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Biomarkers in Amyotrophic Lateral Sclerosis

Facts and Future Horizons

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Abstract

The only specific marker of sporadic amyotrophic lateral sclerosis (ALS) is neuropathologic, namely the presence of inclusions staining positively for ubiquitin and TAR DNA-binding protein (TARDBP, also known as TDP-43) in degenerating motor neurons. Abnormalities in various physiopathologic pathways associated with ALS, such as oxidative stress, inflammation, and excitotoxicity, have been reported in blood, cerebrospinal fluid, and muscle biopsies. A number of studies in ALS patients have indicated that nuclear magnetic resonance (NMR) spectroscopy and diffusion tensor magnetic resonance imaging (MRI) can detect corticospinal lesions. However, because of their relative lack of sensitivity and specificity, these techniques are currently inadequate for use as diagnostic tools in individual patients. Recently, there has been much interest in the use of high-throughput techniques such as transcriptomics, proteomics, and metabolomics for the detection of biomarkers. In the future, a combination of biologic, radiologic, and electrophysiologic markers, rather than a single marker, may prove a useful tool for the diagnosis and follow-up of ALS patients. This article provides an overview of recently described biologic and radiologic markers of the disease.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the combined degeneration of central motor neurons, whose cell bodies are located in the motor cortex and give rise to the pyramidal tract, and peripheral

motor neurons, whose cell bodies are located in the spinal cord and brainstem.[1-4] The only marker specific for sporadic forms of ALS is neuropathologic, namely the presence in degenerating motor neurons of inclusions staining positively for ubiquitin and, as has been demonstrated recently, for the TAR DNA binding protein (TARDBP, also known as TDP-43).[5] In the absence of a specific marker, diagnosis of ALS in clinical practice is based on medical history, clinical examination, electromyography, and exclusion of alternative differential diagnoses.[6] There is therefore a lot of interest in finding a specific marker for ALS, both for diagnostic purposes and for monitoring disease progression. This article evaluates recently described biologic and radiologic markers of the disease. Electrophysiologic markers of ALS in general, and quantification of motor units in particular, are not discussed. We refer the reader to previous reviews of this specific topic, and particularly to a recent review highlighting the usefulness of new consensus electrophysiologic criteria (the 'Awaji criteria').[7]

Relevant citations were identified through PubMed up to October 2008. We also used personal collections of references and reference lists of articles. There were no constraints based on language or publication status.

1. Significance of Markers in Amyotrophic Lateral Sclerosis (ALS)

The phenotypic expression of ALS is heterogeneous, including topographically restricted forms, slowly or rapidly evolving forms, forms associated with temporo-frontal dementia, and sporadic and familial forms. From a physiopathologic point of view, this raises the question of whether ALS is a single disease entity or a syndrome of different clinical entities arising from distinct biologic mechanisms. One way to answer this question is to determine whether the different forms of the disease present common or distinct markers. One example is the recent demonstration that TARDBP-positive motor neuron inclusions, which are characteristic of sporadic forms of ALS, are not found in genetic forms linked to the superoxide dismutase (*SOD1*) gene, thereby suggesting that familial and sporadic forms may not share the same pathogenetic pathway.[8] Another example concerns forms of ALS encountered on islands in the Western Pacific. It is now well documented that the histopathologic hallmark of these forms is neuronal accumulation of neurofilaments comprised of tau protein, as seen in Alzheimer's disease.[9] This suggests that these particular forms of ALS should be included in the group of tauopathies and therefore represent a category of motor neuron disease that is distinct from classical ALS.

