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PET Imaging in ALS

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Abstract

Amyotrophic lateral sclerosis is a neurodegenerative disorder that primarily affects the motor system, but extramotor involvement is common. Progressive muscle weakness and wasting, including bulbar and respiratory muscles, limit survival to 2–5 years after disease onset in most patients. The diagnosis is made on clinical grounds and is based on the presence of signs of upper and lower motor neuron loss in different body regions in the absence of other pathologies that can explain the symptoms and signs of the patient. Making an accurate diagnosis can be difficult in early disease stages. ALS is a heterogeneous disorder with variability in age at onset, in phenotypic presentation, in the extent of frontotemporal involvement and in the disease progression rate. There is a high unmet medical need for objective markers that aid in early diagnosis and in predicting disease outcome. In this chapter, the current knowledge about the diagnostic and prognostic value of ¹⁸F 2-fluoro-2-deoxy-D-glucose-PET in ALS is discussed. The potential of other targets and PET tracers to visualize different aspects of ALS disease pathology is described.

Keywords: amyotrophic lateral sclerosis, frontotemporal dementia, extramotor involvement, FDG PET, neuroinflammation, imaging biomarker

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive disorder, however, with considerable variability in phenotype, disease progression and aetiology. Reliable prognostication regarding unusually fast or slow progression is difficult in clinical practice. Since diagnosis is often delayed until patients are already a year into their disease, pharmacological treatment with riluzole is often postponed as well. Numerous therapeutic clinical trials

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© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. using other drugs have failed to show any benefit. These problems highlight the high need for reliable biomarkers in ALS. Ideally, such a biomarker should be easily accessible and affordable, as well as very sensitive and specific for ALS [1]. It should also be of prognostic value and should change with disease progression making it suitable for treatment monitoring [1]. Besides peripheral biomarkers in blood and cerebrospinal fluid, several neuroimaging biomarkers have been proposed, of which several nuclear or molecular imaging targets seem to be very promising. Although radionuclide imaging is not commonly used in clinical practice for ALS, recent studies suggest that various aspects of ALS pathology can be visualized and quantified.

1.1. ALS and FTD: two overlapping disorders

In ALS, the motor system is primarily affected [2]. Degeneration of upper motor neurons (UMNs) in motor cortex and lower motor neurons (LMNs) in the brain stem and spinal cord results in a progressive weakness and wasting of limb, bulbar and respiratory muscles, limiting survival to 2–5 years after disease onset.

In frontotemporal dementia (FTD), the degenerative process starts in the frontal and/or anterior temporal cortex [3]. Depending on the neuroanatomical regions affected, disease presentations include behavioural variant FTD (with changes in behaviour with apathy, loss of empathy, hyperorality, repetitive behaviours or disinhibition and executive dysfunction) or language variants of FTD (such as primary non-fluent aphasia and semantic dementia). The disease progressively affects other cognitive functions. Survival after disease onset ranges from 5 to 8 years.

ALS and FTD are considered to be extremes of a disease spectrum [4]. In about 10% of patients, both diseases co-occur. In another 30–40%, there is some degree of overlap, with mild motor involvement in patients with FTD, or mild cognitive/behavioural impairment in patients with ALS. About 50% of patients have pure FTD or pure ALS.

The only approved therapy for ALS is riluzole, which extends survival by only a few months, whereas for FTD no disease modifying therapies are available. The cornerstone of the management of patients with FTD/ALS remains multidisciplinary care and supportive measures.

Ten percent of ALS patients and 40% of FTD patients have a positive family history. Mutations in a heterogeneous set of genes have been identified to cause this familial form of FTD/ALS [5]. The inheritance pattern usually is autosomal dominant. Mutations in *SOD1, TARDBP* and *FUS* cause ALS and sometimes ALS-FTD, and mutations in *GRN* and *MAPT* cause FTD, but rarely also ALS. However, by far the most common cause for ALS, FTD/ALS and FTD is the recently discovered mutation in *C9ORF72* [6, 7]. It underlies 30–50% of familial ALS (and ALS-FTD) and 20–25% of familial FTD [8].

At the neuropathological level, an overlap between ALS and FTD is present as well. Especially, cytoplasmic accumulations of TDP-43, the protein encoded by the *TARDBP* gene, are central to the overlap between ALS and FTD, as most patients with sporadic ALS and about half of patients with sporadic FTD have such pathology (FTD/ALS-TDP-43) [9]. In addition, several of the genetic forms of FTD/ALS have TDP-43 aggregates, including mutations in *TARDBP*,

C9ORF72 and *GRN*. TDP-43 is an RNA-binding protein with important functions in gene transcription, splicing, RNA transport and stress granule formation. Accumulations of FUS, another RNA-binding protein, with many structural and functional similarities to TDP-43, is also central to FTD/ALS as mutations in *FUS* cause ALS, rarely FTD, and FUS pathology is also observed in about 5% of sporadic FTD patients.

