between concentrations of CSF S100b and disease severity. . Furthermore, changes of S100b have been reported in other neurodegenerative diseases. Therefore, S100b has limited usefulness for disease diagnosing and monitoring disease progression [38].

Blood

Blood is more accessible compared to CSF. In ALS, serum albumin and creatinine are reliable markers of the severity of clinical status and can be used in defining prognosis at the time of diagnosis [39]. The DREAM-Phil Bowen ALS Prediction Prize4Life challenge also confirmed that uric acid and creatinine could be used as potential nonstandard predictors of disease progression, shedding light on ALS pathobiology [40]. A post hoc analysis of subgroup outcomes and creatinine in the phase III clinical trial (EMPOWER) of dexpramipexole in ALS demonstrated that creatinine loss correlated with disease progression [41]. Similar findings also showed that changes to ferritin and creatinine levels with time were associated with ALS progression, suggesting serum creatinine as a candidate biomarker [42].

Muscle

Skeletal muscle is one of the most severely affected by the disease and it is easily accessible to biopsy. Thus, with progressive denervation and atrophy, muscles may represent a valuable source of biomarkers in ALS. Nogo-A was found to be strongly expressed in ALS muscles, and its expression was correlated with amyotrophic lateral sclerosis functional rating scale (ALSFRS) [43, 44]. However, studies questioned that expression of Nogo-A in human muscle fibers may be not specific for ALS [45, 46]. Muscle transcriptome analyses have found that smad1, 5, 8 mRNA and protein levels, as well as Smad phosphorylation, were elevated in ALS muscle. Therefore, muscle smads could serve as potential candidates for ALS biomarkers [47].

Physiological biomarkers (Table 2)

While biochemical markers may provide clues for the specific cellular or signaling alterations that occur in ALS, a number of global physiological features can be assessed that might differentiate ALS from other neurological diseases and enable the monitoring of disease progression. The presence of fibrillation potentials and positive sharp waves on needle electromyography indicates ongoing LMN degeneration or axonal loss, and prolonged and polyphasic motor units are considered to be a consequence of reinnervation. However, electromyography has a limited sensitivity (60 %) for the diagnosis of ALS, and the characteristics measured, including motor unit duration, amplitude and phase do not systematically change with disease progression. A measure of motor unit loss that is reproducible, noninvasive, rapidly obtained, and amenable to repeated evaluation over time would be highly desirable.

Table 2 Candidate physiological biomarkers for ALS

	Evaluated biomarkers
Measures of LMN loss	Motor unit number estimation (MUNE)
	Axonal excitability
	Electrical impedance myography (EIM)
	Muscle ultrasound (MUS)
Measures of UMN loss	Transcranial magnetic stimulation (TMS)

Measures of LMN Loss

Motor unit number estimation (MUNE)

Motor unit number estimation (MUNE) is a neurophysiological tool that was developed to quantify residual motor axons supplying a muscle, by estimating the contribution of individual motor units to the maximum response amplitude. Longitudinal studies of changes in MUNE in ALS have correlated loss of motor neurons with survival [48]. A number of MUNE techniques for estimating the average amplitude of single motor units have been developed, but most of them have been limited by sampling bias and lack of reproducibility [49]. Recently, multipoint incremental MUNE was found to have excellent test-retest reliability. The rate of decline was more sensitive than that of MRC sum score and ALSFRS-R [50, 51]. Other new MUNE methods, including Bayseian MUNE and motor unit number index (MUNIX), the latter was considered to be a reliable electrophysiological biomarker to track lower motor neuron loss in ALS [52]. Bayesian MUNE could be used to show differing rate of loss of motor units in subgroups of ALS [53].

Axonal excitability

Motor axonal dysfunction has been demonstrated in ALS patients using threshold-tracking technology, with increased persistent conduction in sodium channels and reduced conduction in potassium channels [54, 55]. Changes in axonal excitability evolve with disease progression [56], and may be used as a predictor of survival in ALS patients [57]. Axonal excitability parameters could be used as biomarkers of axonal degeneration.

Electrical impedance myography (EIM)

EIM is an emerging technology in which a high-frequency, low-intensity electrical current is applied to a localized area of muscle and the consequent surface voltages measured [58]. EIM assesses the integrity and structure of the muscle. Recently, a multicenter study compared EIM directly to the ALSFRS-R, MUNE, and handheld dynamometry, and found that EIM outperformed the other measures in terms of its ability to