From a clinical perspective, biomarkers would be extremely useful for diagnosis and monitoring of the disease. Diagnosis of ALS is often difficult because of the phenotypic heterogeneity of the disease and conditions mimicking ALS that represent about 7% in population-based studies.[10] Clinical studies[10,11] and some review articles[12,13] have drawn attention to the limitations of current diagnostic criteria (the 'El Escorial criteria')[1] as a way of identifying different forms of disease. These difficulties may explain, at least in part, the frequent delay in diagnosis.[14,15] The median delay between the appearance of the first symptoms and diagnosis of ALS is between 8 and 11 months.[15-17] This diagnostic delay is detrimental to the management of the disease and precludes early initiation of neuroprotective treatment aimed at preserving surviving neurons.[18] Because of the extreme variability in the clinical course of the disease in terms of both functional degradation and survival, which has been described in many natural history studies and therapeutic trials, it is impossible in practice to establish a vital or functional prognosis for an individual patient. Life expectancy is extremely variable, ranging from 6 months to more than 20 years after the appearance of the first signs of the disease. This variability contributes to the difficulty in identifying variables that may predict the course of the disease and indeed, in studies performed to date, no clinical marker has demonstrated a predictive power of at least 80%.[19]

As far as therapeutic trials are concerned, the absence of disease markers has two principal negative consequences. First, the phenotypic heterogeneity of the disease limits the inclusion of patients in therapeutic trials.[14,15,17] In particular, the absence of clinical signs of upper motor neuron degeneration is an obstacle to including patients in clinical trials; this requires patients to fulfil the revised El Escorial criteria.[17] On the other hand, this variability makes it necessary to include a large number of patients in a trial in order to achieve sufficient statistical power to demonstrate a clinical effect of disease-modifying treatments, particularly on survival. The need to set up large and expensive trials thus limits the number of molecules that can be evaluated in humans at any one time. This problem highlights the interest of finding and using surrogate markers of disease activity, as has proved useful in other neurologic diseases.

2. Biologic Markers of ALS

2.1 Markers in Blood and Cerebrospinal Fluid

To date, the only known specific biologic markers of ALS are the causal mutations identified in familial forms of the

Table I. Classification of familial forms of amyotrophic lateral sclerosis (ALS)

Gene	Locus	Transmission	Phenotype
SOD1	21q22.1	AD	Classical ALS
ALS2	2q33	AR	Juvenile ALS
SETX	9q34	AD	Onset juvenile, slow evolution, presence of sensory signs, absence of bulbar problems
VAPB	20q13	AD	ALS with shaking
TARDBP	1p36	AD	Classical ALS
FUS/TLS	16p11.2	AD	Classical ALS

AD = autosomal dominant; **ALS2** = juvenile ALS (alsin protein); **AR** = autosomal recessive; **FUS/TLS** = protein fused in sarcoma/translocation in liposarcoma; **SETX** = senataxin; **SOD1** = superoxide dismutase; **TARDBP** = TAR DNA-binding protein (TDP-43); **VAPB** = VAMP (vesicle-associated membrane protein)-associated protein B

disease. Familial cases of ALS represent around 10–20% of patients.[20] The causal gene is known in only a minority of cases and most often corresponds to the gene encoding superoxide dismutase type 1 (SOD1), which is responsible for around 10–20% of familial forms. Mutations in the *SOD1* gene can be considered as a genetic marker of ALS and not as a polymorphism, where it has been demonstrated that the mutation is pathogenic or co-segregates with disease within a family.[21] Other genes have been associated with rare familial forms of ALS (table I). Recently, mutations in the gene coding for the protein TARDBP[22] and the protein FUS/TLS (fused in sarcoma/translocated in liposarcoma)[23,24] have been identified in familial and sporadic cases. An increased risk of sporadic ALS has been associated with a number of other genes such as *SMN1* (survival of motor neuron 1, telomeric), *ANG* (angiogenin), *HFE* (hemochromatosis) and *PON1/2* (paraoxonase).[25]

Various biologic changes have been described in the blood or cerebrospinal fluid (CSF) of ALS patients (table II). Several studies have demonstrated anomalies in various physiologic pathways implicated in ALS, such as oxidative stress (for example, increase in 4-hydroxy-2,3-nonenal levels),[26] trophic factors (increase in transforming growth factor-β1),[27] excitotoxicity (increase in glutamate),[28] or inflammation (increase in monocyte chemoattractant protein-1).[26,29] Interpretation of these findings should take sufficient account of the methodologic limitations of many of these studies, which have involved small numbers of patients with poorly defined control populations. Furthermore, many of these changes have been published by a single group and have not been replicated independently. In all cases, even where the studies are methodologically robust, the biologic anomalies observed cannot be considered to be sensitive and specific markers of the disease. A correlation with the clinical severity of ALS has only been demonstrated in a minority of cases, such as with serum levels of certain markers of oxidative stress, inflammation or glutamate (tables II and III).

