Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving upper and lower motor neurons, extra-motor neurons, microglia and astrocytes. The neurodegenerative process results in progressive muscle paralysis and even in cognitive impairment. Within the complex diagnostic work-up, positron emission tomography (PET) represents a valuable imaging tool in the assessment of patients with ALS. PET, by means of different radiotracers (i.e., $^{18}$F-fluorodeoxyglucose, $^{6}$-[F]$^{18}$F-fluoro-L-dopa, $[^{11}]$C-flumazenil) can assess the status of the wide range of brain regions and neural circuits, which can be affected by ALS. Furthermore, experimental radiocompounds have been developed for the evaluation of white matter, which plays a role in the progression of the disease. Here we present a comprehensive review including in different sections the most relevant PET studies: studies investigating ALS and ALS-mimicking conditions (especially primary lateral sclerosis and other neurodegenerative diseases), articles selecting specific subsets of patients (with bulbar or spinal onset), studies investigating patients with familial type of ALS, studies evaluating the role of the white matter in ALS and papers evaluating the diagnostic sensitivity of PET in ALS patients.

**KEY WORDS:** Amyotrophic lateral sclerosis - Positron-emission tomography - Bulbar - Spinal.

Amyotrophic lateral sclerosis (ALS), is a neurodegenerative process characterized by a progressive impairment of motor function at the bulbar and spinal levels with a not yet fully understood aetiology, resulting in progressive muscle paralysis. ALS involves motor neurons allocated in primary motor cortex, brainstem, and spinal cord, but also astrocytes and microglia, which contribute to the spread of the initial damage and disease progression. The neuropathological hallmark of ALS is, along with corticospinal tract degeneration, a selective loss of the anterior horns cells of the spinal cord and of cells in lower motor cranial nerve nuclei. In two-thirds of patients the onset is at the spinal level (with hypotrophy and weakness of upper or lower limbs muscles); in one-third of patients dysphagia and dysarthria are the first symptoms (bulbar onset). The latter group shows in a variable percent of cases a moderately to severe impairment in frontal lobe functions. The main clinical variants of ALS include Primary Muscular Atrophy (PMA), Primary Lateral Sclerosis (PLS), and Progressive Bulbar Palsy (PBP).

Diagnostic work-up is very complex and should include clinical history and examination, electrodiagnostic tests (such as conventional electromy-
ography, nerve conduction studies),\textsuperscript{9} neuroimaging including computed tomography (CT) or magnetic resonance imaging (MRI) of the brain and spinal cord myelogram,\textsuperscript{9, 10} clinical laboratory tests, muscle and/or nerve biopsy, and genetic testing.\textsuperscript{6}

Appropriate investigations are needed to distinguish ALS from its variants, especially PLS \textsuperscript{11, 12} and PMA \textsuperscript{13, 14} or from diseases as frontotemporal dementia (FTD) with similar cognitive symptomatology. Unrelated disorders, such as multifocal motor neuropathy, Kennedy’s disease or X-linked spinobulbar muscular atrophy, may present clinical features similar to ALS or its variants \textsuperscript{6, 15} and are considered to be responsible for diagnostic error in 5-10% of cases.\textsuperscript{16}

Combination of upper motor neuron (UMN) and lower motor neuron (LMN) impairment that cannot be explained by any other disease process by means of the diagnostic work-up, together with progression, is suggestive of ALS.\textsuperscript{15}

In the last decades, neuroimaging by Positron Emission Tomography (PET) has contributed to the knowledge of functional changes occurring in ALS in combination with both cognitive and motor impairment.\textsuperscript{17} Several studies on the use of PET in ALS have been published so far, mainly using \textsuperscript{18}F-fluorodeoxyglucose ([\textsuperscript{18}F]FDG-PET).\textsuperscript{18-21} Most imaging studies focusing on ALS used normal controls as a comparison group to highlight disease neuropathophysiology, which is essential to validate specific ALS related metabolic patterns.\textsuperscript{3, 16, 18, 20, 21, 25-27} Patients with ALS usually demonstrate at PET a diffusely decreased [\textsuperscript{18}F]FDG uptake in primary motor cortex, premotor cortices and supplementary motor areas, but eventually also in parietal and occipital lobes.\textsuperscript{26-28} Clusters of relative hypermetabolism have been demonstrated in the mesiotemporal regions but also in the occipital cortex, cerebellum, and upper brain stem of patients with ALS, compared to normal controls.\textsuperscript{20, 26, 27} All these findings reflect a complex neuropathophysiology of the disease involving degeneration of gray matter and areas of reactive microglial activation.\textsuperscript{29, 30}

