

Neuroimaging in primary lateral sclerosis

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Neuroimaging in Primary Lateral Sclerosis

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Abstract

Increased interest in the underlying pathogenesis of primary lateral sclerosis (PLS) and its relationship to amyotrophic lateral sclerosis (ALS) has corresponded to a growing number of CNS imaging studies, especially in the past decade. Both its rarity and uncertainty of definite diagnosis prior to four years from symptom onset have resulted in PLS being less studied than ALS. In this review, we highlight most relevant papers applying magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), and positron emission tomography (PET) to analysing CNS changes in PLS, often in relation to ALS. In patients with PLS, mostly brain, but also spinal cord has~~ve~~ been evaluated since significant neurodegeneration is essentially restricted to upper motor neuron (UMN) structures and related pathways. Abnormalities of cortex and subcortical white matter tracts have been identified by structural and functional MRI and MRS studies, while metabolic and cell-specific changes in PLS brain have been revealed using various PET radiotracers. Future neuroimaging studies will continue to explore the interface between the PLS-ALS continuum, identify more changes unique to PLS, apply novel MRI and MRS sequences showing greater structural and neurochemical detail, as well as expand the repertoire of PET radiotracers that reveal various cellular pathologies. Neuroimaging has the potential to play an important role in the evaluation of novel therapies for patients with PLS.

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1 Introduction

Despite the relatively low incidence of primary lateral sclerosis (PLS), a number of dedicated imaging studies have been undertaken in recent years to characterise its unique structural, functional, and metabolic signatures. The clinically pure upper motor neuron (UMN) nature of PLS lends itself to *in vivo* structural and functional studies made possible through developments in CNS imaging. Regarded as an extreme end of a continuum of amyotrophic lateral sclerosis (ALS), the much more protracted clinical course of UMN degeneration in PLS contributes to its most recognized neuroimaging changes in the primary motor cortex (PMC, or precentral gyri). These include: (a) focal atrophy and corticospinal and callosal white matter tract degeneration, as revealed by magnetic resonance imaging (MRI), and (b) cortical hypometabolism, neuronal receptor changes, and neurogliosis, as revealed by various positron emission tomography (PET) radioligands. Early imaging studies in PLS were primarily used, as in ALS, to rule out alternative causes of pure UMN dysfunction at brain or spinal cord levels. However, as MRI and PET technologies have advanced over the years, studies began examining deeper aspects of pathogenesis in PLS.

A formal literature review using PubMed was conducted of PLS and keywords related to MRI and PET in September 2020 in accordance with the PRISMA guidelines. The primary search terms of 'PLS', 'motor neuron disease', 'primary lateral sclerosis' were individually paired with 'neuroimaging', 'imaging', 'radiology', 'MRI', 'magnetic resonance imaging', 'PET', 'SPECT', 'morphometry', 'cortical thickness', 'DTI', 'spectroscopy', 'fMRI'. Only articles published in English were reviewed, but both single case reports and case series were considered. Identified papers were systematically reviewed for sample size, control groups, primary imaging modality and main study findings. A total of 117 PLS imaging and related papers were reviewed.

It is noteworthy that imaging studies in PLS over the years have used differing criteria that vary in symptom duration required to establish diagnosis. The 1945 PLS diagnostic criteria (1) suggested a minimum of 5 year symptom duration, while the 1992 Pringle criteria

(2) proposed a minimum symptom duration of 3 years for reliable diagnosis. The 2006 Gordon criteria (3) advocated for a symptom duration of 4 years to establish the diagnosis. Finally, recent 2020 consensus diagnostic criteria (4), recognising the implications of diagnostic delay, introduced a category of 'probable PLS' for patients with isolated UMN symptoms for 2-4 years. Existing imaging studies have primarily utilized the Pringle or Gordon criteria to select patients, although recent reports demonstrate the rationale for the new 2020 consensus criteria by finding that 'probable PLS' patients already exhibit radiological changes consistent with PLS of longer symptom duration (5,6).

This review sought to identify key papers that have contributed to the characterisation of PLS-associated pathology *in vivo* using MRI and PET. ~~Publications using quantitative protocols and well-characterised patient cohorts were highlighted, but multiple case reports and case series were also identified. Identified papers were individually reviewed and annotated for cohort numbers, study design, imaging methods, anatomical focus, clinical or pathological correlations.~~ Future neuroimaging research directions in PLS can be defined based on ALS-imaging studies, which have presented viable methodological, conceptual or technological frameworks not yet been applied to PLS cohorts. While several recent PLS imaging studies have been published that discussed findings in varying detail (7–9), the focus of this paper is to provide an overview of the contribution of imaging to field of PLS research and discuss potential future clinical applications.

MRI in PLS

Background

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1 MRI protocols enable the quantitative appraisal of grey and white matter integrity in
2 comparative and correlative statistical models, and are uniquely useful in the broader
3 spectrum of motor neuron disease (MND) where slowly progressive neurodegenerative
4 changes take place (10). The practical appeal of MRI-based imaging in MND includes non-
5 invasive data acquisition, cost effectiveness, data interpretation at both individual and group
6 levels (11), the widespread availability of MRI scanners (12), the ability to distinguish MND
7 phenotypes (13,14)~~30/03/2020 12:53:00~~, the opportunity to track longitudinal changes
8 (15,16), existing protocols for data harmonisation (17), and the availability of large
9 international data repositories (18). The majority of MRI studies in MND however focus on
10 ALS (19), and relatively few MRI studies have been dedicated to describe PLS-associated
11 imaging changes (8). The technological, methodological, and logistical lessons of ALS
12 imaging are directly transferrable to PLS and many of the techniques pioneered in ALS can
13 be readily applied to PLS cohorts.

14 **Diagnosis of PLS is notoriously difficult as it may initially resemble any one of several**
15 **conditions causing progressive UMN dysfunction or degeneration, including hereditary**
16 **spastic paraparesis (HSP), corticobasal degeneration, multiple sclerosis, etc. Because HSP**
17 **often presents clinically like PLS, a detailed family history is obtained and HSP-associated**
18 **mutations are frequently screened. In a clinical setting, routine spinal cord and brain imaging**
19 **is performed and qualitatively evaluated to rule out structural abnormalities (e.g.,**
20 **compressive myelopathy, neoplasm), or inflammatory conditions (e.g., multiple sclerosis,**
21 **transverse myelitis).**

22
23 **Case reports and case series**

24 A number of case reports and small case series preceded the publication of large
25 prospective MRI studies in PLS. Focal atrophy of pericentral gyri in PLS brain is often visible
26 macroscopically (Figure 1), and may even have a ‘knife edge’ appearance of regional gyri

(2,20). Long disease duration of PLS may not be the only explanation for the prominent cortical atrophy because it is unusual to observe in even advanced longstanding cases of ALS. Furthermore, significant atrophy in PLS, including of underlying white matter, has also been noted to extend more anteriorly (21,22), with MRI changes sometimes linked to cognitive impairment (23,24).

Qualitative findings of corticospinal tract (CST) hyperintensities in brain (25) as well as spinal cord (26) have been observed on T2- (18, 19), and FLAIR-weighted images (27). Such intracranial CST hyperintensity occasionally seen in PLS can resemble a “wine glass” when viewed coronally at the diencephalic level (28), although this and its “funnel”-like appearance when viewed sagittally are more frequently seen in UMN-predominant ALS (Figure 2) (29). Case reports of single PLS patients have also demonstrated CST reductions in fractional anisotropy on diffusion tensor imaging (DTI) (30,31), white matter degeneration (32), and progressive extramotor atrophy (20). However, these qualitative changes are seen in only some cases of PLS and also can occur in ALS (8).