2.2 Muscular Markers

Another approach to the identification of biomarkers in ALS is based on the demonstration of muscular abnormalities. Recent arguments suggest that destabilization of the neuromuscular junction is an early event in the disease, with degeneration of motor neurons occurring in a retrograde fashion ('dying back').[53-55] Signals originating in the muscles seem to be involved in this process, and there has been particular interest in the expression of neurite outgrowth inhibitor (Nogo-A; also known as reticulon 4 [RTN4]) in muscles. This protein is an inhibitor of axonal growth, which is expressed by oligodendrocytes and is not normally detected in muscles. In one study, ectopic expression of Nogo-A in muscles was observed in patients with ALS but not in patients with myopathy or peripheral neuropathy.[56] Recently, Nogo-A has received attention as an early diagnostic marker of ALS in patients who present with isolated lower motor neuron lesions.[57] The presence of Nogo-A in muscular biopsy specimens was predictive of an evolution towards typical ALS, with a positive predictive value of 88% and a negative predictive value of 94%.[57] Furthermore, a correlation was also observed between the level of muscular Nogo-A and functional state,[58] suggesting that this protein could also act as a marker of disease progression (figure 1). Nonetheless, the specificity of Nogo-A as a biomarker for ALS has not been demonstrated unequivocally. Nogo-A has been detected in muscle biopsies from patients with myopathies or peripheral neuropathies in one study[60] but not in biopsies from patients with inclusion-body myositis in another.[61] Technical considerations (biopsy protocols, protein extraction, and the

Table II. Non-exhaustive list of blood parameters that have been reported to be altered in amyotrophic lateral sclerosis

	Parameters	Phenotype
Oxidative stress	HNE ↑[26]	Higher levels associated with higher disability (Appel score)
	GSH-Px activity ↑[30]	
	SOD activity ↓[31]	
	Hydroxyl radicals ↑[31]	
	Coenzyme-Q ↑[32]	Higher levels associated with longer duration
	Calcineurin ↓[33]	
	Bilirubin ↓[34]	NS
Growth factors	VEGF ↑[35]	Higher levels associated with shorter duration
	TGFβ-1 ↑[36]	Higher levels associated with longer duration
	Endoglin ↓[37]	NS
	CNTF ↑[38]	NS
Cytokines	TRAIL ↓[39]	NS
Inflammation	MCP-1 ↑[26]	Higher levels associated with higher disability (Appel score)
	PGE_2 ↑[40]	NS
	MMP-9 ↑[41]	
Other	Cholesterol and LDL ↑[42]	Higher levels associated with longer survival
	Reverse transcriptase activity[43]	
	Apo E[44]	Higher levels associated with shorter survivable and faster decline of functional scores

Apo E = apolipoprotein E; **CNTF** = ciliary neurotrophic factor; **GSH-Px** = selenium-independent glutathione peroxidase; **HNE** = 4-hydroxy-2,3-nonenal; **LDL** = low-density lipoprotein; **MCP-1** = monocyte chemoattractant protein-1; **MMP-9** = matrix metalloproteinase-9; **NS** = non-significant; **PGE_2** = prostaglandin E_2; **SOD** = superoxide dismutase; **TGFβ-1** = transforming growth factor-β1; **TRAIL** = tumor necrosis factor-related apoptosis-inducing ligand; **VEGF** = vascular endothelial growth factor; ↓ indicates decrease; ↑ indicates increase.

source and specificity of antibodies) may explain such discrepancies.[62] Further studies are necessary to determine the role of Nogo-A as a diagnostic marker in ALS.