We will review the scientific literature reporting PET studies in ALS patients discussing the findings in five separate sections: i) studies aiming to differentiate ALS from ALS-mimicking conditions; ii) studies focusing on ALS patients with spinal onset; iii) studies focusing on ALS patients with bulbar onset; iv) studies investigating the impact of white matter on the disease progression; v) papers evaluating the diagnostic sensitivity of PET in ALS patients.

PET studies in patients with ALS-mimicking conditions

[\textsuperscript{18}F]FDG-PET has not been used yet in clinical routine for the evaluation of patients with ALS, but has recently been proposed as a potential biomarker of the disease.\textsuperscript{26, 27}

Neurodegeneration in ALS involves also extra-motor cerebral neurons, including frontal and temporal lobes. Cognitive impairment in ALS shows clinical, pathological and genetic overlap with frontotemporal dementia (FTD).\textsuperscript{10} Abrahams et al.\textsuperscript{3, 18} assessed the cerebral blood flow (rCBF), during activation studies performed by word generation and word repetition (Table I). The authors demonstrated that ALS patients with cognitive impairment show reduced activation in cortical and subcortical regions involving the dorsolateral prefrontal, premotor, medial prefrontal, and insular cortices as well as the anterior thalamic nuclear complex. Conversely, nondemented ALS patients presented a relatively normal pattern of activation. These data are expression of an extramotor neuronal involvement in some nondemented ALS patients that develops probably along a thalamo-frontal association pathway.

Renard et al.\textsuperscript{31} investigated the [\textsuperscript{18}F]FDG-PET findings in 10 patients: 6 ALS and 4 ALS-FTD subjects. The authors reported hypometabolism in the prerolandic medial frontal and orbitofrontal cortices as well as in the anterior temporal lobe of the ALS patients. The anterolateral area was the most preserved part of the frontal lobe in ALS patients, compared to ALS-FTD patients. Conversely in the ALS-FTD subgroups, frontal and temporal regions were highly hypometabolic with relatively spared perirolandic metabolism. Hypometabolism resulted unrelated to the disease duration. No differences were evidenced at [\textsuperscript{18}F]FDG-PET regarding metabolism between patients with symptom onset in the limbs and patients with bulbar onset.\textsuperscript{31}

