and control subjects both in cervical (13•99±1•42 mL vs 13•53±1•60 mL, respectively, p=ns) and in dorsal segments (32•60±3•22 mL vs 32•81±4•25 mL, respectively, p=ns) (figure 2B). As for spinal canal, ideal body weight was directly correlated with overall SC volume in control subjects although this relationship was relatively less evident in ALS patients (Figure 2F).

*Spinal Canal and Spinal Cord Metabolic Activity*

At visual inspection, radioactivity distribution within SC was relatively homogeneous without any focal area of enhanced uptake in both control subjects and ALS patients (Figure 3A-B). As shown in Figure 3C-E, average NSUV was not significantly different in the two populations in the whole spinal canal as well as in cervical and dorsal districts. As expected, this value was significantly lower with respect to corresponding SC\_NSUV in the same districts in both groups (Figure 3C). By contrast, a different pattern was observed when SC radioactive content was analysed. In fact, FDG uptake was higher in the SC of ALS patients with respect to controls. This difference reached the statistical significance for metabolic pattern in the whole SC (NSUV 0•82±0•28 vs 0•70±0•14 in ALS patients and in controls, respectively, p<0•05) and in cervical segment (NSUV 0•99±0•37 vs 0•85±0•20, respectively, p<0•05, Figure 3C-D). On the other hand, it did not reach the statistical significance when dorsal segment was analysed (NSUV 0•72±0•24 vs 0•62±0•18 in ALS patients and controls, respectively, p=0•08, Figure 3E).

Interestingly, effect of ALS on SC FDG accumulation was independent from demographic and clinical variables. In fact, SC \_NSUV did not correlate with age, sex, ALSFRS-R score, time elapsed from diagnosis to PET/CT or riluzole treatment (Figure 4).

*SC metabolic pattern and patient outcome*

The potential prognostic role of metabolic information was suggested by the observation that the 13 non-survivor patients showed a higher SC\_NSUV compared with the 17 survivors (0•71±0•26 vs 0•55±0•16, respectively, p<0•05) (Figure 4F). This effect was confirmed by Kaplan Meyer analysis, as the 16 patients with NSUV ≤5th decile showed a significantly higher mortality rate with respect to the remaining ones in the follow-up after PET/CT imaging (log-rank test, p<0•01, Figure 5).

At univariate analysis, the only predictive variable for overall survival was SC\_NSUV (Table 2). Multivariate analysis confirmed the independent prognostic role of SC metabolic pattern (HR = 24•3, 95% CI 2•2-262•8) although the strength of association should be verified in a large sample. No association with prognosis was observed for age, ALSFRS-R score, time elapsed from diagnosis to PET scanning or presence/absence of riluzole treatment, while a significantly increased risk of death was found for male gender (HR = 4•43, 95% CI: 0•87 - 22•5). Associations of borderline statistical significance were observed with age (p=0•055) and ALSFRS-R score (p=0•082) but no trend in risk with increasing or decreasing age or ALSFRS-R score was present.

**Discussion**

The present study represents the first attempt to estimate the metabolic pattern of spinal canal and SC from whole body PET/CT images. Obtained data indicate that this computational approach might represent a new window to explore SC status in different conditions besides its potential to complement the routine analysis of ALS patients.

*Recognition and measurement of spinal canal and spinal cord by CT analysis*

The estimation of the canal space throughout the whole spine was based on the assumption that compact bone is the structure with the highest X-ray attenuation coefficient among human tissues and can thus be easily identified and extracted in each CT slice. This approach is commonly used in commercially available software for 3D skeleton representation, while its potential has been previously validated in our lab to characterize intraosseous volume and bone marrow metabolic activity14,22-23. However, the heuristic definition of Hounsfield units is a relatively inaccurate method to recognize the spinal canal that is not directly surrounded by osseous tissue in all CT slices. To overcome this problem, we developed a pattern recognition method based on the Hough transform that allowed the identification of canal shape in the connection segments between the different vertebral bodies. This approach permitted to extract PET data and to analyse FDG uptake throughout the whole SC in a systematic fashion.

The most relevant feature of our algorithm is its fully deterministic nature with the user being asked to only identify the occipital skull border and the caudal face of D12. This plane was arbitrarily set as SC caudal edge, due to limited resolution of CT images that prevented the accurate evaluation of more distal segments. On the other hand, the operator-independent nature of this method virtually abolished the need of statistical analysis of its reproducibility measured either by inter-or intra-observer variability.

As a first validation step, we applied this method to a cohort of ALS patients. This model fitted with our purposes due to the pathological evidence of a significant damage to the SC neurons24. However, the potential of this computational approach to PET/CT images can be obviously extended to the evaluation of different conditions affecting SC function. In this line, the possibility to extract the spinal canal and its content might represent a useful tool to precisely evaluate the site of SC injury from whatever cause and mostly to improve the accuracy in monitoring its evolution. This approach will permit to overcome the limitations of visual inspection that so far hampered the evaluation of SC damage in patients with inflammatory diseases25, post-traumatic conditions26, cancer infiltration27 or autoimmune-autotoxicity disorders28,29.