It has thus become clear that ALS is a neurodegenerative disease that primarily affects the motor system, but that a variable degree of extramotor involvement is present in most patients [4].

For treating physicians and for family members, it is important to uncover cognitive problems in ALS, but it is not always easy to perform extensive neuropsychological testing in patients with motor impairments. An imaging biomarker that reliably recognizes and measures both motor and extramotor involvement in ALS patients would be an important achievement for the management of ALS patients and for ALS research.

1.2. Need for an upper motor neuron marker

The diagnosis of ALS requires the presence of both UMN and LMN signs. LMN signs are often readily appreciated on clinical examination and electromyography (EMG) is a very sensitive method to confirm this and even detect subclinical LMN involvement. On the contrary, providing evidence of UMN involvement can be challenging. First, the clinical signs of UMN involvement (hyperreflexia, spasticity, pseudobulbar features, Hoffman's reflex and extensor plantar response) exhibit both a low sensitivity and interrater reliability. Second, there are no reliable tests to show that UMN involvement exists. This lack of a reliable method to detect UMN involvement and track the progressive loss of UMNs is an important blind spot in the ALS field.

Various markers of UMN involvement have been proposed so far. First, the appearance of the motor cortex on magnetic resonance imaging (MRI) has been shown to be altered in ALS patients. At the group level, the thickness of the motor cortex is decreased, especially on 7T MRI [10]. However, at the individual patient level, a clear atrophy can only be demonstrated in around 50% of ALS patients because of considerable overlap with healthy controls [11, 12]. Other parameters like T2 hypointensity and increased quantitative susceptibility mapping (QSM) are present in a high proportion of ALS patients. It probably reflects gliosis due to activated microglia, but still lacks convincing sensitivity and specificity [10, 13, 14]. Second, the appearance of the corticospinal tract (CST) on MRI has also been proposed as a potential UMN marker. T2 hyperintensity of the CST in the posterior limb of the internal capsule is present in almost 50% of ALS patients, while it is absent in other studies [14, 15]. Since only severe hyperintensity seems to be clearly related with ALS and this occurs only late in the disease course, it is of no value in the (early) diagnosis of ALS [16]. Diffusion tensor imaging (DTI) of the CST seemed to be very promising at first, but unfortunately also turned out to lack sensitivity and specificity [16-18]. Third, the functional assessment of the motor cortex and CST by means of transcranial magnetic stimulation (TMS), with motor evoked potentials (MEP) being the most important parameter, has been investigated as a potential UMN biomarker. Literature data are unfortunately discordant: while several studies reported a cortical hypoexcitability [19, 20], others found a clear hyperexcitability [11, 21, 22]. Moreover, several studies showed that TMS cannot discriminate ALS patients from controls [18, 23], so better methods to detect UMN loss in ALS are needed.

1.3. Need for a (differential) diagnostic test

An early and certain diagnosis of ALS is of utmost importance for clinicians and patients. It allows an early initiation of riluzole therapy (as yet the only proven disease-modifying therapy for ALS), an accurate communication about the diagnosis and an early recruitment into clinical trials. Especially, in these early stages of the disease, the disease process may be amenable to therapy.

However, in daily clinical practice, the time between symptom onset and diagnosis (the diagnostic delay) is long, estimated to be 12–14 months in tertiary ALS referral centres [24]. There are various reasons for this delay, including patients-specific and doctor-specific delays. Aspecific presentations and phenotypic variability at onset contributes to the delay. Observation of patients, with repeat clinical examinations and electrodiagnostic testing is a reliable method to correctly identify ALS patients that present early with only mild and focal motor symptoms, but this approach increases the diagnostic delay. Some cases pose a differential diagnostic delay. Since most of these diseases lack abnormalities on CT or MRI imaging, they can impossibly be excluded by conventional imaging. Only a tool that allows to make a positive diagnosis of ALS early in the disease course could solve this problem.

1.4. Need for a prognostic marker

ALS is a heterogeneous disorder. Not only the genetic causes and the age at onset, but also the disease progression is highly variable. The median survival after disease onset is only 33 months and most patients die 2–5 years after disease onset. However, numerous cases of extremely long and extremely short survival have been reported, making up the extreme ends of a wide prognostic spectrum [25]. Making a reliable prognostic estimation is pivotal for both patients and their families and neurologists likewise, but still largely impossible these days. A variety of prognostic factors has been identified, such as age of onset, site of onset, rate of symptom progression, comorbid frontotemporal involvement and nutritional and respiratory status [26–31]. Although they are clearly of value, they all reflect divergent clinical disease parameters and do not directly reflect the underlying disease process. The first prognostic models taking into account the different known prognostic factors are underway [32]. Other, more pathophysiologically relevant biomarkers, such as pNFH levels [33, 34], seem to be promising. But also imaging biomarkers that reliably reflect the extent of motor and extramotor involvement have the potential to become a reliable prognostic determinant.