The group of Turku \textsuperscript{32} reported on a patient, in whom clinical evaluation, MRI and [\textsuperscript{18}F]FDG-PET failed to differentiate between FTD and Alzheimer’s disease (AD), whereas a subsequent \textsuperscript{[11]C}-Pittsburgh compound B ([\textsuperscript{11}C]PiB-PET) investigation was negative for amyloid pathology. The patient later developed ALS symptoms, and post-mortem neuropathological examination was diagnostic of FTD-ALS. Noteworthy, a genetic C9Orf72 expansion was detected. The authors suggested that [\textsuperscript{11}C]PiB-PET may facilitate the early differential diagnosis between AD and FTD, including AD versus C9Orf72-FTD/ALS. Further confirmation
Table I.—Summary of studies evaluating PET in patients with ALS-mimicking conditions.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Patients (N.=)</th>
<th>Controls (N.=)</th>
<th>Disease duration (months)</th>
<th>Reduced uptake</th>
<th>Increased uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pringle et al.</td>
<td>1992</td>
<td>3 PLS</td>
<td>NA</td>
<td>14±55.4</td>
<td>Pericentral cortex.</td>
<td>NA</td>
</tr>
<tr>
<td>Abrahams et al.</td>
<td>1996</td>
<td>6 ALSi</td>
<td>6 ALScu</td>
<td>ALSci: 22.7±15.0</td>
<td>ALS vs ALScu: increased binding in PFC, temporal and parietal cortices.</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 ALSu</td>
<td></td>
<td>ALS: 25.7±17.0</td>
<td>ALS vs HC: dorsolateral PFC, insular cortex and thalamus.</td>
<td>NA</td>
</tr>
<tr>
<td>Le Forestier et al.</td>
<td>2001</td>
<td>5 PLS</td>
<td>NA</td>
<td>91±239.6</td>
<td>Reduced CBF and BZR binding in PMC, prefrontal and ACC.</td>
<td>NA</td>
</tr>
<tr>
<td>Turner et al.</td>
<td>2007</td>
<td>4 PLS</td>
<td>24 ALS</td>
<td>PLS: 51±24</td>
<td>PLS vs HC: reduced BZR in PMC, temporal, parietal cortex and ACC.</td>
<td>NA</td>
</tr>
<tr>
<td>Renard et al.</td>
<td>2011</td>
<td>4ALS-FTD</td>
<td>NA</td>
<td>12-24</td>
<td>PFC, temporal and parietal cortex.</td>
<td>NA</td>
</tr>
<tr>
<td>Yamakawa et al.</td>
<td>2012</td>
<td>2 ALS-AD/FTD</td>
<td>NA</td>
<td>18±8.5</td>
<td>ALS-FTD: frontal and temporal cortex.</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ALS-AD: frontal, temporal and parietal cortex, PCC and precuneus.</td>
<td>NA</td>
</tr>
<tr>
<td>Martikainen et al.</td>
<td>2014</td>
<td>2 C9ORF72-ALS-FTD</td>
<td>NA</td>
<td>24</td>
<td>Frontal, temporal and parietal cortex.</td>
<td>NA</td>
</tr>
<tr>
<td>Van Laere et al.</td>
<td>2014</td>
<td>70 ALS</td>
<td>20 ALS</td>
<td>ALS: 15.2±10.7</td>
<td>PLS vs HC: reduced metabolism in PFC, ACC, pericentral cortex and thalamus.</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 PLS</td>
<td>PLS: 52.5±52.1</td>
<td>ALS vs PLS: more pronounced hypometabolism in ALS in PFC and PCC.</td>
<td>PLS vs HC: cerebellum and occipital cortex.</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 PMA</td>
<td>PMA: 12.5±5.7</td>
<td>ALS vs HC: more pronounced hypometabolism in ALS in sensorymotor cortex.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PLS: primary lateral sclerosis; NA: not available; ALSci: ALS patients with cognitive impairment; ALScu: ALS patients without cognitive impairment; PFC: prefrontal cortex; CBF: cerebral blood flow; BZR: benzodiazepine receptor; PMC: primary motor cortex; ACC: anterior cingulate cortex; HC: healthy control; FTD: frontotemporal dementia; AD: Alzheimer dementia; PCC: posterior cingulate cortex; PMA: primary muscular atrophy.

Regarding the utility of amyloid tracers has been given by Yamakawa et al., in a two-case study of patients with dementia (FTD and AD, respectively) associated with MND. In the patient with FTD, the [11C]PIB-PET was negative, while in the AD patient [11C]PIB-PET documented presence of amyloid accumulation.

Using [11C]-Flumazenil, binding to the subunit of the GABA_A receptor, Le Forestier et al. firstly described a reduction of the synaptic brain function and a reduced neuronal density in the primary motor and anterior cingulate cortex in patients with PLS and ALS. Since PLS and ALS have features in common in respect to the cortical dysfunction, PET can be a useful tool also in discriminating the two diseases. Even though [11C]-Flumazenil patterns are similar in ALS and PLS patients when compared to healthy subjects, differences can be demonstrated by a direct comparison between the [11C]-Flumazenil binding pattern of PLS subjects and both patients with sporadic or familial ALS (homozygous for the 'D90A' mutation). The differentiation, according to Pringle et al., is based on the fact that PLS patients show a more marked neurodegeneration than ALS patients of the neurons located in the primary motor cortex, with the exception of the foot area. Besides, patients with PLS demonstrate the additional involvement of the anterior cingulate gyrus. Lastly, in their large-cohort [18F]FDG-PET study, Van Laere et al. also described more severe hypometabolism in the prefrontal and posterior cingulate cortex of patients with ALS than in patients with PLS.