Quantitative cross-sectional and longitudinal MRI studies in PLS

Over 50 quantitative imaging studies have been published in PLS (8), seven of which had a longitudinal design (33). The cohort sizes of quantitative imaging studies in PLS range from 2 to 33 (9) in single-centre single-protocol studies, single-centre multiprotocol studies that included up to 50 PLS patients, (34), and multicentre studies that included up to 88 PLS patients (35). The majority of PLS imaging studies are MRI studies which typically present morphometric and diffusion tensor imaging analyses. Morphometric structural analyses of PLS brain invariably capture precentral gyrus atrophy (36,37), PMC surface area reductions (21,22) and focal PMC thinning (9,38,39), although subcortical grey matter degeneration has also been reported (40). Existing imaging studies in PLS describe primarily group-level changes. Despite the academic relevance of identifying PLS-associated imaging signatures

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1 from a cohort of patients, the clinical utility of such observations is limited on an individual
2 patient level.

3 HSP also presents similarly clinically, and suspected PLS patients are typically
4 screened for an extensive panel of HSP-associated mutation after a detailed family history is
5 taken. Clinical scans aimed at out ruling structural, neoplastic and inflammatory conditions
6 are typically qualitatively evaluated. TheA goal aspiration of quantitative neuroimaging,
7 particularly if high resolution 3D volumetric 3D-MR data sets are acquired, (T1w/DTI) without
8 slice gaps is- the accurately categorisingation- of individual patients into diagnostic and
9 prognostic categories. Initiatives-Interpreting of individual patient data interpretation have
10 been successfully undertaken in ALS has been reported susing various a variety of machine
11 learning approaches (11,41–45), but these approaches have not yet been applied to in PLS
12 because as very large reference data sets are presently lacking needed to build and validate
13 classification models. Once such reference datasets are available for PLS patients, their
14 images may also be interpreted at an individual level.

15
16 **Extramotor and subcortical MRI studies in PLS**

17 Reports on extra-motor cortical involvement in PLS are inconsistent and range from
18 limited regional pathology to widespread parietal, prefrontal, cerebellar and brainstem
19 degeneration (9,46,47). Over 20 diffusion tensor imaging studies have been published in
20 PLS which consistently capture CST pathology (30–32), but cerebellar (9,48), and corpus
21 callosum (37,49) pathologies have also been described. Some studies have specifically
22 highlighted extra-motor, extra-corpus callosum diffusivity alterations involving the superior
23 and inferior longitudinal fasciculi, fornix, thalamic radiations, and parietal lobes (23,24).

24 Recent imaging studies in PLS focusing on subcortical grey matter degeneration
25 have found that certain structures such as the amygdala may be preferentially affected in
26 ALS but less likely to be affected in PLS (50). However, other subcortical structures like the

thalamus are not only atrophic in PLS (51) but thalamic motor and sensory nuclei are selectively affected in PLS (52,53).

~~Reports on extra-motor cortical involvement in PLS are inconsistent and range from limited regional pathology to widespread parietal, prefrontal, cerebellar and brainstem degeneration (27,35,36). Over 20 diffusion tensor imaging studies have been published in PLS which consistently capture CST pathology (23–25), but cerebellar (27,37), and corpus callosum (31,38) pathologies have also been described. Some studies have specifically highlighted extra-motor, extra-corpus callosum diffusivity alterations involving the superior and inferior longitudinal fasciculi, fornix, thalamic radiations, and parietal lobes (16,17).~~

Specialized magnetic resonance studies in PLS

A smaller proportion of MRI studies in PLS report spectroscopy and functional MRI findings. Proton MR spectroscopy (^1H -MRS) studies of PMC of PLS patients, have reported reduced *N*-acetyl aspartate (NAA)/creatine (Cr) ratios (54–56) and increased *myo*-inositol/Cr ratios (57). These findings are consistent with neuronal dysfunction or loss, and gliosis, respectively. Unlike in ALS, however (58,59), whole-brain multi-voxel MRS techniques have not yet been applied to PLS cohorts (60). The drawback of hypothesis-driven volume of interest (VOI) spectroscopy is that extra-motor metabolic changes cannot be systematically evaluated unless using multivoxel spectroscopic imaging (61). Functional MRI (fMRI) studies reported increased functional connectivity in PLS (62,63), which similarly to ALS studies (64) were interpreted as an adaptive, compensatory process. While spinal cord hyperintensities along the pyramidal tracts are rarely observed in PLS (26), and considerable methodological advances are occurring in spinal imaging in ALS (45,65,66), no robust prospective cord imaging studies have yet been undertaken in PLS.

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Challenges and limitations of MRI studies in PLS

The majority of PLS imaging studies are primarily descriptive because clinico-radiological correlations in MND are often regarded to be contentious (67). Nonetheless, clinical scores have been correlated to grey matter volume reductions (21,36), PMC measures (38,68,69), white matter indices (9,21,70), proton-density alterations (71), and NAA reductions (56,57,72). Disease duration is thought to correlate with imaging measures by some (73) but not all groups (38,49,74). Functional disability in ALS results from concomitant upper and lower motor degeneration rendering correlations analyses between cerebral measures and functional scales controversial. Due to its selective UMN degeneration, PLS is the ideal condition to study the imaging correlates of motor impairment. Functional scale scores, upper- and lower limb tapping rates have been recently linked to white matter degeneration of the corona radiata in a somatotopic pattern (9). With the recent publication of revised consensus diagnostic criteria for PLS (4), and the development of a PLS specific functional rating scale (75), future imaging studies are likely to capture more meaningful clinikoradiologic correlations.

Compared to ALS (15,16), there is a striking lack of longitudinal imaging studies in PLS (76). Existing longitudinal studies in PLS suffer from cohort size limitations, typically being two time-point designs, and varying considerably in follow-up intervals (55,73,74,77). The longitudinal analysis of 12 PLS patients (69) detected no progressive CST changes over time. Interestingly, a study of eight pre-PLS patients who initially did not fulfil diagnostic criteria exhibited progressive precentral gyrus thinning and increasing functional connectivity (78). Other studies of suspected PLS patients showing connectivity and grey/white matter abnormalities before meeting diagnostic criteria (37,78,79) suggested that protracted symptom duration in current diagnostic criteria of PLS does not alone influence underlying pathological changes. The majority of PLS imaging studies use healthy controls or ALS patients (16,21,37,57,68,70,79–81) to describe PLS imaging signatures, and only few rely on alternative neurodegenerative controls such as cohorts of HSP (49,82) or FTD (46) patients.

One of the problems of comparing ALS and PLS cohorts is the considerable differences in symptom duration. Until recently (4), previous diagnostic criteria of PLS required symptom duration of 4 years (3), whereas in ALS symptom duration of 4 years is typically associated with considerable disability (83). Accordingly, studies contrasting established MND patients inevitably compare cohorts of PLS patients with disproportionately longer symptom duration than the participating ALS patients. This may explain reports of lower cortical thickness (16,21,81) and precentral gyrus NAA levels (57) in PLS compared ALS. Other studies have opted to control for symptom duration differences in their statistical models (9). Differences in statistical approaches, imaging modalities, choice of control groups and study designs preclude a conclusive view whether PLS has a unique imaging signature distinct from ALS.

It is clear that precentral gyrus, corpus callosum, CST, cerebellar, brainstem and basal ganglia involvement are shared features of both PLS (34,38,39,46,68,70,82) and ALS (71–76). Some studies (9) suggest the preferential involvement of the splenium of the corpus callosum in PLS in contrast to ALS where the fibres of the forceps minor in the genu are more affected.

PET in PLS

Background

Although many molecular imaging studies using PET in patients with ALS have been published using various radiolabelled ligands (for review, see (84–86)), relatively few PET studies exist in PLS. Accordingly, individual studies will be highlighted. We will not discuss single-photon emission computed tomography (SPECT) studies except to mention a single case report in a patient with PLS of reduced signal in both frontal lobe pericentral areas (87).

