The blood biomarker puzzle – A review of protein biomarkers in neurodegenerative diseases

Christian Barro, MD PhD[1,2], Henrik Zetterberg, MD PhD[3-7]

[1] Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, MA, USA

[2] Department of Neurology, Harvard Medical School, Boston, MA, USA

[3] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[4] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[5] Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK

[6] UK Dementia Research Institute at UCL, London, UK

[7] Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Word count title: 12
Word count abstract: 186
Word count main text: 6633
Number of figures/tables: 1/2

Correspondence to:

Christian Barro, cbarro1@bwh.harvard.edu, 60 Fenwood Road, 10032A Boston, MA, 02115, USA, tel.: +1 617 525 5326.

Key words: neurofilament, neurologic disease, biomarker

Highlights

- Fluid biomarkers for neurodegenerative conditions are an urgent unmet need
- Technical advances enable a highly sensitive detection of biomarkers in blood
- Each biomarker is reflecting different aspects of CNS pathology
- Biomarkers can stratify patients based on biology rather than clinical phenotype

Abstract

Neurodegenerative diseases are heterogeneous in their cause and clinical presentation making clinical assessment and disease monitoring challenging. Because of this, there is an urgent need for objective tools such as fluid biomarkers able to quantitate different aspects of the disease. In the last decade, technological improvements and awareness of the importance of biorepositories led to the discovery of an evolving number of fluid biomarkers covering the main characteristics of neurodegenerative diseases such as neurodegeneration, protein aggregates and inflammation. The ability to quantitate each aspect of the disease at a high definition enables a more precise stratification of the patients at inclusion in clinical trials, hence reducing the noise that may hamper the detection of therapeutical efficacy and allowing for smaller but likewise powered studies, which particularly improves the ability to start clinical trials for rare neurological diseases. Moreover, the use of fluid biomarkers has the potential to support a targeted therapeutical intervention, as it is now emerging for the treatment of amyloid-beta deposition in patients suffering from Alzheimer's disease. Here we review the knowledge that evolved from the measurement of fluid biomarker proteins in neurodegenerative conditions.

Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
BBB	Blood-brain barrier
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
CXCL13	C-X-C Motif Chemokine Ligand 13
ECL	Electrochemiluminescence
ELISA	Enzyme-linked immunosorbent assay
FTD	Frontotemporal dementia
GFAP	Glial fibrillary acidic protein
HD	Huntington's disease
IMR	Immunomagnetic reduction
MCI	Mild cognitive impairment
MMP9	Matrix metalloproteinase 9
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NfL	Neurofilament light chain
(p)NfH	(phosphorylated) Neurofilament heavy chain
PD	Parkinson's disease

PEA	Proximity extension assay
PET	Positron emission tomography
PCR	Polymerase chain reaction
pwMS	Patients with multiple sclerosis
qPCR	Quantitative PCR
RT-QuIC	Real-time quaking-induced conversion
SMA	Spinal muscular atrophy
Simoa	Single molecule array
TDP-43	Transactive response DNA-binding protein 43

1. Introduction

Neurodegenerative diseases are caused by a wide spectrum of biological dysfunctions. The clinical manifestation, the disease course and the therapeutical response is therefore heterogeneous. Biomarkers are tools that enable the detection of pathological processes responsible for these dysfunctions. Sometimes we measure biomarkers that are a byproduct of the disease, other times we measure biomarkers that reflect the damage caused by the disease. In the last few decades, an improvement of immunoassays and mass spectrometry-based methods enabled the robust detection of a number of blood biomarkers that are now initiating a revolution in the way that neurological patients are monitored. Biomarkers originating from the central nervous system (CNS) are present at a higher concentration in the cerebrospinal fluid (CSF) and at multiple fold lower concentration in the blood. Thus, the initial process of biomarker discovery took advantage of CSF biospecimen, particularly from patients suffering from Alzheimer's disease (AD) and amyotrophic lateral sclerosis (Rosengren et al., 1996). These positive findings set a fertile ground for the further development of immunoassays. We may think of them as first generation assays as immunoblot, second generation assays as the enzyme-linked immunosorbent assay (ELISA), third generation using electrochemiluminescence (ECL) technology and the latest fourth generation based on Single molecule array technology (Simoa) (Khalil et al., 2018). The translation of CSF assays into blood was particularly successful with the fourth generation of assays. This category expanded over the last years and now, besides the Simoa (Rissin et al., 2010), it encompasses also proximity extension assays (PEA) (Chitnis et al., 2019), Single Molecule Counting (SMC) (Fischer et al., 2015), immunomagnetic reduction (IMR) (Lue et al., 2019), the Ella platform based on microfluidic channels (Dysinger et al., 2017), and ongoing developments on platforms already used in clinical routine as the ADVIA Centaur and Atellica (Plavina et al., 2019). What was known from the CSF was strengthened and expanded by blood analysis. This exciting new horizon for the biomarker field opens new challenges, including which biomarker suits which disease the best, and what does a fluid biomarker concentration reflect in terms of tissue pathology/pathophysiology and how should it be interpreted. Here we want

to help achieving answers to these questions by reviewing the knowledge pertaining to the use of protein biomarkers in blood for neurodegenerative diseases (**Table 1)**.