**PET studies on patients with spinal ALS**

Patients with spinal onset represent the most studied subgroup of ALS patients so far. 17, 20, 21, 26, 27, 36-38 Dalakas et al. 38 firstly indicated that hypometabo-
Table II.—Summary of studies evaluating PET in ALS patients with spinal onset.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Patients (N=)</th>
<th>Controls (N=)</th>
<th>Disease duration (months)</th>
<th>Reduced uptake</th>
<th>Increased uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalakas et al.</td>
<td>1987</td>
<td>12</td>
<td>11</td>
<td>12-45</td>
<td>Cerebral cortex and basal ganglia.</td>
<td>NA</td>
</tr>
<tr>
<td>Hatazawa et al.</td>
<td>1988</td>
<td>12</td>
<td>11</td>
<td>12-45</td>
<td>Motor-sensory cortex, extra-motor cortex and putamen.</td>
<td>NA</td>
</tr>
<tr>
<td>Ludolph et al.</td>
<td>1992</td>
<td>18</td>
<td>12</td>
<td>16.4±10.5</td>
<td>Entire cortex and regionally in frontal, frontobasal and superior occipital cortex.</td>
<td>NA</td>
</tr>
<tr>
<td>Cistaro et al.</td>
<td>2012</td>
<td>19</td>
<td>20</td>
<td>17.8±16.0</td>
<td>Occipital cortex.</td>
<td>Pons and midbrain.</td>
</tr>
<tr>
<td>Pagani et al.</td>
<td>2013</td>
<td>136</td>
<td>40</td>
<td>4.1</td>
<td>Premotor cortex, PFC and occipital cortex</td>
<td>Midbrain, cerebellum and temporal cortex</td>
</tr>
<tr>
<td>Cistaro et al.</td>
<td>2014</td>
<td>30</td>
<td>40</td>
<td>12.1±8.8</td>
<td>Frontal, superior temporal and insular cortex, ACC, PCC, caudate and thalamus.</td>
<td>Premotor cortex, post-cortical, temporal cortex, midbrain and occipital cortex.</td>
</tr>
<tr>
<td>Van Laere et al.</td>
<td>2014</td>
<td>48</td>
<td>20</td>
<td>15.2±10</td>
<td>PMC, PFC, lateral frontal cortex, ACC and thalamus.</td>
<td>Cerebellum, brain stem, occipital and medial temporal cortex.</td>
</tr>
</tbody>
</table>

Lism at [18F]FDG-PET imaging is a sign of a neuronal malfunctioning in ALS patients (Table II). Hypometabolism appeared to extend beyond the expected regions (primary motor cortex), including also the surrounding healthy neurons. Later studies corroborated the findings of Dalakas, demonstrating that PET abnormalities, besides affecting the primary motor cortex, involve an extensive range of brain regions including the frontal, parietal, and prefrontal regions and even occipital lobe. Hatazawa et al. showed a correlation between altered [18F]FDG metabolism and length of disease in ALS patients. A metabolic asymmetry between right and left lobe, but without preferential side, was observed in patients with ALS with upper and lower motor neuron involvement, whereas a normal or near normal [18F]FDG activity was noted in four ALS patients, who presented with only lower motor neuron involvement.

Another important hallmark of ALS is the correlation between hypometabolism at [18F]FDG-PET in motor cortex and the characteristic clinical features of the disease (progressive muscular weakness and tendon reflex changes). In this respect an inverse correlation has been observed between metabolism in middle frontal gyrus and severity of neurological signs.

Discrepant results have been reported between severity of hypometabolism and disease duration. While Hatazawa et al. reported the degree of hypometabolism in the motor-sensory cortex, assessed in a small group of ALS patients, to be correlated with the disease duration, other authors found no correlations. These contrasting findings may be related to the small sample of the investigated patients and controls.

Cistaro et al. described in 2012 the clinical and [18F]FDG-PET findings of 19 patients with spinal onset part of a group of 32 ALS patients. When compared to healthy controls, the subgroup with spinal onset demonstrated relative hypometabolism in small clusters in bilateral lingual and in right fusiform gyri and demonstrated less severe hypometabolic imaging pattern than patients with bulbar onset. Direct comparison between spinal and bulbar patients showed a less dramatic involvement of prefrontal, frontal and parietal regions in the former group suggesting a different metabolic state between the two conditions. Furthermore, relative hypermetabolism was described in the upper brain stem mainly in patient with spinal onset. Van Laere replicated the hypermetabolic findings in the posterior regions, including the cerebellum, occipital lobe, brain stem, and mesial temporal cortices.