2.3 Contribution of High-Throughput Techniques

Although data about the contribution of high-throughput techniques for the discovery of useful biomarkers in ALS are still scarce, this approach offers considerable potential. Using a mass spectrometry proteomics technique, Pasinetti et al.[63] detected anomalies in three protein species (4.8, 6.7, and 13.4 kDa), which enabled a correct diagnosis of ALS to be made with 91% sensitivity and 97% specificity. Protein sequence analysis identified the 13.4 kDa protein species as cystatin C and the 4.8 kDa protein species as a peptide fragment of the neurosecretory protein VGF. However, these results are yet to be confirmed, and comparisons with other neurologic pathologies, in particular those that can mimic ALS, are necessary. Analysis of the muscular transcriptome carried out in a murine model of ALS[64] also paves the way to the identification of new muscular markers in humans.

3. Radiologic Markers

There has been much interest in radiologic markers of ALS because of the difficulties in observing clinical signs of central motor neuron damage. Clinical upper motor neuron signs are initially absent in 7–10% of cases of ALS.[15,17] Furthermore, the risk of a diagnostic delay of >18 months is increased in patients who present with isolated lower motor neuron signs.[15] These forms pose problems in the differential diagnosis between ALS and other lower motor neuron pathologies such as spinal muscular atrophies or multifocal neuropathies with conduction blocks. The evolution of the disease is suggestive of a diagnosis of ALS when central signs appear; however, this is not the case in all patients, notably because the severity of peripheral signs can mask central signs.

3.1 Conventional Magnetic Resonance Imaging

Conventional cerebral and brainstem magnetic resonance imaging (MRI) has an important place in the differential diagnosis of ALS.[65] Signs indicating degeneration of the

pyramidal tract can sometimes be identified. These may consist of cortical atrophy, which is predominant in the frontal region, or a characteristic T2 or fluid-attenuated inversion recovery (FLAIR) hyposignal at the level of the primary motor cortex.[66,67] Numerous MRI studies, using various techniques (inversion-recuperation; magnetic transfer; diffusion imaging, or T1-, T2-, or fast-spin echo proton density-weighted MRI), have identified a focal hypersignal in the white matter along the corticospinal tract, from the *centrum semiovale* to the brainstem (figure 2).[67-78] However, these anomalies are inconsistent and are not readily quantifiable. Furthermore, no clear association between the speed of progression or the stage of the disease and the presence of this hypersignal has been demonstrated.[79,80]

3.2 Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy is an attractive method because it allows measurement of the neurochemical profile of a particular region of the brain *in vivo*. The main peak is *N*-acetylaspartate (NAA), which is a marker of neuronal integrity. Several studies have demonstrated a decrease in NAA[81,82] and/or ratios of NAA with choline-containing compounds (Cho) and creatine (Cr) [NAA/Cho and NAA/Cr ratios] at the level of the motor cortex of patients with ALS.[81,83] With regard to the diagnosis of ALS mimic syndromes and to the delineation of phenotypes, two small series showed that NMR spectroscopy may distinguish patients with ALS from patients with progressive muscular atrophy.[84,85] However, the diagnostic value of this test is poor because of the considerable overlap between values in healthy subjects and those in ALS patients. The combined measurement of the NAA peak and a marker of astrocyte gliosis, namely *myo*-inositol, has been proposed, but this is not sufficiently reliable to be used as a diagnostic tool.[86]

3.3 Diffusion Tensor Imaging

3.3.1 Principle

Diffusion tensor imaging (DTI) is a promising technique to evaluate the degeneration of white-matter fiber bundles. Diffusion is a process that results from the random movement of molecules *in vivo* (Brownian motion). It is characterized by its speed and by its direction, which depends on the structure of the brain tissue. Diffusion of water in the CSF is isotropic (identical in all directions), whereas in brain tissue it occurs preferentially along the axis of orientation of the white-matter fiber bundles (figure 3). The application of diffusion gradients in several directions in MRI results in a diffusion tensor image. A map of the apparent coefficient of diffusion and an anisotropy map can

Table III. Non-exhaustive list of cerebrospinal fluid parameters that have been reported to be altered in amyotrophic lateral sclerosis