1.5. Need for an in vivo marker of ALS pathophysiology

Conventional neuroimaging has only revealed gross pathological insights by showing atrophy of the motor cortex and alterations of the CST. These imaging modalities can reveal structural

changes in ALS at the group level with high spatial resolution. However, they lack the capacity to provide a reflection of the neuropathological process at the cellular or molecular level and are not applicable at the individual patient level. Imaging biomarkers that can visualize ALS disease pathology, such as neuroinflammation, neuronal death and ideally TDP-43 accumulation would greatly advance the field of ALS research. It would not only be valuable for diagnostic purposes, but would also be useful to monitor the evolution of disease over time and as a readout for treatment effects of disease-modifying therapies.

So far, positron emission tomography (PET) has not been commonly used in ALS. However, recent studies show that various radioligands have potential to be useful imaging biomarkers in ALS. These can be of value in the diagnosis, in predicting outcome and in imaging disease pathology in ALS. In this chapter, an overview of the PET studies in ALS is given and future perspectives on the use of PET in ALS are discussed. On overview of all tracers used in ALS



Figure 1. PET tracers used in ALS. Using PET imaging six different neurological systems or cell populations can be assessed using different tracers. General glucose metabolism in the grey matter is assessed by [¹⁸F]-FDG. Cortical amyloid deposition can be assessed using [¹¹C]-PIB to distinguish ALS from Alzheimer's disease. The extrapyramidal system can be investigated using [¹⁸F]-fluorodopa, [¹⁸F]-FPCIT or [¹²³I]-FP-CIT. Neuronal integrity can be visualized using tracers for the GABA-A receptor ([¹¹C]-flumazenil) or the 5-HT1A receptor ([¹¹C]-WAY100635). Microglial cells can be highlighted using tracers targeting the translocator protein (TSPO), like [¹⁸F]-DPA-174, [¹¹C]-(R)-PK11195 or [¹¹C]-PBR28. Astrocytes are visualized by tracers for the MAO-B enzyme ([¹¹C]-DED).

so far is provided in **Figure 1**. Apart from the commonly used tracers for indirect neuronal functioning, such as glucose metabolism ([¹⁸F]-FDG) and perfusion, more specific receptor or protein deposition tracers used in other neurodegenerative diseases, like Parkinson's disease ([¹⁸F]-FP-CIT) and Alzheimer's disease ([¹¹C]-PIB), have also been investigated in ALS patients. Recently, tracers with an affinity for specific cell types, like neurons ([11C]-flumazenil, [11C]-WAY100635), microglial cells (TSPO ligands) and astrocytes ([11C]-DED), can also highlight specific pathophysiological processes of ALS.

2. FDG PET imaging in ALS

2.1. Early FDG PET and perfusion SPECT studies revealed widespread cortical abnormalities

In the 1980s and 1990s of the previous century a mere handful of studies investigated a small number of ALS patients using ¹⁸F-2-fluoro-2-deoxy-D-glucose PET (FDG PET) imaging. The common denominator of their findings was a generalized cerebral hypometabolism [35–37]. Despite the low spatial resolution of the PET scanners at the time, some studies did reveal a predominance for the motor cortex [36] and even provided preliminary evidence for frontal hypometabolism [37, 38] which was even related to clinical cognitive impairment in one study. A few studies assessing cerebral perfusion using SPECT imaging confirmed a predominant involvement of the motor cortex with extensive frontal hypoperfusion, often related to cognitive impairment [39–41]. These pilot studies were instrumental in redirecting the view of ALS as a disease exclusively affecting motor neurons towards a multisystem neurodegenerative disease as generally accepted nowadays.