Lastly, in 2014, further confirmation of this putative ALS metabolic pattern came from the study of Pagani et al., who recruited 136 patients with spinal ALS. The authors evidenced, as previously described in former studies, a relative hypermetabolism in bilateral midbrain, superior temporal gyrus, and right cerebellum of patients with spinal-onset as well as hypometabolic areas in bilateral primary and associative visual cortex and in bilateral prefrontal and premotor cortex.
PET studies on patients with bulbar ALS

In 1993, Kew et al.41 performed a 15O PET activation study aiming to measure the regional cerebral blood flow (rCBF) in 12 patients with ALS, 7 of them with bulbar onset, comparing them to 6 age-matched controls (Table III). Patients and controls underwent PET at rest and during execution of stereotyped and freely selected movements of a joystick. The ALS patient showed an increased activation in primary sensorimotor cortex during activation tasks, whereas decreased uptake was detected at rest in the sensorimotor and lateral premotor cortices, supplementary motor area, anterior cingulate cortex, paracentral lobule, superior and inferior parietal cortex. Noteworthy, greater activation was observed in ALS patients in the ventral third (face area) of the contralateral primary sensorimotor cortex and in the adjacent contralateral ventral premotor and parietal association regions during freely selected joystick movements. The magnitude of rCBF changes in the contralateral face area during activation was robustly correlated with the response time and the presence of spasticity. The authors argued that these parameters, time of response and conduction velocity along the central motor pathways, are partially dependent on conduction velocity along the central motor pathways. Being spasticity a clinical manifestation of damaged pathways, the relationship of these two parameters with rCBF changes in the face area suggests that a cortical adaptation to pyramidal tract disruption may be responsible for the recruitment of this area.41

Cistaro et al.20 also evaluated, among the 32 patients with ALS, 13 patients with bulbar onset comparing them to 19 spinal ALS patients and 22 controls. Patients with spinal onset showed higher scores in neuropsychological examination than patients with bulbar onset. Furthermore, relative hypermetabolism was described in the upper brain stem also in patients with bulbar onset rough less evident than in spinal patients. In ALS patients with bulbar onset, a significant relative decreased metabolism was found in large prefrontal, frontal and parietal regions as compared with patients with spinal onset, suggesting a differential neuropsychological and metabolic state between the two subgroups of patients. On the other hand, a normal or near normal frontoparietal [18F]FDG activity was observed in ALS patients with a spinal onset. Once the patients number was substantially increased,26 the bulbar subgroup (59 patients) showed, as compared to controls, severe metabolic deficit in motor, premotor, and prefrontal cortex. Such hypometabolic cluster was also found in the comparison with spinal patients, though less extended and with a lesser statistical significance. The severe hypometabolism in frontal and prefrontal regions was confirmed in another recent study in which 59 sporadic ALS patients were investigated (Figure 1) but where no differences in metabolism were found between patients with spinal and bulbar onset.27 These findings speak in favour of a different metabolic distribution in bulbar and spinal patients, stressing an association between frontal hypometabolism and the prodromal or full blown cognitive decline in the former group.

**PET studies in ALS patients carrying specific mutations**

In 2014, Cistaro et al.36 evaluated the [18F]FDG-PET pattern in 15 patients with familial ALS carrying the GGGGCC hexanucleotide repeat expansion in the

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**Table III.—Summary of studies evaluating PET in ALS patients with bulbar onset.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Patients (N=)</th>
<th>Controls (N=)</th>
<th>Disease duration (months)</th>
<th>Reduced uptake</th>
<th>Increased uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kew et al.41</td>
<td>1993</td>
<td>7</td>
<td>6</td>
<td>23</td>
<td>Sensorimotor, lateral premotor cortex, SMA, parietal cortex and ACC.</td>
<td>Upon activation: PMC, insula and ACC.</td>
</tr>
<tr>
<td>Cistaro et al.20</td>
<td>2012</td>
<td>13</td>
<td>20</td>
<td>17.8±16.0</td>
<td>Inferior, middle and superior frontal cortex, ACC and insula.</td>
<td>Amygdala, pons, midbrain.</td>
</tr>
<tr>
<td>Pagani et al.26</td>
<td>2013</td>
<td>59</td>
<td>40</td>
<td>4.1</td>
<td>PMC, premotor cortex and PFC.</td>
<td>Midbrain.</td>
</tr>
<tr>
<td>Van Laere et al.27</td>
<td>2014</td>
<td>21</td>
<td>20</td>
<td>15.2±10</td>
<td>PMC, PFC, lateral frontal cortex, ACC and thalamus.</td>
<td>Cerebellum, brain stem, occipital and medial temporal cortex.</td>
</tr>
</tbody>
</table>