	Parameters	Phenotype
Oxidative stress	HNE ↑[26]	
	GSH-Px activity ↑[30]	
	SOD activity ↓[45]	
	Nitrate ↑[45]	
	8-OHdG ↑[31]	
	CML ↑[46]	
Growth factors	TGFβ-1 ↑[27]	Higher levels in patients with long duration
	PEDF ↑[47]	
	VEGF ↓[48] or ↑[49]	
Cytokines	Flt3 ligand ↑[50]	NS
Inflammation	MCP-1 ↑[29,51]	
	PGE_2 ↑[40]	NS
Other	Glutamate ↑[28]	Higher levels associated with lower limb functional scores and faster rate of deterioration of manual muscle testing
	cGMP ↓[52]	NS

8-OHdG = 8-hydroxy-2′-deoxyguanosine; **cGMP** = cyclic guanosine 5′monophosphate; **CML** = Nε-(carboxymethyl)lysine; **Flt3** = fms-like tyrosine-kinase 3; **GSH-Px** = selenium-independent glutathione peroxidase; **HNE** = 4-hydroxy-2,3-nonenal; **MCP-1** = monocyte chemoattractant protein-1; **NS** = non-significant; **PEDF** = pigment epithelium-derived factor; **PGE_2** = prostaglandin E_2; **SOD** = superoxide dismutase; **TGFβ-1** = transforming growth factor-β1; **VEGF** = vascular endothelial growth factor; ↓ indicates decrease; ↑ indicates increase.

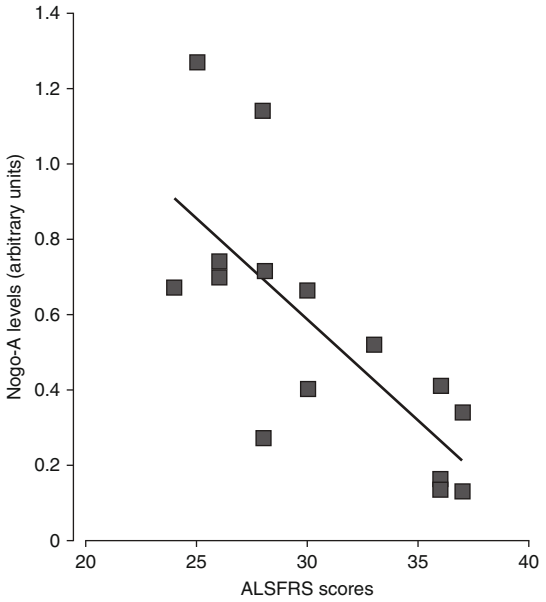

Fig. 1. Correlation between levels of Nogo-A (Western blot) in muscle biopsies (spot intensities are expressed as arbitrary units of optical density) and functional state measured on the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)[59]

thus be created, which provides useful information about the microstructural organization of the source tissue.

3.3.2 Abnormalities in ALS

Many studies have demonstrated an increase in the coefficient of diffusion (mean diffusivity) and particularly a decrease in fractional anisotropy (FA) in different areas of the intracranial portion of the corticospinal tract (subcortical white matter, internal capsule, and brainstem).[69,80,87-93] One study has also revealed a significant decrease in mean FA at the level of the cervical spinal cord.[80] In one study, abnormalities were also observed in patients who had no clinical signs of upper motor neuron involvement at the time of MRI investigation but developed pyramidal tract symptoms later in the course of their disease.[87] Thus the authors suggested that tensor MRI can be used to assess upper motor neuron involvement in ALS patients before clinical symptoms of corticospinal tract lesions become apparent, and that it may therefore contribute to earlier diagnosis of motor neuron disease. The use of tractography methods in association with DTI, which enables reconstruction of the three-dimensional geometry of the pyramidal tract, appears promising.[94,95] This type of approach makes it possible to establish an FA profile along the pyramidal tract.[95] Furthermore, by using a voxel-by-voxel approach, which consists of comparing groups of patients without an *a priori* predefined region of interest, it has been demonstrated that the anomalies on DTI exist outside the primary motor regions, thereby confirming that ALS is a multisystem degenerative disorder (figure 4).[89] This diffusion of lesions has also been observed using other neuroradiologic techniques such as voxel-by-voxel morphometry (VBM).[96]