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[¹⁸F]-FDG PET studies in PLS

[¹⁸F]-fluoro-2-deoxy-D-glucose PET ([¹⁸F]-FDG PET), which assesses glucose metabolism by neurons and neuroglia, has been the most commonly used molecular imaging modality in ALS and PLS. One of the earliest [¹⁸F]-FDG PET reports of brain changes in PLS appeared in a book chapter in 1990 (88) and subsequently part of the seminal paper by Pringle and colleagues (2) of clinical features, neuropathology, and diagnostic criteria in eight PLS patients. PET scans, which were performed in only three patients, detected reduced glucose uptake in the pericentral cortex of two with the most prominent clinical features of PLS; PET findings in the one with reportedly mild signs could not be distinguished from normal. Nonetheless, their proposed diagnostic criteria included “decreased glucose consumption in pericentral region on PET scan” under findings “additionally suggestive of PLS”. Subsequent PET studies in PLS have all included less than double-digit patient numbers until recently.

When [¹⁸F]-FDG hypometabolism is restricted to the PMC, it can be very conspicuous, and has been termed the “stripe sign”. First reported in 3 patients with PLS who had spastic dysarthria and varying degrees of lower or upper limb spasticity, hypometabolism was particularly prominent along the hemisphere’s lateral convexity in one patient (89). Too few patients with similar distributions of spasticity were studied to determine whether somatotopic (homuncular) representation of the pericentral hypometabolism occurred, although the one patient with only unilateral arm spasticity had a more prominent stripe sign in the appropriate contralateral hemisphere. Interestingly, a case report of a PLS patient with only upper limb spasticity and normal lower limb exam revealed bilateral pericentral [¹⁸F]-FDG PET hypometabolism that relatively spared parasagittal PMC where lumbosacral spinal cord-projecting corticomotoneurons would be expected to reside (90) (Figure 3).

The ability for [^{18}F]-FDG PET to distinguish PLS and ALS brains based on their metabolic signatures is poor. An earlier report of 7 PLS patients compared to 70 with ALS (comparing both to 20 healthy controls) suggested that hypometabolism was slightly more prominent in prefrontal cortex and posterior cingulate cortex of ALS patients (91). However, a subsequent prospective study by the same group with a new cohort of patients using volume of interest (VOI)-based discriminant analysis found no differences between PLS ($n=10$) and ALS ($n=105$) patient groups (92). Although both groups showed the most prominent hypometabolism in prefrontal and premotor cortices, as well as hypermetabolism in medial temporal cortex, cerebellum, and upper brainstem compared to 20 healthy controls, 9 of 10 PLS patients could not be distinguished from those with ALS. The authors' final consensus was that [^{18}F]-FDG PET cannot differentiate between brain metabolic signal changes in PLS and ALS patients (92). Recent advances in metabolic imaging suggest that combined cord and brain [^{18}F]-FDG PET may differentiate ALS from PLS (93).

Mills syndrome is a very rare form of UMN degeneration characterised by slowly progressive ascending hemiparesis and ipsilateral spasticity (94) that may be considered clinically as a unilateral variant of PLS (95). Two [^{18}F]-FDG PET studies of patients with clinical features consistent with Mills syndrome showed differences in extent of hemispheric hypometabolism. The first of a 63 year-old male with 10 years of slowly progressive left body hemiparesis and spasticity revealed significant hypometabolism in motor and premotor regions of both hemispheres, compared to 45 healthy controls, although slightly worse on the right (96). In contrast, the other of 3 females with right body pure UMN signs showed significantly reduced [^{18}F]-FDG PET binding in the PMC and adjacent areas in only the left hemisphere (92). Such variability in concordance of hemispheric hypometabolism with clinical UMN deficits supports the suggestion that Mills syndrome is useful only as a descriptive clinical term (97).

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6 2 **Ligand PET studies in PLS**
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9 3 ***Neuronal GABA_A receptor alterations in PLS brain***
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12 4 [11C]-flumazenil binds to the benzodiazepine receptor subunit of the GABA_A receptor
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14 5 (R) localized to brain neurons. A study of 9 PLS patients included [11C]-flumazenil PET at a
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16 6 single time-point in 5 patients alongside longitudinal clinical and electrophysiologic
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18 7 evaluations (98). PET measurement of regional cerebral blood flow (rCBF), an index of
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20 8 synaptic brain function, was reduced in the precentral gyrus (fronto-opercular region),
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22 9 ventrolateral prefrontal region, and anterior cingulate cortex. GABA_A-R density, a potential
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24 10 surrogate for neuronal cell body and proximal dendrite integrity, was not as diffusely
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26 11 decreased but localized within the foci of rCBF reduction. Whether reduced [11C]-flumazenil
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28 12 binding represents neuronal loss, changes in GABA_A-R functional properties, or both, is
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30 13 uncertain. However, this study confirmed the motor system dysfunction in PLS is similar to
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32 14 that in ALS, at least for rCBF.
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36 15 A subsequent study with [11C]-flumazenil PET compared 4 PLS patients with ALS
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38 16 patients due to either sporadic disease (*n*=24) or a homozygous SOD1 (D90A) mutation
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40 17 (*n*=10) resulting in slowly progressive UMN-predominant disease (*n*=10), and controls
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42 18 (*n*=24) (99). All patient groups (relative to controls) displayed significantly decreased [11C]-
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44 19 flumazenil PET signal in bilateral motor cortices and the right parietal lobe. Radioligand
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46 20 binding was significantly lower in both ALS patient groups (especially homD90A SOD1 after
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48 21 cluster correction, *P*<0.05) than in PLS patients in bilateral anterior frontal and orbitofrontal
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50 22 regions. Only PLS patients showed additional [11C]-flumazenil binding reductions in bilateral
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52 23 anterior cingulate gyri and the left superior temporal lobe. Although the significance of this
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54 24 regional involvement is unclear in PLS (99), lower [11C]-flumazenil binding has been
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56 25 observed in similar brain areas of ALS patients with mild cognitive impairment (100).
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Neuroglial binding is increased in PLS brain

PET using other radioligands identifying astrocytes and/or microglia has substantiated *in vivo* the occurrence of gliosis in MND brain as initially shown *post mortem*. The first study of cerebral inflammation in patients with ALS ($n=10$) compared to healthy controls ($n=14$) used [^{11}C]-(R)-PK11195, which binds to peripheral benzodiazepine receptors expressed only by activated microglia. Despite this first-generation ligand having high nonspecific binding and poor signal-to-noise ratio (101), the study revealed significantly increased signal in PMC, thalamus, and pons (102). Of note, the degree of binding was strongly proportional to the amount of UMN burden on clinical examination ($r=0.73$, $P=0.009$). This set the stage for the first study of cerebral neuroinflammation by the same group using the same radioligand in each of three patients with prominent UMN dysfunction arising from 3 different conditions: PLS, Mills syndrome, or inflammatory cervical myelopathy (97). In the PLS patient, [^{11}C]-(R)-PK11195 binding was higher in PMC of the hemisphere contralateral to most affected side of the body. However, in the patient with Mills syndrome, similarly high binding was localized to the superior frontal lobe anterior to the PMC and supplementary motor area (parasagittal mesial cortex) only of the hemisphere contralateral to affected limbs. This corresponded to cortical regions where greatest UMN dysfunction would be expected based on clinical findings. Not surprisingly, the patient with cervical myelopathy showed no increased cerebral [^{11}C]-(R)-PK11195 BP values, although they were significantly increased in the bilateral thalami and pons of all 3 patients. Microglial activation in these latter subcortical regions of may occur because of axonal connectivity with the respective sites of primary neuronal damage, whether cortical or spinal (97).

A more recently developed PET radiotracer used in studies of ALS and PLS brains is [^{11}C]-PBR28, which binds to a 18pKD translocator protein (TSPO) expressed in mitochondria of activated microglia and reactive astrocytes (103). Increased binding [^{11}C]-PBR28 in the CNS does not discriminate between glial cell type (103) or neuroglial function, and is influenced by a polymorphism (Ala147Thr) in the TSPO gene requiring genotyping of

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1 individuals prior to PET scanning (104). Identifying PET radioligands that specifically label
2 only astrocytes or microglia, particularly those activated into a neurotoxic state, could assist
3 in identifying effective experimental therapies that target neuroinflammation.