2.2. Regions involved on recent FDG PET imaging

More recent FDG PET studies in ALS patients, using the next-generation PET scanners with higher spatial resolution, could confirm the presence of hypometabolism in the primary motor cortex, which is hence thought to be the signature of ALS on FDG PET imaging [42–44]. Besides the primary motor cortex, other peri-Rolandic regions like the premotor cortex and primary sensory cortex have been found to be affected on FDG PET [42–45]. In keeping with the clinical and pathophysiological overlap with FTD, several prefrontal (dorsolateral prefrontal cortex, orbitofrontal cortex, anterior frontal cortex) and temporal (anterior temporal lobe, fusiform gyrus) regions frequently display hypometabolism in ALS patients [42–45]. Some studies even report hypometabolism of primary and associative visual cortices in ALS patients [42, 45]. Examples of typical FDG PET patterns seen in ALS are given in **Figure 2**. Patient 1 is an example of an ALS patient with modest hypometabolism in the peri-Rolandic areas on FDG PET. In patient 2, extensive hypometabolism in the motor cortex can be noted. In about 10% of patients, extensive regions of hypometabolism are also present in the frontal and/or anterior temporal lobes. Patient 3 is an example of an ALS patients with extensive areas of hypometabolism in the frontal areas. Patient 4 has pronounced anterior temporal hypometabolism.



Figure 2. Commonly affected regions on FDG PET in ALS patients. Three regions frequently and typically display hypometabolism on FDG PET in ALS patients; motor cortex, prefrontal cortex and anterior temporal lobe. The three left images depict the stereotactic surface projections of brain FDG PET uptake. The three images on the right show the corresponding Z-score images (comparing patient to healthy volunteers). In patient 1 and 2, hypometabolism in the Rolandic area (including parts of the motor cortex) is noted. While this is only mild in patient 1, patient 2 has extensive hypometabolism. In patient 3, an obvious hypometabolism in the prefrontal cortex is noted. In patient 4, extensive hypometabolism of the anterior temporal lobe is noted.

Recently, several studies also reported the presence of (relative) hypermetabolism on FDG PET in certain regions. Hypermetabolism in ALS patients seems to be most obvious in the infratentorial region, like midbrain, pons and cerebellum [44–46]. This hypermetabolism is thought to be the reflection of increased astrocytosis along the course of the CST [45]. Also, hypermetabolism in mesial temporal structures, like hippocampus and amygdala, has been reported [44, 45].

2.3. Detection of frontotemporal involvement on FDG PET

As explained above, a clear link between ALS and FTD has been established over the last years. This is also backed up by the early studies showing frontotemporal hypometabolism using FDG PET and rCBF measures [37, 38]. Based on the extent of frontotemporal versus motor neuron involvement, patients across the ALS-FTD spectrum can be divided into five categories [47]. Pure ALS (without any evident cognitive abnormality) and pure FTD (without any obvious motor abnormality) are located at the opposite ends of this spectrum. Patients who meet diagnostic criteria for both ALS as FTD are considered to have 'ALS-FTD'. ALS patients with mild behavioural dysfunction are classified as having ALS with behavioural impairment ('ALSbi'), whereas patients with mild executive and language dysfunction are said to have ALS with cognitive impairment ('ALSci'). Patients with a diagnosis of FTD and some motor neuron involvement are said to have 'FTD-MND'.

The signature of frontotemporal involvement on PET imaging constitutes hypometabolism mainly focused on the prefrontal cortex, often extending to the entire frontal cortex and anterior temporal cortex and even to the thalamus [48, 49]. Contrary to FTD patients, this frontotemporal hypometabolism is often more symmetric in ALS-FTD patients [49]. Importantly, this hypometabolism is often already present in otherwise normal frontotemporal lobes on MRI, leading to the general view that hypometabolism precedes atrophy [50]. This makes FDG PET a very sensitive tool to detect frontotemporal involvement even in a very early clinical stage. Hence, frontotemporal hypometabolism on FDG PET has extensive potential to become an important diagnostic marker, and this independently from hypometabolism in the primary motor cortex.

Several studies performing both PET imaging as neuropsychological testing in ALS patients found a correlation between extent of frontotemporal hypometabolism and neuropsychological performance [37, 38, 43]. A recent large study indeed confirmed that ALS patients with mild cognitive impairment ('ALSci') exhibit moderate frontotemporal hypometabolism, which is clearly more pronounced in real 'ALS-FTD' patients and less pronounced to even absent in 'pure ALS' patients [48]. So, when patients are stratified along the ALS-FTD spectrum, there seems to be a proportionate correlation between clinical cognitive involvement and fronto-temporal hypometabolism on PET imaging.

Frontotemporal hypometabolism on PET not only has the potential to become a diagnostic marker, it has also been shown to provide important prognostic information. In a considerably large study of ALS patients, extensive prefrontal hypometabolism was associated with significantly shorter survival [51].