2. Highly sensitive platforms

Technological advances have made it possible to quantify CNS proteins in the blood (**Figure 1 and Table 2)**. This mostly started with the third generation of immunoassays based on ECL (Khalil et al., 2018), which is a kind of luminescence produced during electrochemical reactions in solutions. This technology builds on the sandwich immunoassay principle in which one antibody captures the analyte and another antibody detects it, but instead of an enzymatic reaction producing the detection signal, electrochemiluminescent labels are conjugated to detection antibodies. At the reading phase an electrical stimulus activates the labels that emit a quantifiable signal. The use of a an electrical instead of optical signal (as in the ELISA) conferred to the ECL a higher sensitivity (Kuhle et al., 2016).

The advent of the fourth-generation assays characterized by a deeper sensitivity began with the Simoa technology. One of the core characteristics is the formation of immunocomplexes on paramagnetic beads instead of the bottom of a well. This allows to separate the immunocomplexes at the reading stage in spatially distant micro-wells. Hence, the signal originating from one immunocomplex is not spilling into the signal originating from other immune complexes. It allows for the optical detection of signal emitted from the single beads. The ratio between the wells with and wells without signal plotted against a calibrator curve results in the concentration of the targeted protein (Rissin et al., 2010). Most of the current literature on blood biomarkers for neurodegenerative diseases have been produced by using this technology.

A more recent fourth-generation platform is Ella. The platform is based on the use of microfluidic circuits. The sample loaded in a cartridge flows through the microfluidic channels presenting a pre-coating capture antibody that binds the target protein. The antibody is coated on three separate areas, thus allowing for three technical replicates. A system of pumps allows

the flow of the detector antibody, wash solution and the fluorescent dye through the channels. The level of fluorescence is compared to pre-analyzed calibrator curve (Dysinger et al., 2017).

A different concept is brought by the PEA. Two antibodies used to recognize the targeted protein present a specific sequence of oligonucleotide (a DNA “barcode”). The DNA barcodes have specific annealing sites that hybridize if in close proximity, *i.e.*, bound to the same protein, this is followed by an extension and initial amplification by PCR. The PCR is per se non-quantitative. The quantitative read-out is obtained by qPCR or by sequencing. The specificity of the DNA barcodes allows for an incredible number of simultaneously measured proteins (multiplex) and the amplification cycles at the qPCR stage allow for the detection of low abundant proteins (Assarsson et al., 2014).

SMC is a fourth-generation assay characterized by a spatially defined excitation area. More precisely, the sample is first incubated with capture and fluorescently labelled detector antibodies; unbound antibodies are removed by washing. The sample is scanned by a laser able to excite the fluorophore present on the immune complex. The concentration of the target protein is calculated by comparing the count of excitations in the sample and the count in the calibrators (Todd et al., 2007).

The measurement of proteins by IMR could be seen as the equivalent of doing a magnetic resonance imaging (MRI) scan of the samples. Paramagnetic beads coated with an antibody specific to the targeted protein are incubated with the sample and stimulated by alternate magnetic field. The device detects the magnetic pulse originating by the rotating beads. The presence of immunocomplexes on the bead causes a decrease in the rotation speed of the bead, which reflects the quantity of targeted protein present in the sample (Lue et al., 2019).

A further approach is the real-time quaking-induced conversion (RT-QuIC) that is employed for the quantification of prion-like protein seeds. Aggregation-prone proteins, like prion protein and α-synuclein, can adapt a certain conformation that can stimulate further misfolding and aggregation of normally folded proteins. Tissue extracts have been found to contain such seeds and recently they have been found in cerebrospinal fluid (CSF) from patients with, *e.g.*,

prion disease or Parkinson's disease. The assay principle is surprisingly simple: to detect misfolded seeds, recombinant protein (*e.g.*, prion protein) is added into the sample that is incubated over days with regular shaking. Seeded misfolding is detected with fluorescent thioflavin T sensitive to the β-sheet folding of the target protein. The fluorescence is measured over time and compared with reference material of positive and negative controls and the final output is a qualitative result (Cramm et al., 2016; Scialò et al., 2020). An RT-QuIC assay for prion inclusions uses recombinant prion protein, an assay for α-synuclein inclusions uses recombinant α-synuclein, etc. The technique does not work for blood yet.