The diagnostic value of DTI remains limited because of overlap between the values measured in ALS patients and those in control subjects. In one study, measurement of FA in the internal capsule to detect central motor neuron lesions compared with healthy subjects had a sensitivity of 95%, but the specificity was only 71%, with a positive predictive value of 82%.[78]

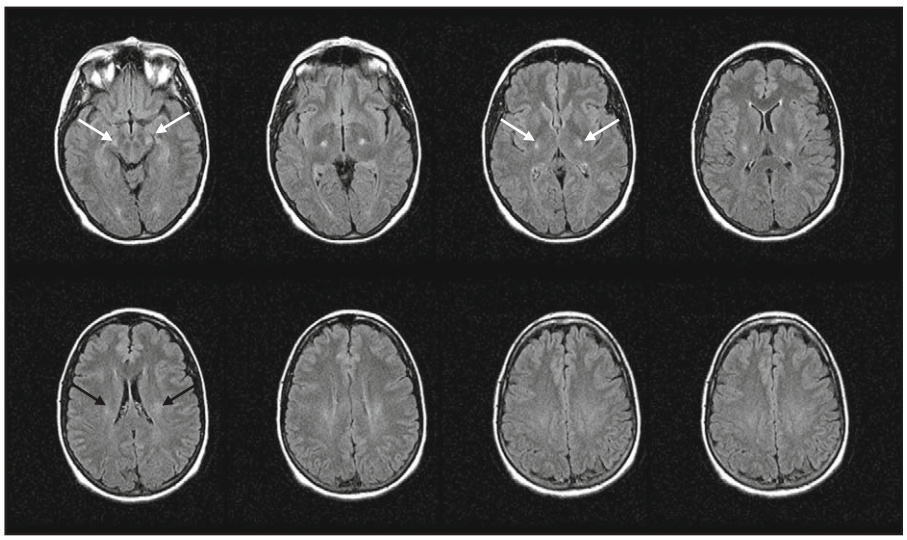

Fig. 2. Conventional magnetic resonance imaging (MRI) in amyotrophic lateral sclerosis. Hypersignal of the corticospinal tract on fluid-attenuated inversion recovery (FLAIR) sequences. This hypersignal (arrows) is visible along the corticospinal tract from the *centrum semiovale* to the brainstem.

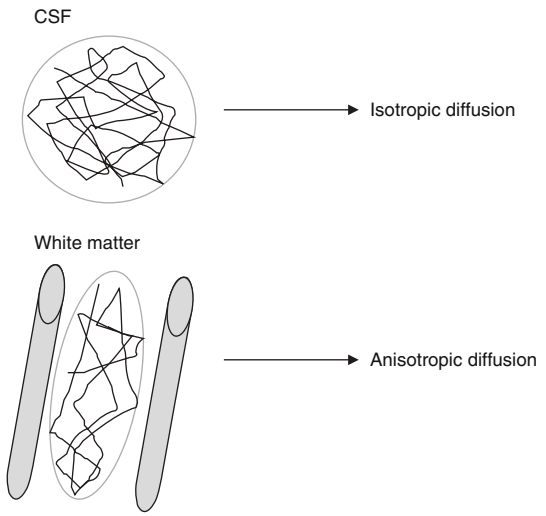

Fig. 3. Principle of diffusion tensor magnetic resonance imaging (MRI). Diffusion of water molecules in the cerebrospinal fluid (CSF) is identical in all directions (isotropy). In the white matter, the diffusion takes place preferentially along the axis of orientation of the fiber bundles (anisotropy).