2.4. FDG PET as a (differential) diagnostic marker

Extensive studies demonstrating high diagnostic accuracy are a prerequisite for any imaging biomarker to become incorporated into the diagnostic criteria of ALS. Recently, four large studies assessed the sensitivity and specificity of FDG PET in ALS patients compared to healthy controls. The first study made use of a 'region of interest method' [42]. When all brain regions were taken into account, FDG PET was able to discriminate ALS patients from controls with a sensitivity of 89% and specificity of 82.5%. When only a specific set of regions was used, an even higher sensitivity (95%) with the same specificity (82.5%) could be obtained. The second study analysed FDG PET images using a method evaluating disease-specific spatial patterns on a voxel-based manner, trying to disclose the networks with the highest diagnostic value [52]. When all relevant networks were taken into account an astonishing accuracy of 99.0% was achieved. Remarkably, when only the most discriminating network (i.e. bilateral cerebellum and midbrain) was used, accuracy was still 96.3%. The third study reported an accuracy of 89.7% using a VOI-based discriminant analysis, further increasing to 95% if a discriminant analysis based on a support vector machine (SVM) approach was used [44]. In the fourth study, a prospective validation of the diagnostic algorithm based on SVM was carried out in 105 novel cases and a sensitivity and specificity of 100% was obtained [51]. All these studies suggest that FDG PET is a very sensitive marker of ALS pathology that can be used in a clinical setting.

There is, however, one major limitation of these studies. While these studies report a high sensitivity using healthy controls, it is clear specificity may be lower since ALS patients need to be discriminated from patients with ALS mimicking diseases. Unfortunately, no large studies using FDG PET in such disease mimics exist. A study performing FDG PET in ten patients with Kennedy's disease even surprisingly revealed the presence of frontal hypometabolism [53]. This means that FDG PET may be insufficient to discriminate ALS from certain ALS mimicking diseases.

2.5. FDG PET as a prognostic marker

Besides its capacities as a diagnostic marker, FDG PET is increasingly proposed as a potential marker for prognosis. So far, only two studies assessed the value of FDG PET in predicting the prognosis of ALS [44]. Assessing 70 ALS patients, extensive prefrontal hypometabolism was associated with a significantly shorter survival. This is in line with previous studies that reported concomitant clinical FTD or cognitive/behavioural impairment with a worse prognosis [4]. More recently, a larger prospectively collected cohort of 175 (including the initial 70 patients) was studied [51]. It was confirmed with longer follow-up that extensive hypometabolism in the prefrontal or anterior temporal lobe was associated with a shorter survival. In a Cox regression, taking into account other prognostic factors, such as age of onset, site of onset, diagnostic delay, FVC and slope in the ALS FRS-R, extensive frontotemporal hypometabolism was significantly correlated with shortened survival (**Figure 3**).



Figure 3. Relation of ALS survival with degree of frontotemporal hypometabolism. Kaplan-Meier survival plot of all ALS patients (ALS group 1 + ALS group 2) with (red line, n = 34) and without (blue line, n = 141) extensive hypometabolism in the frontal and/or temporal cortex (n = 175, p < 0.001) (This research was originally published in *JNM*. Van Weehaege et al. prospective validation of 18F-FDG brain PET discriminant analysis methods in the diagnosis of amyotrophic lateral sclerosis. *J Nucl Med*. 2016;vol:pp-pp. © by the Society of Nuclear Medicine and Molecular Imaging, Inc.) [51].

3. Other tracers

3.1. Tracers assessing the extrapyramidal system

The exact role of the extrapyramidal system in the pathophysiology of ALS is still under debate. Although several large clinical studies reported the increased presence of extrapyramidal symptoms and signs (increased tonus, postural instability and backward falls) in ALS patients [54, 55], others even reported an increased incidence of Parkinson's disease in the ALS population [56]. The presence of extrapyramidal symptoms in ALS is correlated with the presence of a hexanucleotide repeat expansion in *C90rf72* in many patients [57, 58]. PET studies have tried to make a significant contribution in elucidating a possible link between ALS and extrapyramidal symptoms.

[¹⁸F]-fluorodopa was one of the first radioligands used to assess the integrity of the nigrostriatal tract with PET by quantifying dopadecarboxylase activity presynaptically, while nowadays dopamine transporter imaging due to its better access using [¹²³I]-FP-CIT SPECT is the most widely used method to assess the extrapyramidal system. More novel and specific tracers such as [¹⁸F]-PE2I for PET dopamine transporters are already in use at several centres worldwide [59].

Literature data regarding extrapyramidal radionuclide imaging in ALS patients is still sporadic and seems to be discordant. While two earlier studies reported a dopaminergic deficit in sporadic and familial ALS patients without clinical extrapyramidal disease [60, 61], this was contradicted in a more recent small study investigating ALS patients with concomitant clinical parkinsonism ('ALS-parkinsonism') [62]. Another study did report dopaminergic deficits in two ALS-parkinsonism patients assessed by [¹⁸F]-FP-CIT PET [63]. These discrepancies are believed to be due to the heterogeneity of ALS-parkinsonism, and larger datasets are needed.

