detect deterioration [59], and EIM can serve as a meaningful measure of disease severity in ALS [60]. One advantage of EIM is its ability to assess a variety of muscles and to measure specifically the area of the body where the disease is progressing most rapidly. The other advantage is that EIM of tongue musculature could distinguish patients with ALS from healthy controls. The demonstrated relationship between tongue function and Ph supports further testing of EIM of the tongue as a potential biomarker in ALS [61]. Large studies are needed to further refine the technique for easy use and validate it as a biomarker for future clinical trials.

Muscle ultrasound (MUS)

Ultrasound may also detect changes in the thickness and echogenicity in muscles with and without clinical weakness [62]. Muscle ultrasound differentiated between ALS and mimics with 96 % sensitivity and 84 % specificity, and it is a sensitive tool to screen for regional lower motor neuron involvement [63]. The most established role of MUS in the ALS clinic relates is the identification of fasciculations. The sensitivity and specificity of MUS in diagnosing ALS was almost equivalent to those of EMG, and combined use of EMG and MUS enhanced the diagnostic accuracy compared to EMG alone [64].

Measures of UMN Loss

Transcranial magnetic stimulation (TMS)

TMS is a neurophysiological test that measures UMN functional integrity, and it is able to improve the sensitivity of ALS diagnosis by demonstrating evidence of subtle subclinical UMN dysfunction, as well as clarify the relationship between ALS and its variants [65], such as PMA. It is used to study the excitability and conductivity of the corticospinal system. Changes in cortical excitability may precede the development of muscle weakness in ALS [66, 67]. Single pulse evoked TMS amplitude could used to objectively discriminate ALS from neurological controls and assess the progression of ALS [68–70]. The threshold tracking TMS technique could used to differentiate ALS from non-ALS disorders with a sensitivity of 73.21 % and specificity of 80.88 % at an early stage in the disease. It may represent a useful diagnostic investigation to prove UMN dysfunction at early stages of ALS when combined with the Awaji criteria [71].

Neuroimaging biomarkers (Table 3)

Imaging offers a noninvasive approach to biomarker discovery and disease monitoring. If neuroimaging biomarkers were validated, they could be easily integrated into routine clinical evaluation of patients with suspected ALS, and revealed disease mechanisms that might aid the discovery of novel drug targets.

Table 3 Candidate neuroimaging biomarkers for ALS

	Evaluated biomarkers
Radionuclide imaging	SPECT
	PET
Magnetic resonance imaging (MRI)	Voxel & surface-based MRI morphometry (VBM&SBM)
	Diffusion tensor imaging (DTI)
	Functional MRI (fMRI)
	Magnetic resonance spectroscopy (MRS)
	Spinal cord MRI

Radionuclide imaging

Single photon emission computed tomography (SPECT) is a practical and potentially widely applicable form of radionuclide imaging. It was at the forefront of the now established concept of a continuum between ALS and frontotemporal dementia (FTD) [72]. Positron emission tomography (PET) has greater resolution than SPECT. Pivotal 'activation' PET studies, using tracers sensitive to blood flow and metabolism, provide in vivo evidence for a consistent extramotor cerebral pathology in ALS [73], while 'ligand' PET is used to identify specific cerebral neuronal receptor changes in ALS. The PET ligand 11C-PK11195 binds to the peripheral benzodiazepine receptor, which are expressed by activated microglia. A study provided *in vivo* evidence of widespread corticospinal tract and extra-motor microglial activation in ALS patients [74]. A serotonin 5-HT1A receptor PET ligand 11C-WAY100635 showed marked reductions in binding in a group of nondepressed ALS patients [75]. Loss of binding was mainly located in frontotemporal regions. These locations are similar in distribution to a subsequent study in patients with FTD [76], and this striking reduction in serotonin-1A receptor binding was confirmed histologically [77]. The future value of PET in ALS will depend on the development of ligands with relevance to pathogenic hypotheses, e.g., more specific neuroinflammatory or protein markers.

Magnetic resonance imaging (MRI)

The observation of corticospinal tract hyperintensity lacks sensitivity and specificity for the diagnosis of ALS. Routine clinical MRI has limited value as a source of biomarkers in ALS, e.g., the marked precentral gyrus atrophy was demonstrated in rare cases of PLS. Thus, the advanced analysis methods have greater potential in this regard.

Voxel & surface-based MRI morphometry

Automated and unbiased whole-brain analysis techniques have been developed to quantify and segment grey and white matter (WM) morphology using T1-weighted images, and this advanced analysis techniques