SMA: supplementary motor area.
The C9Orf72 gene (Table IV). The authors compared the 15 patients with familial ALS with a group of 12 patients with ALS and comorbid FTD without the C9Orf72 expansion (ALS-FTD), with 30 cognitively normal ALS patients and with 40 normal controls. Among the 4 groups, patients carrying the C9Orf72 mutation showed the more severe involvement of the central nervous system, with significant hypometabolism in the anterior and posterior cingulate cortex, insula, caudate, cortices thalamus and the left frontal and superior temporal cortices, and hypometabolism in the midbrain, bilateral occipital cortex, globus pallidus, and left inferior temporal cortex.

In the same year Van Laere investigated the possible presence of specific metabolic signature at [18F]FDG-PET imaging in a large group (N.=81) of patients with suspected diagnosis of ALS. The diagnosis of pure ALS was made in seventy subjects, among which 11 carried the C9orf72 mutation. Whereas overall hypometabolism was evidenced in the periorbital region and with variable degree in the prefrontal cortex in the majority of patients, patients with C9orf72 mutation demonstrated greater relative hypometabolism in the thalamus, prefrontal and posterior cingulate cortices when compared with C9orf72-negative ALS patients.

Taken together these studies concordantly suggest a more severe central nervous system involvement in patients carrying the C9orf72 mutation as compared to both sporadic ALS and ALS-FTD patients underscoring the correlation between changes at [18F]FDG-PET and the severity of the clinical picture. Since genetic testing has become part of the diagnostic workup, also atypical cases beyond the common ALS phenotype have been identified. Adeli et al. reported on an atypical case of C9Orf72-FTD/ALS, with detailed longitudinal follow-up. The patient presented mild cognitive impairment which evolved into Alzheimer’s disease (AD) and later development of ALS, without behavioural or personality changes in his course, followed by motor neuron dysfunction about six years after the onset of cognitive dysfunction. The first PET study at presentation had showed mild hypometabolism in the medial frontal and lateral temporal cortices (with more prominent involvement of the left side), which became more severe during follow-up.

Previous studies have also dealt with ALS patients carrying mutation of SOD1, a gene encoding the enzyme copper, zinc superoxide dismutase protein (Cu/Zn-SOD). Cu/Zn-SOD, widely expressed in numerous subsets of cells of central and peripheral nervous systems, plays a fundamental role in protecting cells against reactive oxygen species, and mutated forms of the protein can result in apoptotic death of motor neuron cells. With regard to the evidence that spinal motor neurons and dopaminergic neurons are among the cells with the highest expression of Cu/Zn-SOD, Przedborski et al. postulated a subclinical nigrostriatal dysfunction in SOD1-mutated patients and investigated by 6-[18F] fluoro-L-dopa ([18F]FDOPA) PET 14 familial ALS (FALS) patients (7 with and 7 without SOD1-mutation). Despite the absence of statistically significant

Figure 1.—PET findings in 119 ALS patients compared with 30 controls. Statistically significant differences (P<0.05 FDR) are highlighted on a MRI T1 template. Hypermetabolism: left: sagittal view; center and right: coronal views. Significant corrected clusters are seen in midbrain, medial temporal lobes and cortisplian tracts.
differences between the two groups of patients regarding the striato-occipital ratios (SOR), five FALS patients showed abnormal $[^{18}F]$FDOPA uptake, confirmed at post-mortem by nigrostriatal degeneration. Two out of 5 patients with abnormal $[^{18}F]$FDOPA-PET imaging presented clinical features of Parkinson’s disease (bradykinesia), and 2/5 patients without clinical signs showed SOR values within the range of early Parkinson’s disease. Furthermore, the authors did not find any correlation between abnormal SOR values, age at onset or duration of symptoms. This last finding is in contrast with those of Takahashi et al. who found an inverse correlation between reduced $[^{18}F]$FDOPA uptake and duration of symptoms but in a population of sporadic ALS patients.