While in some cases the extrapyramidal signs are thought to be caused by true degeneration of the extrapyramidal system, in other cases, they are believed to be the result of cortical lesions. The latter concept is confirmed by a more recent study investigating UMN-ALS patients using [¹²³I]-FP-CIT SPECT [64]. While a dopaminergic deficit was indeed evident in the majority of patients which even correlated with disease duration, there was no correlation with functional extrapyramidal scores (like UPDRS). This means that, although the neuropathological process in ALS extends towards the extrapyramidal system, some of the extrapyramidal signs noted in ALS patients are probably due to spasticity, which is a typical UMN feature.

3.2. Tracers for neuroinflammation

Neurons rely on several supportive cells, commonly named glial cells, to survive and exert their normal function. These glial cells, including astrocytes, microglia and oligodendrocytes, provide nutritional and trophic support to neurons, especially motor neurons. ALS is characterized by a neuroinflammatory reaction consisting of an activation of astrocytes and microglia. Several studies using ALS animal models have taught us that dysfunction of these cell types significantly contributes to motor neuron death, independent of intrinsic motor neuron dysfunction, leading to the view of ALS as a non-cell autonomous disease [65].

Two targets have been used to visualize this 'neuroinflammation' in ALS patients using radionuclide imaging. [¹¹C]-DED, a deprenyl derivative, selectively binds the MAO-B enzyme which is primarily though not exclusively located in astrocytes. Because of the high background activity of this ligand and newer derivatives, they are limited to research applications. The second, more frequently used target is the activated microglial cell, the macrophages of the central nervous system [66]. One of the most studied targets for activated microglia is the translocator protein (TSPO), a mitochondrial protein that is highly overexpressed in activated microglia [67]. Several radioligands such as [¹¹C]-(R)-PK11195, [¹⁸F]-DPA-174 and [¹¹C]-PBR28 can be used to quantify TSPO binding.

The major finding in TSPO PET studies in ALS is an increased binding in the motor cortex, highlighting this region as the primary focus of neuropathology [68–70]. The degree of neuroinflammation in the motor cortex is positively correlated with clinical UMN scores and probably negatively correlated with ALS-FRS. Other frequently involved regions are the prefrontal cortex, thalamus, pons and CST [68–71]. The latter two probably reflect the secondary neuroinflammation due to degeneration of the CST. Interestingly, some studies have shown that the inflammation can be detected on the individual patient level.

Thus, PET imaging assessing neuroinflammation has a high potential to become a specific UMN marker which can be used at the single patient level. Moreover, it could be an interesting method to monitor the effect of treatment selectively targeting the neuroinflammatory process.

3.3. Tracers reflecting neuronal loss and/or dysfunction

Although the neurodegenerative process in ALS is non-cell autonomous, markers of selective motor neuron death would be of significant value. FDG PET assesses glucose metabolism in general, hence encompassing various processes like neuronal dysfunction, atrophy, microgliosis and astrocytosis. Therefore, the capabilities of FDG PET to selectively assess motor neuron degeneration are limited.

Two tracers have been used so far to specifically assess neuronal loss and/or dysfunction. [¹¹C]flumazenil, which selectively binds GABA-A receptors expressed by neurons, is the most widely used. It is, however, unclear whether this ligand primarily visualizes pyramidal neurons (like motor neurons) or interneurons. Although an early study reported an almost generalized decreased signal in the cortex [72], more recent studies revealed a more selected involvement of primary motor and motor association cortices, which was even correlated with a clinical UMN score [73, 74]. One study used the radioligand [¹¹C]-WAY100635 targeting the 5-HT1A serotonin receptor which is expressed in pyramidal neurons [75]. They also reported a generalized cortical decrease, which was most pronounced in the motor cortex and frontotemporal regions.

So, these neuronal radioligands seem to be able to specifically highlight the primary pathophysiological process of ALS, which is degeneration and dysfunction of the UMNs. Hence, it seems promising to further investigate the diagnostic and prognostic value of these potential UMN markers.

4. Correlation with phenotype

4.1. ALS subtypes

The pattern of motor neuron involvement in ALS is highly heterogeneous. Depending on the relative upper and LMN involvement and depending on the neuroanatomical region within the motor system with the most extensive pathology, different subtypes of motor neuron degeneration have been defined. While in some patients UMN features (spasticity, hyperreflexia) dominate the clinical picture, LMN features prevail in other patients. It remains unclear if the pure UMN disorder, PLS (primary lateral sclerosis), and the pure LMN disorder, progressive muscular atrophy (PMA), should be regarded as separate disease entities or merely as the extreme ends of the ALS spectrum [25]. Similarly, the site of onset is also highly variable, with onset in a limb ('spinal onset') being more frequent than onset in the bulbar musculature ('bulbar onset') [25]. The neuroimaging signature of these subtypes has not yet been extensively studied using PET. The challenge will be to find commonalities and differential representations of the different endophenotypes.