4 In a study comparing patients with ALS ($n=53$) and PLS ($n=11$) against healthy
5 controls ($n=21$), [^{11}C]-PBR28 PET and 3T MRI were concurrently performed using the same
6 integrated scanner at 2 time points (105). Whole brain voxelwise analyses revealed
7 increased ligand binding in cortical and subcortical regions of motor cortices in both MND
8 patient groups compared to controls, although this was significantly higher in subjacent white
9 matter of patients with PLS. It is unknown whether this is due to longer disease duration in
10 the PLS group (~ 12.2 years) compared to the ALS group (~ 2.2 years) or because of disease
11 mechanism differences between the two MND's. On the other hand, surface-based analyses
12 between the patient groups showed no differences in [^{11}C]-PBR28 uptake over the PMC,
13 although cortical thickness was significantly lower in PLS patients. Fractional anisotropy of
14 white matter below the PMC and mean diffusivity in both PMC and subjacent white matter
15 were significantly more abnormal in patients with PLS. Although a longitudinal study, only
16 ALS patients ($n=10$) were studied six months later with no overall change in [^{11}C]-PBR28
17 binding after whole brain voxelwise analysis. Relatively slow disease progression in these
18 ALS patients was an explanation for this, as reflected by the revised ALS functional rating
19 scale (ALSFRS-R) progression rate of 0.5 points/month. If this were the case, detecting any
20 change of [^{11}C]-PBR28 uptake over time in PLS patients would be even less likely
21 considering their much slower progression rate (e.g. ALSFRS-R progression rate of PLS
22 patients in same paper = 0.19 points/month). Nonetheless, the relevance of all these
23 findings was enhanced by the study's multimodal approach of clinical measures and
24 concomitantly acquiring both radiotracer and structural MRI data to allow precise anatomical
25 localization of the PET changes and relationship with cortical thickness and tract diffusivity
26 (105). A study of brain [^{11}C]-PBR28 uptake and concomitant structural MRI performed by
27 the same group focused only on patients with PLS ($n=10$) compared to healthy controls

($n=10$) at a single time-point, more precisely identified the highest [^{11}C]-PBR28 binding to reside in subcortical white matter beneath the PMC (106). Unlike the ALS patients in the previous study (105), PLS patients in this study showed no correlations between ligand uptake and ALSFRS-R or UMN burden scores. This may have resulted from limitations of each scale to detect differences at such high degrees of UMN dysfunction, or clinical changes in protracted stages of PLS did not correspond to the imaging metrics (106).

Discussion

changes

Magnetic resonance and PET imaging studies have already contributed considerable insights into the pathogenesis of PLS. However, most studies are limited by small sample sizes and being cross-sectional in nature with surprisingly few published longitudinal studies. Key future research directions include the characterisation of suspected PLS patients who don't fulfil current diagnostic criteria (78), combined PET-MRI studies (106), use of novel PET radioligands to identify specific anatomy (e.g., microglia only, synaptic terminals) or neurochemistry (e.g., misfolded proteins), combined brain-spinal cord imaging studies (66), and the adaptation of longitudinal study designs (76). The harmonisation of acquisition protocols across multiple centres and studies spearheaded by international consortia such as NiSALS may pave the way for large multicentre studies, which will overcome the sample size limitations of current single centre studies (18). Furthermore, continued advances in hardware, software development, and post-processing techniques will expedite imaging data acquisition and analysis, and enhance overall neuroimaging quality. Applying such advances to CNS neuroimaging in patients with PLS will enhance our ability to better characterise and track disease progression, as well as to evaluate pharmacologic and other novel therapies in clinical trials of PLS.

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Neuroimaging in Primary Lateral Sclerosis

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Abstract

Increased interest in the underlying pathogenesis of primary lateral sclerosis (PLS) and its relationship to amyotrophic lateral sclerosis (ALS) has corresponded to a growing number of CNS imaging studies, especially in the past decade. Both its rarity and uncertainty of definite diagnosis prior to four years from symptom onset have resulted in PLS being less studied than ALS. In this review, we highlight most relevant papers applying magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), and positron emission tomography (PET) to analysing CNS changes in PLS, often in relation to ALS. In patients with PLS, mostly brain, but also spinal cord has been evaluated since significant neurodegeneration is essentially restricted to upper motor neuron (UMN) structures and related pathways. Abnormalities of cortex and subcortical white matter tracts have been identified by structural and functional MRI and MRS studies, while metabolic and cell-specific changes in PLS brain have been revealed using various PET radiotracers. Future neuroimaging studies will continue to explore the interface between the PLS-ALS continuum, identify more changes unique to PLS, apply novel MRI and MRS sequences showing greater structural and neurochemical detail, as well as expand the repertoire of PET radiotracers that reveal various cellular pathologies. Neuroimaging has the potential to play an important role in the evaluation of novel therapies for patients with PLS.

1 Introduction

Despite the relatively low incidence of primary lateral sclerosis (PLS), a number of dedicated imaging studies have been undertaken in recent years to characterise its unique structural, functional, and metabolic signatures. The clinically pure upper motor neuron (UMN) nature of PLS lends itself to *in vivo* structural and functional studies made possible through developments in CNS imaging. Regarded as an extreme end of a continuum of amyotrophic lateral sclerosis (ALS), the much more protracted clinical course of UMN degeneration in PLS contributes to its most recognized neuroimaging changes in the primary motor cortex (PMC, or precentral gyri). These include: (a) focal atrophy and corticospinal and callosal white matter tract degeneration, as revealed by magnetic resonance imaging (MRI), and (b) cortical hypometabolism, neuronal receptor changes, and neurogliosis, as revealed by various positron emission tomography (PET) radioligands. Early imaging studies in PLS were primarily used, as in ALS, to rule out alternative causes of pure UMN dysfunction at brain or spinal cord levels. However, as MRI and PET technologies have advanced over the years, studies began examining deeper aspects of pathogenesis in PLS.

A formal literature review using PubMed was conducted of PLS and keywords related to MRI and PET in September 2020 in accordance with the PRISMA guidelines. The primary search terms of 'PLS', 'motor neuron disease', 'primary lateral sclerosis' were individually paired with 'neuroimaging', 'imaging', 'radiology', 'MRI', 'magnetic resonance imaging', 'PET', 'SPECT', 'morphometry', 'cortical thickness', 'DTI', 'spectroscopy', 'fMRI'. Only articles published in English were reviewed, but both single case reports and case series were considered. Identified papers were systematically reviewed for sample size, control groups, primary imaging modality and main study findings. A total of 117 PLS imaging and related papers were reviewed.

It is noteworthy that imaging studies in PLS over the years have used differing criteria that vary in symptom duration required to establish diagnosis. The 1945 PLS diagnostic criteria (1) suggested a minimum of 5 year symptom duration, while the 1992 Pringle criteria

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3 1 (2) proposed a minimum symptom duration of 3 years for reliable diagnosis. The 2006
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5 2 Gordon criteria (3) advocated for a symptom duration of 4 years to establish the diagnosis.
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7 3 Finally, recent 2020 consensus diagnostic criteria (4), recognising the implications of
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9 4 diagnostic delay, introduced a category of ‘probable PLS’ for patients with isolated UMN
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11 5 symptoms for 2-4 years. Existing imaging studies have primarily utilized the Pringle or
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13 6 Gordon criteria to select patients, although recent reports demonstrate the rationale for the
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15 7 new 2020 consensus criteria by finding that ‘probable PLS’ patients already exhibit
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17 8 radiological changes consistent with PLS of longer symptom duration (5,6).
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21 9 This review sought to identify key papers that have contributed to the
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23 10 characterisation of PLS-associated pathology *in vivo* using MRI and PET. Future
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25 11 neuroimaging research directions in PLS can be defined based on ALS-imaging studies,
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27 12 which have presented viable methodological, conceptual or technological frameworks not yet
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29 13 been applied to PLS cohorts. While several recent PLS imaging studies have been
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31 14 published that discussed findings in varying detail (7–9), the focus of this paper is to provide
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33 15 an overview of the contribution of imaging to field of PLS research and discuss potential
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35 16 future clinical applications.
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41 18 **MRI in PLS**

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44 19 **Background**

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47 20 MRI protocols enable the quantitative appraisal of grey and white matter integrity in
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49 21 comparative and correlative statistical models, and are uniquely useful in the broader
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51 22 spectrum of motor neuron disease (MND) where slowly progressive neurodegenerative
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53 23 changes take place (10). The practical appeal of MRI-based imaging in MND includes non-
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55 24 invasive data acquisition, cost effectiveness, data interpretation at both individual and group
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57 25 levels (11), the widespread availability of MRI scanners (12), the ability to distinguish MND
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59 26 phenotypes (13,14), the opportunity to track longitudinal changes (15,16), existing protocols
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for data harmonisation (17), and the availability of large international data repositories (18). The majority of MRI studies in MND however focus on ALS (19), and relatively few MRI studies have been dedicated to describe PLS-associated imaging changes (8). The technological, methodological, and logistical lessons of ALS imaging are directly transferrable to PLS and many of the techniques pioneered in ALS can be readily applied to PLS cohorts.