Among the several known SOD1 gene mutations, the ‘D90A’ mutation is associated with a slower progression than usual of the disease and a longer survival. Patients with D90A mutation seem to show a different pattern of cortical neuronal loss or dysfunction at $[^{11}C]$flumazenil PET imaging, compared to patients with sporadic ALS (premotor and motor cortices and posterior motor associative areas). Turner detected a decreased uptake in the frontal lobe of the dominant hemisphere and in anterior cingulate gyrus in 10 symptomatic patients carrying D90A mutation and a smaller cluster of decreased of $[^{11}C]$flumazenil binding at the left-fronto-temporal junction, which he named “the clinical horizon”, possibly reflecting cortical modifications appearing before the onset of the clinical signs. There is evidence that patients with sporadic ALS demonstrate a relative reduced cortical inhibition at $[^{11}C]$flumazenil-PET a detectable in motor and motor associative areas, compared to patients with D90A mutation supporting the role of excitotoxic pathogenic mechanisms in determining the different survival between the two groups. The diverse cortical vulnerability of D90A-mutated patients was also explored in a comparative study with a small group of PLS patients, highlighting a relative decreased $[^{11}C]$flumazenil binding pattern in the anterior and orbital-frontal regions of D90A-mutated patients.

Studies on the impact of white matter and neuroinflammation on the evolution of ALS disease

There are many data in literature suggesting an active role of white matter in the progression of ALS disease. Some authors have postulated that the increased number of astrocytes found in ALS brain may be due to the colonization of the empty spaces
left by the dead neurons,\textsuperscript{22, 52} whereas a few studies have showed a link between increased astrocytosis in areas of neurodegeneration in ALS patients and disease progression.\textsuperscript{19, 53}

These findings are in agreement with the evidence that glucose is not consumed only by neurons and that its uptake therefore does not exclusively reflect neural activity. Conversely astrocytes also play an important role in the regulation of glucose utilization being the major \textsuperscript{18}F]FDG uptake determinant in the astrocyte-neuron functional unit.\textsuperscript{54, 55}

Turner \textit{et al.}\textsuperscript{23} performed a study in 10 patients with ALS and demonstrated an extensive microglial activation by means of \textsuperscript{11}C-(R)-PK11195, a ligand for the \textsuperscript{18}kDa translocator protein (TSPO) (formerly known as the peripheral benzodiazepine binding site), expressed on the mitochondrial membrane of activated microglia (Table V). Uptake was found in the motor cortex, pons and thalamus, and appeared to be related with the burden of upper motor neuron clinical signs.\textsuperscript{23} The group of Johansson provided evidence that astrocytosis may be depicted \textit{in vivo} in ALS patients and highlighted the utility of \textsuperscript{11}C-(L)-deprenyl-D2 (DED), targeting the enzyme MAO-B, primarily located in astrocytes.\textsuperscript{56} The authors documented uptake of DED in pons and white matter in 7 ALS patients and emphasized the need of future studies combining \textsuperscript{[11]}C(R)-PK11195 and \textsuperscript{[11]}CDED PET to clarify the spatial and temporal relationship between microglial activation and astrocytosis in ALS.

A further study with a glial tracer was performed by Corcia \textit{et al.}\textsuperscript{57} who evaluated neuroinflammation in ALS patients using \textsuperscript{[18]F}-DPA-714, a more recently developed TSPO radioligand with high signal-to-noise ratio. A significant increase of distribution volume ratio values, corresponding to microglial activation, was shown in the primary and supplementary motor and temporal cortices, thus suggesting the use of this tracer as a promising biomarker of neuroinflammation.\textsuperscript{57}

Table V.—Summary of PET studies evaluating white matter in ALS patients.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Patients (N.=)</th>
<th>Controls (N.=)</th>
<th>Disease duration (months)</th>
<th>Reduced uptake</th>
<th>Increased uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turner \textit{et al.}\textsuperscript{23}</td>
<td>2004</td>
<td>10</td>
<td>14</td>
<td>25.3±11.6</td>
<td>NA</td>
<td>PMC, pons, dorsolateral PFC, thalamus.</td>
</tr>
<tr>
<td>Johansson \textit{et al.}\textsuperscript{56}</td>
<td>2007</td>
<td>7</td>
<td>7</td>
<td>27.6±13.6</td>
<td>NA</td>
<td>Pons and WM.</td>
</tr>
<tr>
<td>Corcia \textit{et al.}\textsuperscript{57}</td>
<td>2012</td>
<td>10</td>
<td>7</td>
<td>11.7; 5-27</td>
<td>NA</td>
<td>PMC, SMA and temporal lobe.</td>
</tr>
</tbody>
</table>

WM: white matter.