4.1.1. PLS and Mills' syndrome

PLS is a variant of ALS with selective UMN signs for several years [76]. Mills' syndrome is an unusual unilateral variant of PLS, which eventually spreads to the contralateral side after a variable time period [25]. On postmortem examination, there is no difference in the essential pathological processes (e.g. TDP-43 positive intraneuronal inclusions) [77]. However, to explain this phenotypic variability, there must be a difference in the focal initiation and spreading pattern of the neurodegeneration. PET imaging has several advantages to assess this question, like in vivo usability and availability of specific tracers. Unfortunately, since no large studies investigating PET in PLS/Mills' patients have been undertaken so far, we need to rely on a handful of small case series. Four studies in PLS patients performed either FDG PET or [¹¹C]-flumazenil PET and mainly found similar abnormalities as seen in ALS patients [44, 51, 78, 79]. However, in one study involvement of the primary motor cortex seemed to be more severe than in pure ALS [79]. On the other hand, some specific regions like the prefrontal cortex and posterior cingulate seem to be spared in PLS [44, 78]. So, based on these PET findings, neuropathology in PLS may be more restricted to the motor cortex.

Only five cases of Mills' patients with PET imaging have been reported so far. While one patient had an asymmetric involvement of the motor cortices, an almost unilateral pattern of hypometabolism (FDG PET) or hypercaptation (TSPO radioligand) has been noticed in the four remaining patients [80–82]. So, compared to PLS, neuropathology in Mills' syndrome seems to be focused even more on one unilateral motor cortex, suggesting a more restricted contiguous spread of disease in this endophenotype.

4.1.2. PMA

PMA is a motor neuron disease with selective involvement of the LMN, and probably has to be seen as an unusual variant of ALS [25]. Literature regarding FDG PET in PMA patients is

very limited. Only two early studies performing FDG PET in ALS patients performed a subanalysis in patients with only LMN signs. In keeping with the clinical absence of UMN signs, no to only very mild cerebral hypometabolism is present in these patients [35, 36]. Larger studies are required to establish if peri-Rolandic hypometabolism is present in a proportion of PMA patients and if this predicts progression to ALS or disease outcome.

In comparison, a few MRI-based imaging studies investigating UMN involvement in PMA remained inconclusive as well. One study found no evidence for thinning of the motor cortex on high-resolution MRI [83]. While one study found modest though clear abnormalities of the CST on DTI imaging [84], this was contradicted by another earlier study [85]. Finally, an fMRI study revealed modest prefrontal activation abnormalities in PMA patients [86].

So based on several imaging modalities, it is so far unclear whether significant measurable UMN involvement is present in all PMA patients.

4.1.3. Spinal versus bulbar

In most ALS patients, the disease starts with asymmetric weakness of a limb, and hence, this classical form of ALS is called 'spinal onset ALS'. In about 20% of ALS patients, however, weakness starts in the bulbar muscles, this form is called 'bulbar onset ALS' [25]. While both endophenotypes clearly have a distinct disease initiation and disease course, they eventually converge into a common phenotype of generalized weakness. The pathological substrates underlying these initial differences are largely not understood. PET imaging has tried to elucidate some aspects of this enigma.

While one study using FDG PET suggested a differential pattern of involvement between spinal and bulbar onset ALS in frontal and parietal regions [45], this was not confirmed by others [42]. A study investigating perfusion (regional cerebral blood flow, rCBF) in ALS patients reported a significantly lower rCBF of the frontal lobe in bulbar onset patients compared to spinal onset [87]. Based on TSPO PET imaging, one study found evidence for increased neuroinflammation in the brainstem of bulbar onset ALS patients, whereas neuro-inflammation in the motor cortex seemed to be less pronounced [70]. So, the specific functional imaging correlate of these two endophenotypes has not been clearly established yet.

4.2. Severity of disease

An independent objective marker for severity and spreading pattern of disease pathology is highly needed. None of the imaging biomarkers so far has been able to reflect local disease severity and disease spreading in a longitudinal fashion. A limited amount of studies investigated the link between PET imaging and clinical scores. First, although several FDG PET studies in ALS patients found no correlation with parameters of disease severity in general, one study did reveal a correlation of prefrontal hypometabolism with a reduced ALS-FRS R (ALS functional rating scale revised version) [44]. Second, PET imaging of microglia, via TSPO radioligands, was not correlated with ALS-FRS R in two studies [68, 69]. However, one of these studies found a relation with clinical burden of UMN signs [68]. Third, abnormalities on [¹¹C]-flumazenil PET in one study was not correlated with ALS-FRS, whereas it was associated with

the UMN score [74]. So in general, findings with the currently available PET targets show very little, if any, clear correlation with clinical severity of disease. Longitudinal studies are required to find out if PET imaging is of value in tracking disease progression.