Diagnosis of PLS is notoriously difficult as it may initially resemble any one of several conditions causing progressive UMN dysfunction or degeneration, including hereditary spastic paraparesis (HSP), corticobasal degeneration, multiple sclerosis, etc. Because HSP often presents clinically like PLS, a detailed family history is obtained and HSP-associated mutations are frequently screened. In a clinical setting, routine spinal cord and brain imaging is performed and qualitatively evaluated to rule out structural abnormalities (e.g., compressive myelopathy, neoplasm), or inflammatory conditions (e.g., multiple sclerosis, transverse myelitis).

Case reports and case series

A number of case reports and small case series preceded the publication of large prospective MRI studies in PLS. Focal atrophy of pericentral gyri in PLS brain is often visible macroscopically (Figure 1), and may even have a 'knife edge' appearance of regional gyri (2,20). Long disease duration of PLS may not be the only explanation for the prominent cortical atrophy because it is unusual to observe in even advanced longstanding cases of ALS. Furthermore, significant atrophy in PLS, including of underlying white matter, has also been noted to extend more anteriorly (21,22), with MRI changes sometimes linked to cognitive impairment (23,24).

Qualitative findings of corticospinal tract (CST) hyperintensities in brain (25) as well as spinal cord (26) have been observed on T2- (18, 19), and FLAIR-weighted images (27).

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3 1 Such intracranial CST hyperintensity occasionally seen in PLS can resemble a “wine glass”
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5 2 when viewed coronally at the diencephalic level (28), although this and its “funnel”-like
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7 3 appearance when viewed sagittally are more frequently seen in UMN-predominant ALS
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9 4 (Figure 2) (29). Case reports of single PLS patients have also demonstrated CST reductions
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11 5 in fractional anisotropy on diffusion tensor imaging (DTI) (30,31), white matter degeneration
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13 6 (32), and progressive extramotor atrophy (20). However, these qualitative changes are seen
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15 7 in only some cases of PLS and also can occur in ALS (8).
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22 9 **Quantitative cross-sectional and longitudinal MRI studies in PLS**

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24 10 Over 50 quantitative imaging studies have been published in PLS (8), seven of which
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26 11 had a longitudinal design (33). The cohort sizes of quantitative imaging studies in PLS range
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28 12 from 2 to 33 (9) in single-centre single-protocol studies, single-centre multiprotocol studies
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30 13 that included up to 50 PLS patients, (34), and multicentre studies that included up to 88 PLS
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32 14 patients (35). The majority of PLS imaging studies are MRI studies which typically present
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34 15 morphometric and diffusion tensor imaging analyses. Morphometric structural analyses of
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36 16 PLS brain invariably capture precentral gyrus atrophy (36,37), PMC surface area reductions
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38 17 (21,22) and focal PMC thinning (9,38,39), although subcortical grey matter degeneration has
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40 18 also been reported (40). Existing imaging studies in PLS describe primarily group-level
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42 19 changes. Despite the academic relevance of identifying PLS-associated imaging signatures
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44 20 from a cohort of patients, the clinical utility of such observations is limited on an individual
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46 21 patient level.
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51 22 A goal of quantitative neuroimaging, particularly if high resolution 3D volumetric MR
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53 23 data sets are acquired, is accurately categorising individual patients into diagnostic and
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55 24 prognostic categories. Interpreting individual patient data in ALS has been reported using
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57 25 various machine learning approaches (11,41–45), but not in PLS because large reference
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59 26 data sets are presently lacking to build and validate classification models. Once such
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reference datasets are available for PLS patients, their images may also be interpreted at an individual level.

Extramotor and subcortical MRI studies in PLS

Reports on extra-motor cortical involvement in PLS are inconsistent and range from limited regional pathology to widespread parietal, prefrontal, cerebellar and brainstem degeneration (9,46,47). Over 20 diffusion tensor imaging studies have been published in PLS which consistently capture CST pathology (30–32), but cerebellar (9,48), and corpus callosum (37,49) pathologies have also been described. Some studies have specifically highlighted extra-motor, extra-corpus callosum diffusivity alterations involving the superior and inferior longitudinal fasciculi, fornix, thalamic radiations, and parietal lobes (23,24).

Recent imaging studies in PLS focusing on subcortical grey matter degeneration have found that certain structures such as the amygdala may be preferentially affected in ALS but less likely to be affected in PLS (50). However, other subcortical structures like the thalamus are not only atrophic in PLS (51) but thalamic motor and sensory nuclei are selectively affected in PLS (52,53).

Specialized magnetic resonance studies in PLS

A smaller proportion of MRI studies in PLS report spectroscopy and functional MRI findings. Proton MR spectroscopy (¹H-MRS) studies of PMC of PLS patients, have reported reduced *N*-acetyl aspartate (NAA)/creatine (Cr) ratios (54–56) and increased *myo*-inositol/Cr ratios (57). These findings are consistent with neuronal dysfunction or loss, and gliosis, respectively. Unlike in ALS, however (58,59), whole-brain multi-voxel MRS techniques have not yet been applied to PLS cohorts (60). The drawback of hypothesis-driven volume of interest (VOI) spectroscopy is that extra-motor metabolic changes cannot be systematically

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3 1 evaluated unless using multivoxel spectroscopic imaging (61). Functional MRI (fMRI) studies
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5 2 reported increased functional connectivity in PLS (62,63), which similarly to ALS studies (64)
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7 3 were interpreted as an adaptive, compensatory process. While spinal cord hyperintensities
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9 4 along the pyramidal tracts are rarely observed in PLS (26), and considerable methodological
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11 5 advances are occurring in spinal imaging in ALS (45,65,66), no robust prospective cord
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13 6 imaging studies have yet been undertaken in PLS.
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20 8 **Challenges and limitations of MRI studies in PLS**

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22 9 The majority of PLS imaging studies are primarily descriptive because clinico-
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24 10 radiological correlations in MND are often regarded to be contentious (67). Nonetheless,
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26 11 clinical scores have been correlated to grey matter volume reductions (21,36), PMC
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28 12 measures (38,68,69), white matter indices (9,21,70), proton-density alterations (71), and
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30 13 NAA reductions (56,57,72). Disease duration is thought to correlate with imaging measures
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32 14 by some (73) but not all groups (38,49,74). Functional disability in ALS results from
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34 15 concomitant upper and lower motor degeneration rendering correlations analyses between
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36 16 cerebral measures and functional scales controversial. Due to its selective UMN
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38 17 degeneration, PLS is the ideal condition to study the imaging correlates of motor impairment.
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40 18 Functional scale scores, upper- and lower limb tapping rates have been recently linked to
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42 19 white matter degeneration of the corona radiata in a somatotopic pattern (9). With the recent
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44 20 publication of revised consensus diagnostic criteria for PLS (4), and the development of a
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46 21 PLS specific functional rating scale (75), future imaging studies are likely to capture more
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48 22 meaningful clinikoradiologic correlations.
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53 23 Compared to ALS (15,16), there is a striking lack of longitudinal imaging studies in
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55 24 PLS (76). Existing longitudinal studies in PLS suffer from cohort size limitations, typically
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57 25 being two time-point designs, and varying considerably in follow-up intervals (55,73,74,77).
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59 26 The longitudinal analysis of 12 PLS patients (69) detected no progressive CST changes over
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time. Interestingly, a study of eight pre-PLS patients who initially did not fulfil diagnostic criteria exhibited progressive precentral gyrus thinning and increasing functional connectivity (78). Other studies of suspected PLS patients showing connectivity and grey/white matter abnormalities before meeting diagnostic criteria (37,78,79) suggested that protracted symptom duration in current diagnostic criteria of PLS does not alone influence underlying pathological changes. The majority of PLS imaging studies use healthy controls or ALS patients (16,21,37,57,68,70,79–81) to describe PLS imaging signatures, and only few rely on alternative neurodegenerative controls such as cohorts of HSP (49,82) or FTD (46) patients.