Figure 2.—Three-dimensional rendering showing those regions in which \textsuperscript{[18]F]FDG uptake was significantly higher in CTR (N.=20) as compared with ALS patients (N.=59) (threshold P<0.0001\textsubscript{uncorrected}). Top row left: medial left view; top row right: medial right view; second row left: posterior view; second row right: frontal view; third row left: right-side view; third row right: left-side view; bottom row left: view from below; bottom row right: view from above.
On the other hand, both Cistaro et al.\(^{20}\) and Pagani et al.\(^{26}\) speculated that the hypermetabolism found in midbrain and corticospinal tracts (Figure 2) might be due to astrocytosis resulting in a relatively higher glucose uptake as compared to the normal condition in which the amount of astrocytes in such two structures is very limited.

**Diagnostic sensitivity analyses**

As in other neurodegenerative disease,\(^{58}\) the use of machine learning tools is rapidly increasing, in order to differentiate ALS from mimicking syndromes. Machine learning identifies complex patterns automatically and may help nuclear medicine physicians make intelligent decisions on the analysis of PET images, which may be combined with other information (structural, clinical, genetic), also known as radiomics.\(^{59}\)

In 2010, Filippini et al. used in a MRI study discriminant analysis, combining radial diffusivity, fractional anisotropy and voxel-based morphometry, in a group of ALS patients reporting 92% sensitivity, 88% specificity, and 90% accuracy.\(^{59}\)

Only two studies analyzed the diagnostic performance of PET in ALS patients due to the rarity of disease and the small sample in most studies.

Van Laere et al.\(^{27}\) evaluated the diagnostic ability of \(^{18}\)F-FDG-PET in a large cohort of 81 patients with suspected diagnosis of ALS. Without a priori knowledge PET correctly classified 100% of controls, 89% of ALS patients and 71% patients with PLS.\(^{27}\) A direct comparison between ALS and PLS, using a support vector machine approach and 88 VOIs, showed a high accuracy (89.7%), the prefrontal cortex, thalamus, posterior cingulate, and anterior cingulate being the most discriminating regions.

Recently Pagani, et al.\(^{26}\) reported for \(^{18}\)F-FDG-PET implementing the generalized linear discriminant model a sensitivity of 95% and a specificity of 83% in discriminating ALS patients from healthy controls in the largest study published so far, including 195 ALS subjects and 40 controls. The study analyses were base on 51 cortical and subcortical predefined volumes of interest and documented a mixed hypermetabolic-hypometabolic pattern, whereby marked hypometabolism was demonstrated in frontal, pre-motor, and occipital cortices, whereas relative hypermetabolism was described in the midbrain, superior temporal gyrus and hippocampus.

**Future perspectives and conclusions**

Despite the lack of multicentre studies to evaluate the utility of neuroimaging in differentiating ALS from ALS-mimicking conditions, an appropriate assessment of sensitivity and specificity of \(^{18}\)F-FDG-PET is now possible due to the large patients cohorts recruited by some of the centres involved in ALS research.\(^{20, 27}\) A high accuracy in diagnosing ALS patients and possibly discriminating them from ALS-mimicking diseases patients will be essential to recruit appropriate subjects for clinical trials, to develop new therapies and to identify possible disease familiarity allowing early interventions.

Functional imaging is likely to increment in the next future its role in the evaluation of patients affected by ALS and probably \(^{18}\)F-FDG-PET/CT will continue to be an imaging tool of outstanding importance in the evaluation of ALS in the next decade. Last but not least, the introduction of PET/MRI scanners in clinical setting would contribute to better understand the ALS pathophysiology combining morphological and functional information, which may also include novel markers for neuroinflammation or effects on neurotransmission.\(^{61}\)

**References**


Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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