5. Correlation with genotype

While most cases of ALS are sporadic, about 10% are caused by a variety of genetic deficits, with alterations in the *C9orf72* and *SOD1* gene being the most frequent and most studied [88]. A few PET studies have been performed in genetic subtypes of ALS.

5.1. C9orf72

Two recent studies investigated 26 patients in total with *C9orf72*-related ALS using FDG PET and found in general more severe hypometabolism [44, 46]. Remarkably, both studies also reported hypometabolism in the thalamus and parts of the limbic system to be present almost uniquely in *C9orf72* patients. An example of FDG PET findings in a *C9orf72* ALS patient is



Figure 4. PET abnormalities in a C9orf72 ALS patient. Example of Z-score images of FDG PET imaging of a *C9orf72* patient with ALS revealing a remarkable hypometabolism of the thalamus, as noted on axial (A and B) and sagittal (C and D) sections.

provided in **Figure 4**. This involvement of thalamus, extrapyramidal system and limbic system is probably the neuroanatomical correlate of the increased incidence of phenocopies of several other neurodegenerative diseases in *C9orf72* mutation carriers. Among others, phenocopies of Parkinson's disease, Huntington's disease, corticobasal degeneration, Alzheimer's disease and several neuropsychiatric diseases (psychosis, schizophrenia) have been reported in *C9orf72* mutation carriers [57].

5.2. SOD1 (D90A)

Worldwide, the D90A is the most common *SOD1* mutation, although it is not the most studied one [89]. This mutation can be inherited in an autosomal recessive or dominant way, which is quite unusual in ALS. Two studies performing [¹¹C]-Flumazenil-PET in a total of 21 D90A patients revealed that these patients showed a specific pattern of reduced tracer binding confined to the left frontotemporal junction and anterior cingulate gyrus, without involvement of the motor and premotor cortices [73, 74]. Larger studies on other *SOD1* mutations or other genetic subtypes of ALS are largely lacking.

6. Future perspectives

More than a decade PET imaging has seen a revival in the ALS field using either new hardware and software technologies or novel tracers. In the near future, PET will hopefully find applications in both clinical practices, namely clinical trials and neurobiological research.

First, PET imaging could become of value in the ALS diagnosis in the future. Although conventional imaging (e.g. MRI) is only intended to rule out other diseases, FDG PET has the potential to be used as a positive argument to make a diagnosis of ALS at the single patient level. Both hypometabolism in the motor cortex as in the frontotemporal cortex will be of diagnostic value and could be considered to incorporate in clinical criteria for ALS, similar to the inclusion of frontotemporal hypometabolism in the diagnostic criteria of FTD [90]. However, additional research comparing ALS patients with ALS mimicking diseases is needed to reliably assess the sensitivity and especially the specificity in the real-life clinical setting of early diagnosis.

Second, PET imaging will be further investigated as a biomarker of disease. More studies assessing the correlation with severity of disease need to be undertaken. Similarly, more longitudinal studies are needed to relate early PET abnormalities with clinical course, hopefully fulfilling the high need for a prognostic marker in the clinic.

Third, PET imaging could become valuable in clinical trials of ALS patients. By increasing the diagnostic yield, especially early in the disease, it will be possible to include patients early after disease onset, hence increasing the power to obtain positive results in pharmaceutical trials. Additionally, several tracers (neuroinflammation, neuronal loss ...) could be used as a read out to demonstrate target engagement or even to assess the effect of treatments. Of particular

interest, a tracer for TDP-43 would revolutionize the field, as PET detection and quantification of misfolded proteins have done in Alzheimer's disease with beta-amyloid and tau imaging.

Fourth, tracers assessing neuroinflammation will be further investigated, and aside from TSPO other targets such as type 2 cannabinoid receptors, the purinergic receptor P2X7, and matrix metalloproteinases are investigated currently. The power of protein or receptor tracers mainly lies in the selectivity of their target, that is they selectively reflect one aspect of ALS pathogenesis which increases with disease progression. Hence, in contrast to FDG PET which reflects general glucose metabolism, these tracers have the potential to be used as 'positive tracers' for disease severity and progression. They are also of high interest to gain insight in the pathophysiology of ALS. Probably, several tracers assessing other aspects of ALS pathology will be developed and investigated as well.

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