3.4 Nuclear Imaging Techniques

Positron emission tomography (PET) and monophotonic emission tomography (single photon emission computerized tomography [SPECT]) are nuclear imaging techniques, which use various tracers to either reveal neuron dysfunction or loss directly, or are associated with a pathogenic mechanism involved in the disease. In SPECT, after injection of hexamethylpropyleneamine oxime labelled with technetium-99m (^{99m}Tc), several studies have demonstrated a decrease in cerebral blood flow, reflecting variations in underlying neuronal activity in the primary motor cortex of ALS patients,[97-100] which can also extend in an anterior fashion into the frontal lobes, particularly in patients with associated cognitive problems (figure 5).[101] In PET with 2-fluoro-2-deoxy-glucose, a variable decrease in cerebral glucose metabolism at rest has also been observed in ALS.[102,103] Recently, one PET study using a specific marker for serotonin $(5\text{-HT})_{1A}$ receptors has identified a global cortical decrease in fixation of the marker, predominantly in the frontal and temporal lobes.[104] Complementary studies are, however, necessary to determine the role of this technique in detecting cortical lesions in these patients. Microglial activation is known to be involved in the physiopathology of ALS, and another study using a ligand expressed by activated microglia has detected microglial activation in the motor cortex, as well as the thalamus, protuberance and prefrontal dorsolateral cortex of ALS patients.[105] This tool could be useful for measuring the effect of treatment targeting inflammation in ALS.

3.5 Functional Imaging Techniques

Functional PET and MRI (fMRI) imaging techniques are research tools that can be used to study cortical reorganization. Regional modifications in cerebral blood flow have been studied during hand motor tasks.[106,107] Interestingly, some studies have demonstrated that cerebral activation involved more extensive cortical regions than in control subjects and also involved the controlateral cortex. This phenomenon is interpreted as a functional compensation mechanism for motor cortical lesions in ALS.

3.6 Radiologic Markers of Disease Progression

Several studies have provided consistent evidence for correlations between clinical variables reflecting the severity of the disease, and radiologic markers such as the NAA peak in spectroscopy[90] or the extent of FA measured by DTI.[88,89] In contrast, there have been few longitudinal studies, and those that have been undertaken have often provided contradictory findings. Some studies have demonstrated a decrease in spectroscopy[108] and DTI[88] parameters over time, but this has not been confirmed in other studies where no significant modifications were observed.[90,93] These discrepancies could, at least in part, be accounted for by the heterogeneity of the

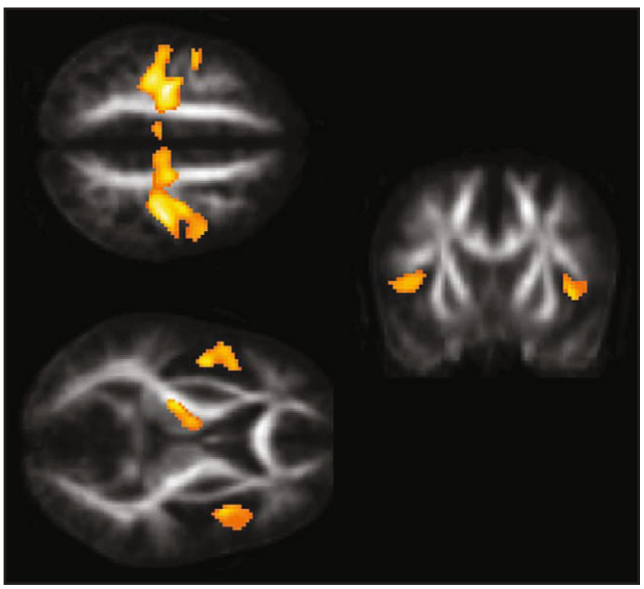

Fig. 4. Cerebral magnetic resonance imaging (MRI), diffusion tensor imaging (DTI), voxel-by-voxel analysis of amyotrophic lateral sclerosis (ALS) patients compared with control subjects.[89] The colored areas correspond to the brain regions where DTI parameters differ significantly between the group of ALS patients and the group of control subjects. This study demonstrates that the radiologic anomalies in ALS are not limited to the primary motor system.