One of the problems of comparing ALS and PLS cohorts is the considerable differences in symptom duration. Until recently (4), previous diagnostic criteria of PLS required symptom duration of 4 years (3), whereas in ALS symptom duration of 4 years is typically associated with considerable disability (83). Accordingly, studies contrasting established MND patients inevitably compare cohorts of PLS patients with disproportionately longer symptom duration than the participating ALS patients. This may explain reports of lower cortical thickness (16,21,81) and precentral gyrus NAA levels (57) in PLS compared ALS. Other studies have opted to control for symptom duration differences in their statistical models (9). Differences in statistical approaches, imaging modalities, choice of control groups and study designs preclude a conclusive view whether PLS has a unique imaging signature distinct from ALS.

It is clear that precentral gyrus, corpus callosum, CST, cerebellar, brainstem and basal ganglia involvement are shared features of both PLS (34,38,39,46,68,70,82) and ALS (71–76). Some studies (9) suggest the preferential involvement of the splenium of the corpus callosum in PLS in contrast to ALS where the fibres of the forceps minor in the genu are more affected.

PET in PLS

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Background

Although many molecular imaging studies using PET in patients with ALS have been published using various radiolabelled ligands (for review, see (84–86)), relatively few PET studies exist in PLS. Accordingly, individual studies will be highlighted. We will not discuss single-photon emission computed tomography (SPECT) studies except to mention a single case report in a patient with PLS of reduced signal in both frontal lobe pericentral areas (87).

[¹⁸F]-FDG PET studies in PLS

[¹⁸F]-fluoro-2-deoxy-D-glucose PET ([¹⁸F]-FDG PET), which assesses glucose metabolism by neurons and neuroglia, has been the most commonly used molecular imaging modality in ALS and PLS. One of the earliest [¹⁸F]-FDG PET reports of brain changes in PLS appeared in a book chapter in 1990 (88) and subsequently part of the seminal paper by Pringle and colleagues (2) of clinical features, neuropathology, and diagnostic criteria in eight PLS patients. PET scans, which were performed in only three patients, detected reduced glucose uptake in the pericentral cortex of two with the most prominent clinical features of PLS; PET findings in the one with reportedly mild signs could not be distinguished from normal. Nonetheless, their proposed diagnostic criteria included “decreased glucose consumption in pericentral region on PET scan” under findings “additionally suggestive of PLS”. Subsequent PET studies in PLS have all included less than double-digit patient numbers until recently.

When [¹⁸F]-FDG hypometabolism is restricted to the PMC, it can be very conspicuous, and has been termed the “stripe sign”. First reported in 3 patients with PLS who had spastic dysarthria and varying degrees of lower or upper limb spasticity, hypometabolism was particularly prominent along the hemisphere’s lateral convexity in one patient (89). Too few patients with similar distributions of spasticity were studied to determine whether somatotopic (homuncular) representation of the pericentral

hypometabolism occurred, although the one patient with only unilateral arm spasticity had a more prominent stripe sign in the appropriate contralateral hemisphere. Interestingly, a case report of a PLS patient with only upper limb spasticity and normal lower limb exam revealed bilateral pericentral [^{18}F]-FDG PET hypometabolism that relatively spared parasagittal PMC where lumbosacral spinal cord-projecting corticomotoneurons would be expected to reside (90) (Figure 3).

The ability for [^{18}F]-FDG PET to distinguish PLS and ALS brains based on their metabolic signatures is poor. An earlier report of 7 PLS patients compared to 70 with ALS (comparing both to 20 healthy controls) suggested that hypometabolism was slightly more prominent in prefrontal cortex and posterior cingulate cortex of ALS patients (91). However, a subsequent prospective study by the same group with a new cohort of patients using volume of interest (VOI)-based discriminant analysis found no differences between PLS ($n=10$) and ALS ($n=105$) patient groups (92). Although both groups showed the most prominent hypometabolism in prefrontal and premotor cortices, as well as hypermetabolism in medial temporal cortex, cerebellum, and upper brainstem compared to 20 healthy controls, 9 of 10 PLS patients could not be distinguished from those with ALS. The authors' final consensus was that [^{18}F]-FDG PET cannot differentiate between brain metabolic signal changes in PLS and ALS patients (92). Recent advances in metabolic imaging suggest that combined cord and brain [^{18}F]-FDG PET may differentiate ALS from PLS (93).

Mills syndrome is a very rare form of UMN degeneration characterised by slowly progressive ascending hemiparesis and ipsilateral spasticity (94) that may be considered clinically as a unilateral variant of PLS (95). Two [^{18}F]-FDG PET studies of patients with clinical features consistent with Mills syndrome showed differences in extent of hemispheric hypometabolism. The first of a 63 year-old male with 10 years of slowly progressive left body hemiparesis and spasticity revealed significant hypometabolism in motor and premotor regions of both hemispheres, compared to 45 healthy controls, although slightly worse on the right (96). In contrast, the other of 3 females with right body pure UMN signs showed

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3 1 significantly reduced [¹⁸F]-FDG PET binding in the PMC and adjacent areas in only the left
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5 2 hemisphere (92). Such variability in concordance of hemispheric hypometabolism with
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7 3 clinical UMN deficits supports the suggestion that Mills syndrome is useful only as a
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9 4 descriptive clinical term (97).

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15 6 **Ligand PET studies in PLS**

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18 7 ***Neuronal GABA_A receptor alterations in PLS brain***

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21 8 [¹¹C]-flumazenil binds to the benzodiazepine receptor subunit of the GABA_A receptor
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23 9 (R) localized to brain neurons. A study of 9 PLS patients included [¹¹C]-flumazenil PET at a
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25 10 single time-point in 5 patients alongside longitudinal clinical and electrophysiologic
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27 11 evaluations (98). PET measurement of regional cerebral blood flow (rCBF), an index of
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29 12 synaptic brain function, was reduced in the precentral gyrus (fronto-opercular region),
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31 13 ventrolateral prefrontal region, and anterior cingulate cortex. GABA_A-R density, a potential
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33 14 surrogate for neuronal cell body and proximal dendrite integrity, was not as diffusely
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35 15 decreased but localized within the foci of rCBF reduction. Whether reduced [¹¹C]-flumazenil
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37 16 binding represents neuronal loss, changes in GABA_A-R functional properties, or both, is
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39 17 uncertain. However, this study confirmed the motor system dysfunction in PLS is similar to
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41 18 that in ALS, at least for rCBF.