*FDG and spinal cord involvement in ALS.*

In the present study, spinal onset ALS was associated with a slight, yet significant, increase in FDG uptake whose distribution was relatively inhomogeneous being most evident in SC districts located at the cervical spine segment. This finding at least partially conflicts with the expected reduction in tissue metabolic rate caused by the neuronal loss that has been described not only in the motor cortex but also in SC anterior horns of ALS patients24. Nevertheless, a large body of literature extensively documented that neuro-inflammation represents a key-signalling event in ALS11. This concept originated from pathology studies documenting the activation of microglia and astrocytes, as well as the presence of lymphocytes and macrophages in post-mortem tissue harvested from motor cortex and SC of both patients and experimental models of ALS2,7. As a common interpretation, these studies suggested that activated microglia might accumulate within the degenerating areas and might contribute to propagate and sustain the tissue damage through the release of free radicals and other neurotoxic substances such as glutamate28–30. More recently, this mechanism has been documented to also occur in the early disease phases. In fact, different studies documented inflammatory microglial activation in various cortical areas of ALS patients using PET imaging and different tracers targeting the translocator protein TSPO8,31,32. In this line, our observation of a relative increase in SC FDG uptake might extend to lower motor neurons the previously documented pattern of motor cortex damage in ALS33,34 and might reflect inflammatory mechanisms rather than the expected consequences of motor neuron loss and subsequent SC atrophy.

Obviously, FDG uptake cannot be considered per se a specific marker of microglia activation9,10. Nevertheless, the relevance of inflammatory mechanisms on ALS progression is supported by follow-up evaluation. In fact, Kaplan-Meyer analysis indicated that higher FDG uptake significantly predicted a higher mortality rate. Multivariate analysis confirmed this finding and documented the independent prognostic role of SC metabolism.

The observed difference in prognosis between patients with high and low FDG uptake was striking (HR=24). Nevertheless, the effective clinical potential of SC FDG uptake in outcome prediction cannot be assessed in the present study because of the limited number of patients, the retrospective nature of our evaluation and, mostly, the fact that this hypothesis was generated by our study and needs to be validated on independent datasets. A similar consideration also applies to the exclusion of patients with bulbar onset disease: this decision – justified by the focus of our computational algorithm on SC metabolism –prevents the capability to define the clinical value of this information.

Nevertheless, whether confirmed in larger prospective studies, the prognostic significance of SC metabolic pattern would indicate a relevant role for SC inflammatory response in the progression of ALS pathology. Also, this tool might be applied to other neurodegenerative or inflammatory diseases affecting SC function. The relevance of this consideration relies on the fact that spinal canal extraction can be obviously applied to PET/CT images obtained with other tracers such as those selectively targeting TSPO protein. Accordingly, the computational analysis of SC tracer uptake might permit to understand the role of SC microglia activation in ALS progression. The availability of this biomarker could be invaluable for the development of new therapeutic approaches, especially in early phase clinical trials in which current entry criteria only considering phenotype, disability severity and disease duration markedly hamper the correct identification of target patients.

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**Figure legends**

*Figure 1.* A: Detection of spinal canal and spinal cord at different heights of the vertebral column. The HT-based procedure with respect to the curve with 3 convexities allows the spinal canal identification (blue line), while spinal cord is detected by the HT-based procedure with respect to the ellipse (green line).

B: Variation of the 3-convexity curve graph with respect to different values of the parameter *b*. From left to right: *b = 0.03*; *b = 0.1*; *b = 0.27*. The parameter *a* is kept fixed to *0.5*.

C: From left to right: edge detection of a CT slice in the vertebral region; edge points inside the region bounded by the curve with 3 convexities; the curve with 3 convexities (blue line) and the ellipse (green line) detected by applying the Hough transform-based procedure.

*Figure 2.* A: Sagittal plane of CT whole body scan (left) and its corresponding counterpart representing extraction of cervical (red) and dorsal (green) spinal canal. B: Total spinal canal (solid columns) and spinal cord (dashed columns) volumes in the control population (CTR) and in the 30 ALS patients. The same representation is proposed for the cervical (C) and dorsal (D) segments. No differences could be observed between control subjects and ALS patients. Panel E represents the scatterplot and the regression lines of spinal canal volume as a function of ideal body weight. Panel F displays the same analysis for SC volume.

*Figure 3.* A: MIP projection of whole body CT scan co-registered with the extracted PET data within SC in a control subject (CTR); PET data alone for the corresponding SC displaying average SUV normalized for the corresponding liver value (average NSUV); pictorial representation of cervical and dorsal segments. B: The same as in A for an ALS patient. C: Average NSUV for the whole spinal canal (solid columns) and SC (dashed columns) in control subjects (CTR) and 30 ALS patients. FDG uptake was significantly lower in the SC of control patients (\*=p<0.05). This same difference occurred for cervical SC segments (D) while it did not reach the statistical significance in the dorsal one (E).

*Figure 4:* Scatterplots reporting the absence of a significant correlation between SC\_NSUV and patient age (A), time gap between diagnosis and imaging (C) and ALSFR-S (E). SC metabolic pattern was not significantly different between male and female patients (B); similarly, it was not significantly affected by riluzole treatment (D). By contrast, SC\_NSUV was higher (\*=p<0.05) in non survivors with respect to the remaining patients.

*Figure 5:* Kaplan Meyer curves representing overall survival in patients with SC\_NUSV > 5th decile (red) and in the remaining ones (green). High FDG uptake in the whole SC was associated with a higher mortality rate.