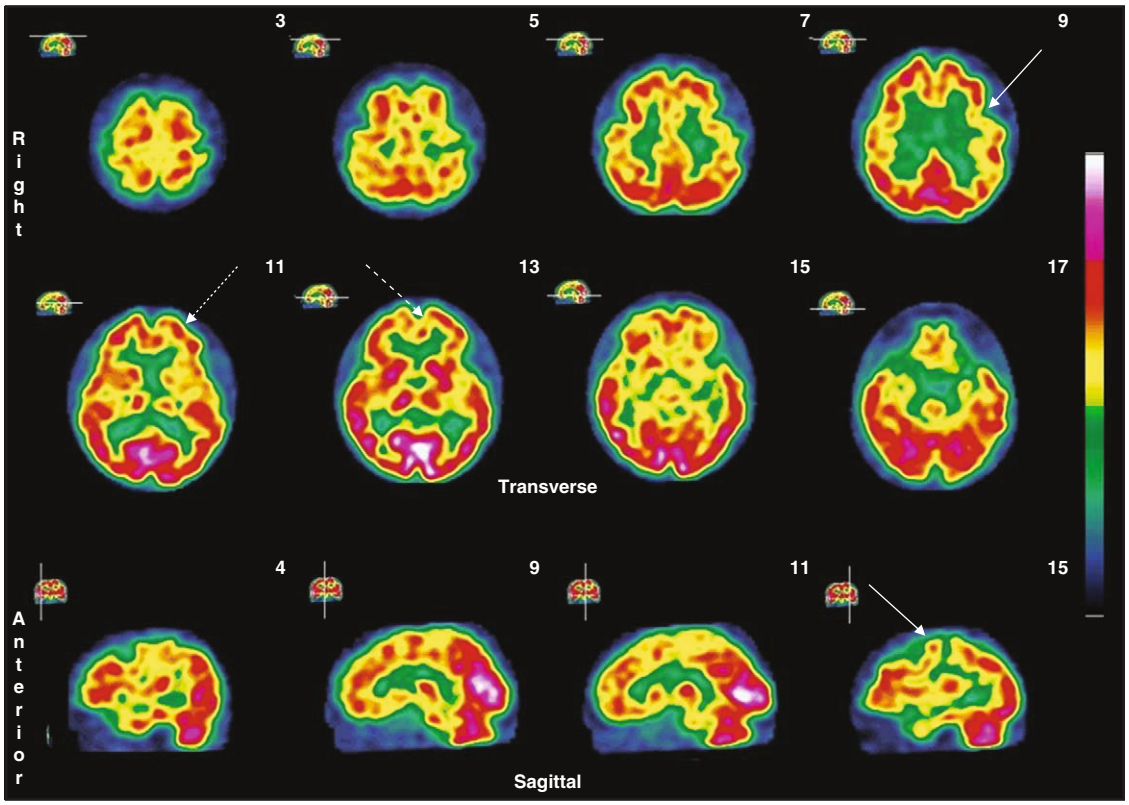

Fig. 5. Perfusion assessed by ^{99m}Tc-ethyl cysteinate dimer single proton emission computerized tomography (SPECT) imaging. A severe decrease in perfusion in the operculo-insular and rolandic regions (solid arrows) and a more moderate decrease in the mesial (dashed arrow) and dorsolateral prefrontal (dotted arrow) cortices can be observed (courtesy of Dr M-O Habert, Department of Nuclear Medicine, Hôpital de la Pitié Salpêtrière, Paris, France).

patient populations studied, notably with respect to the clinical stage of the disease. It has been suggested that these techniques may only detect temporal modifications at an early stage of the disease.[90,93]

4. Conclusion

It is important to consider the future of biologic and radiologic markers in the diagnosis and evaluation of ALS. The sensitivity and specificity of these markers, which are currently insufficient to be used as reliable diagnostic tools, could be improved by technologic advances. These advances concern both radiologic markers – for example, the development of high-field MRI or tractography techniques – and biologic markers such as high-throughput techniques. In the future, it is likely that the combined use of several markers will provide a suitable diagnostic tool for use in clinical practice, associating biologic, radiologic, and electrophysiologic parameters.[90] Furthermore, a combination of biomarkers may be of value for monitoring disease progression and as surrogate endpoint markers in clinical trials testing disease-modifying drugs.

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