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45 19 A subsequent study with [¹¹C]-flumazenil PET compared 4 PLS patients with ALS
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47 20 patients due to either sporadic disease (*n*=24) or a homozygous SOD1 (D90A) mutation
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49 21 (*n*=10) resulting in slowly progressive UMN-predominant disease (*n*=10), and controls
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51 22 (*n*=24) (99). All patient groups (relative to controls) displayed significantly decreased [¹¹C]-
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53 23 flumazenil PET signal in bilateral motor cortices and the right parietal lobe. Radioligand
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55 24 binding was significantly lower in both ALS patient groups (especially homD90A SOD1 after
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57 25 cluster correction, *P*<0.05) than in PLS patients in bilateral anterior frontal and orbitofrontal
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59 26 regions. Only PLS patients showed additional [¹¹C]-flumazenil binding reductions in bilateral

1 anterior cingulate gyri and the left superior temporal lobe. Although the significance of this
2 regional involvement is unclear in PLS (99), lower [^{11}C]-flumazenil binding has been
3 observed in similar brain areas of ALS patients with mild cognitive impairment (100).

5 ***Neuroglial binding is increased in PLS brain***

6 PET using other radioligands identifying astrocytes and/or microglia has
7 substantiated *in vivo* the occurrence of gliosis in MND brain as initially shown *post mortem*.
8 The first study of cerebral inflammation in patients with ALS ($n=10$) compared to healthy
9 controls ($n=14$) used [^{11}C]-(R)-PK11195, which binds to peripheral benzodiazepine receptors
10 expressed only by activated microglia. Despite this first-generation ligand having high
11 nonspecific binding and poor signal-to-noise ratio (101), the study revealed significantly
12 increased signal in PMC, thalamus, and pons (102). Of note, the degree of binding was
13 strongly proportional to the amount of UMN burden on clinical examination ($r=0.73$,
14 $P=0.009$). This set the stage for the first study of cerebral neuroinflammation by the same
15 group using the same radioligand in each of three patients with prominent UMN dysfunction
16 arising from 3 different conditions: PLS, Mills syndrome, or inflammatory cervical myelopathy
17 (97). In the PLS patient, [^{11}C]-(R)-PK11195 binding was higher in PMC of the hemisphere
18 contralateral to most affected side of the body. However, in the patient with Mills syndrome,
19 similar high binding was localized to the superior frontal lobe anterior to the PMC and
20 supplementary motor area (parasagittal mesial cortex) only of the hemisphere contralateral
21 to affected limbs. This corresponded to cortical regions where greatest UMN dysfunction
22 would be expected based on clinical findings. Not surprisingly, the patient with cervical
23 myelopathy showed no increased cerebral [^{11}C]-(R)-PK11195 BP values, although they were
24 significantly increased in the bilateral thalami and pons of all 3 patients. Microglial activation
25 in these latter subcortical regions of may occur because of axonal connectivity with the
26 respective sites of primary neuronal damage, whether cortical or spinal (97).

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1 A more recently developed PET radiotracer used in studies of ALS and PLS brains is
2 [¹¹C]-PBR28, which binds to a 18pKD translocator protein (TSPO) expressed in mitochondria
3 of activated microglia and reactive astrocytes (103). Increased binding [¹¹C]-PBR28 in the
4 CNS does not discriminate between glial cell type (103) or neuroglial function, and is
5 influenced by a polymorphism (Ala147Thr) in the TSPO gene requiring genotyping of
6 individuals prior to PET scanning (104). Identifying PET radioligands that specifically label
7 only astrocytes or microglia, particularly those activated into a neurotoxic state, could assist
8 in identifying effective experimental therapies that target neuroinflammation.

9 In a study comparing patients with ALS (*n*=53) and PLS (*n*=11) against healthy
10 controls (*n*=21), [¹¹C]-PBR28 PET and 3T MRI were concurrently performed using the same
11 integrated scanner at 2 time points (105). Whole brain voxelwise analyses revealed
12 increased ligand binding in cortical and subcortical regions of motor cortices in both MND
13 patient groups compared to controls, although this was significantly higher in subjacent white
14 matter of patients with PLS. It is unknown whether this is due to longer disease duration in
15 the PLS group (~12.2 years) compared to the ALS group (~2.2 years) or because of disease
16 mechanism differences between the two MND's. On the other hand, surface-based analyses
17 between the patient groups showed no differences in [¹¹C]-PBR28 uptake over the PMC,
18 although cortical thickness was significantly lower in PLS patients. Fractional anisotropy of
19 white matter below the PMC and mean diffusivity in both PMC and subjacent white matter
20 were significantly more abnormal in patients with PLS. Although a longitudinal study, only
21 ALS patients (*n*=10) were studied six months later with no overall change in [¹¹C]-PBR28
22 binding after whole brain voxelwise analysis. Relatively slow disease progression in these
23 ALS patients was an explanation for this, as reflected by the revised ALS functional rating
24 scale (ALSFRS-R) progression rate of 0.5 points/month. If this were the case, detecting any
25 change of [¹¹C]-PBR28 uptake over time in PLS patients would be even less likely
26 considering their much slower progression rate (e.g. ALSFRS-R progression rate of PLS
27 patients in same paper = 0.19 points/month). Nonetheless, the relevance of all these

findings was enhanced by the study's multimodal approach of clinical measures and concomitantly acquiring both radiotracer and structural MRI data to allow precise anatomical localization of the PET changes and relationship with cortical thickness and tract diffusivity (105). A study of brain [^{11}C]-PBR28 uptake and concomitant structural MRI performed by the same group focused only on patients with PLS ($n=10$) compared to healthy controls ($n=10$) at a single time-point, more precisely identified the highest [^{11}C]-PBR28 binding to reside in subcortical white matter beneath the PMC (106). Unlike the ALS patients in the previous study (105), PLS patients in this study showed no correlations between ligand uptake and ALSFRS-R or UMN burden scores. This may have resulted from limitations of each scale to detect differences at such high degrees of UMN dysfunction, or clinical changes in protracted stages of PLS did not correspond to the imaging metrics (106).

Discussion

Magnetic resonance and PET imaging studies have already contributed considerable insights into the pathogenesis of PLS. However, most studies are limited by small sample sizes and being cross-sectional in nature with surprisingly few published longitudinal studies. Key future research directions include the characterisation of suspected PLS patients who don't fulfil current diagnostic criteria (78), combined PET-MRI studies (106), use of novel PET radioligands to identify specific anatomy (e.g., microglia only, synaptic terminals) or neurochemistry (e.g., misfolded proteins), combined brain-spinal cord imaging studies (66), and the adaptation of longitudinal study designs (76). The harmonisation of acquisition protocols across multiple centres and studies spearheaded by international consortia such as NiSALS may pave the way for large multicentre studies, which will overcome the sample size limitations of current single centre studies (18). Furthermore, continued advances in hardware, software development, and post-processing techniques will expedite imaging data acquisition and analysis, and enhance overall neuroimaging quality. Applying such advances to CNS neuroimaging in patients with PLS will enhance our ability to better characterise and

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1 track disease progression, as well as to evaluate pharmacologic and other novel therapies in
2 clinical trials of PLS.

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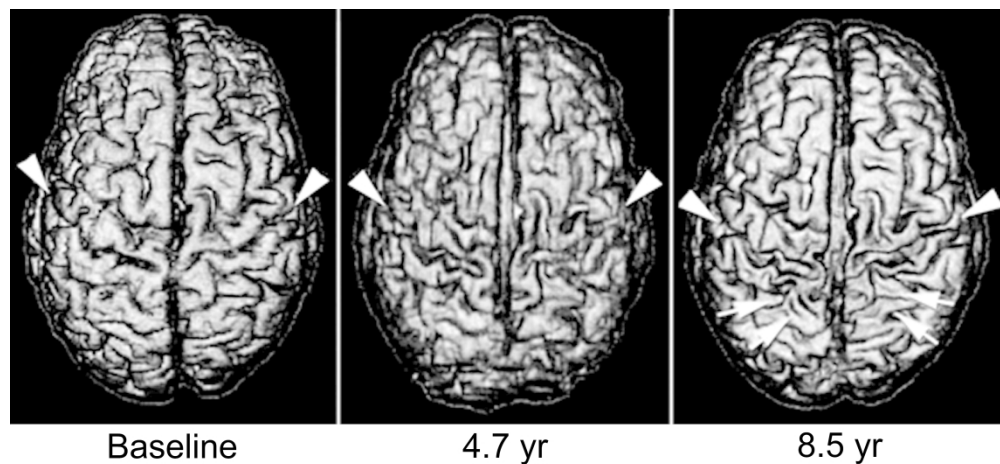


Figure 1 - Cortical surface renderings from 3-D MRI of a patient with PLS at baseline (left), 4.7 yr later (middle), and 8.5 yr later (right). Progressive atrophy of pericentral gyri and widening of the central sulcus (arrowheads) are noted until atrophy is finally apparent of the superior parietal region in the latest scan (double arrows). Adapted with permission from ref (20).

206x95mm (600 x 600 DPI)

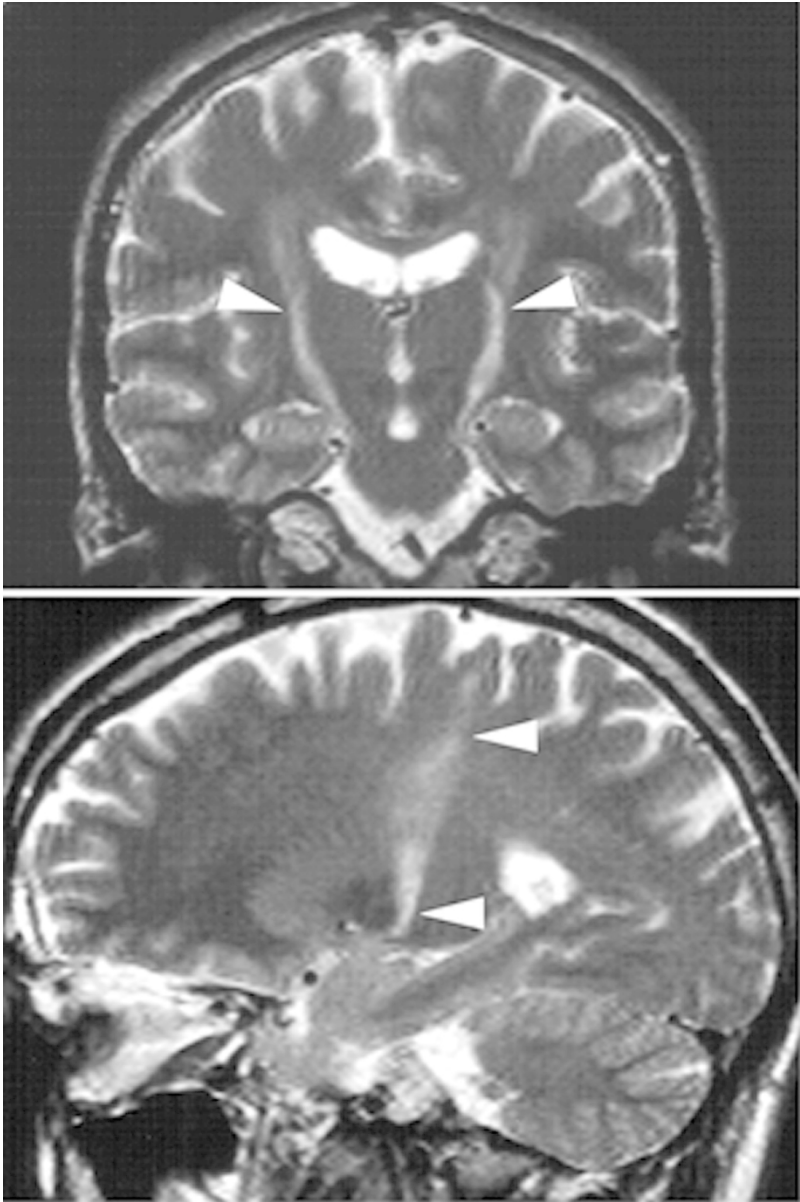


Figure 2 - Corticospinal tract hyperintensity seen on T2-weighted brain MRI of a 35 year-old man with upper motor neuron predominant ALS. This takes on a “wine glass” appearance on coronal view (arrowheads in top) and resembles a “funnel” or “cone” on sagittal view (arrowheads in bottom). Adapted from ref (29).

104x157mm (600 x 600 DPI)

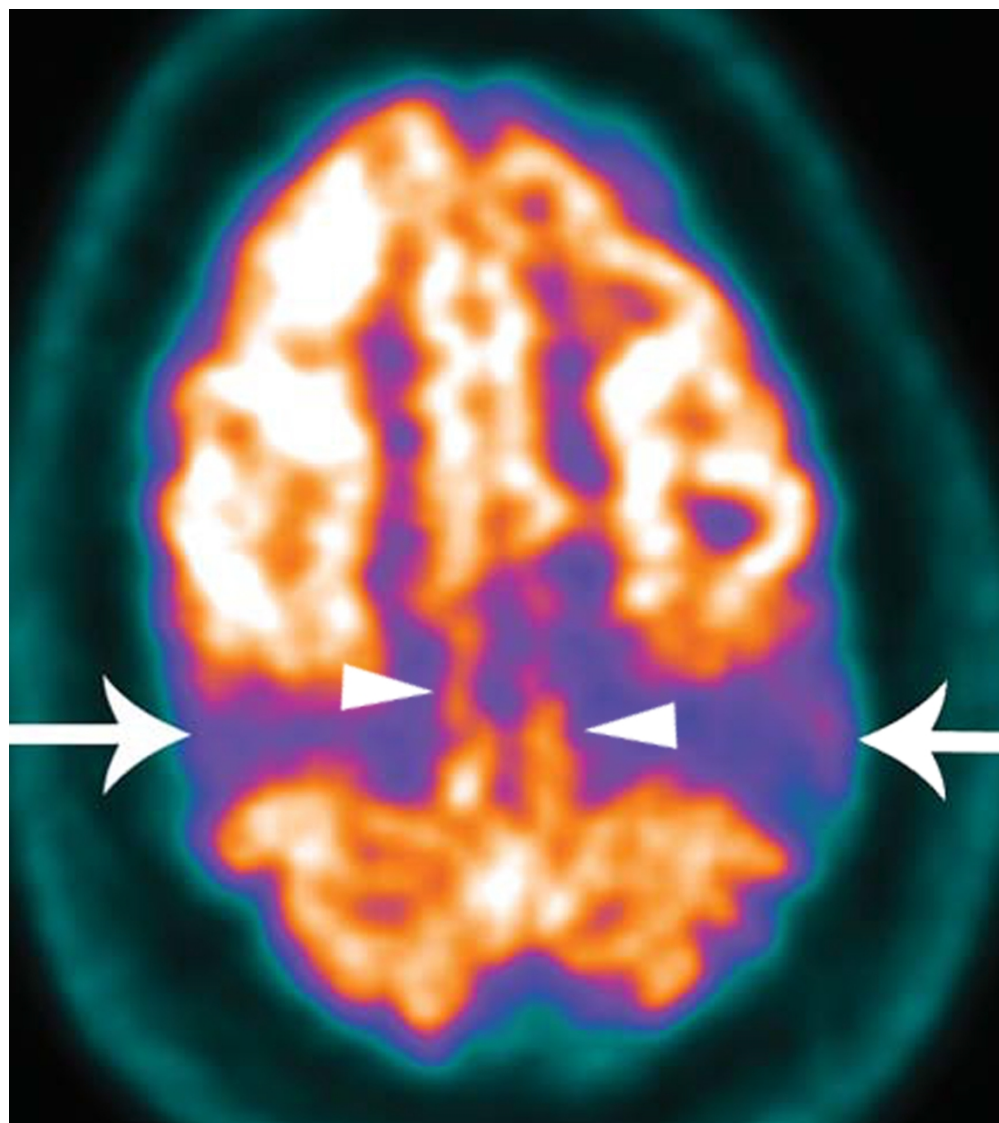


Figure 3 - Axial view shows FDG PET hypometabolism most prominently along primary motor cortex ('stripe sign') in both hemispheres (arrows), although parasagittal cortex appears less affected (arrowheads). Adapted with permission from ref (90).

101x113mm (600 x 600 DPI)