Finally, mass spectrometry-based techniques for protein concentration determination in blood are getting increasingly common. For example, immunoprecipitation mass spectrometry (IP-MS) is the assay type that results in the most reliable quantification of amyloid β (Aβ) peptides in plasma to date.

Regarding inter-platform comparisons, a practical example was brought by Gauthier and colleagues who showed how the blood levels of neurofilament light chain (NfL) measured by Simoa (NfL advantage kit) and Ella are highly correlated (Gauthier et al., 2021), hence the findings obtained by Ella translate to Simoa and vice versa. However, the absolute NfL level was almost half in the Simoa measurements, despite the fact that both use the same pair of antibodies. The main reason for differences in absolute concentration in such cases is that the assays use different calibrators that have not been standardized to each other. A certified reference material that can be used by assay manufacturers to "calibrate their calibrators" can result in standardization of biomarker assays.

3. Alzheimer's disease (AD)

The marvelous success of biomarkers in this disease shaped the landscape of biomarker discovery in neurologic diseases. Analysis of postmortem tissue and later PET scans evidenced the presence of aggregates of beta amyloid and neurofibrillary tangles in the brain

of AD patients (Masters et al., 2015). In chronological order, the disease appears to start with an overproduction of an amyloid beta isoform that is 42 amino acids long (Abeta42, A) followed by an accumulation of neurofibrillary tangles rich in truncated and phosphorylated tau (T), which results in neurodegeneration (N) (Masters et al., 2015). From a biomarker perspective, these three domains are part of the ATN biomarker classification for neurologic diseases (Jack et al., 2016).

A. Presence or absence of Aβ pathology in the brain can be estimated through the analysis of the levels of Aβ peptides in the CSF (Blennow and Zetterberg, 2018). The fluid biomarker gold standard has become the ratio of 42 (aggregation-prone) to 40 (more soluble) amino acid-long Aβ in the CSF (Aβ42/Aβ40 ratio) (Schindler et al., 2019). The quantification of Aβ42 in CSF showed a high agreement with the Aβ deposition seen on PET scan (Blennow et al., 2015; Palmqvist et al., 2014). Biological limitations hampered the translation of the Aβ assays in the blood (Janelidze et al., 2016); the levels are low, Aβ sticks to other plasma proteins and there is also peripheral production in, *e.g.*, blood platelets, which leads to noise in the assays (Citron et al., 1994). Anyhow, new mass spectrometry-, Simoa- and ECL-based techniques are now making it possible to reproduce the well-established CSF Aβ42/Aβ40 reduction in individuals who are Aβ-positive according to amyloid positron emission tomography (PET) also in blood (Nakamura et al., 2018; Ovod et al., 2017; Palmqvist et al., 2019). The reduction of the Aβ42/Aβ40 ratio is around 50% in CSF but only about 10-15% in blood since it occurs on the background of non-CNS Aβ.

T. It is fascinating that the presence of tau in the CSF/blood of AD patients may not only be a consequence of neuronal damage, but an active release from the neurons in response to the accumulation of Aβ (Kanmert et al., 2015; Maia et al., 2013; Sato et al., 2018). This hypothesis is further supported by the higher magnitude of increase in CSF tau seen in AD patients compared to patients suffering from other tauopathies (diseases with both neurodegeneration and neurofibrillary tangles) (Skillbäck et al., 2015; Zetterberg, 2017a). The quantification of total-tau (t-tau; measured using assays directed at the N-terminal and mid-regions of the

protein, which do not discriminate between different isoforms) in the blood remains challenging, but with Simoa and some other techniques it is now possible to quantify different phosphorylation states of tau and fragments of tau in the blood. Specifically, blood pTau181 discriminated with an accuracy of 100% AD patients from Aβ- conditions like frontotemporal dementia patients (FTD), and discriminated with an accuracy of more than 80% AD patients from patients with mild cognitive impairment (MCI), progressive supranuclear palsy, corticobasal syndrome, vascular dementia, primary progressive aphasia, Parkinson's disease and multiple systems atrophy (Karikari et al., 2020). Even more strikingly, blood pTau181 was able to discriminate asymptomatic Aβ+ individuals from asymptomatic Aβ- individuals (Karikari et al., 2020). These findings were extended and strengthen by two parallel seminal works from Thijssen and colleagues (Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) investigators et al., 2020) and Janelidze and colleagues (Janelidze et al., 2020). Strikingly, blood pTau231, another phosphorylation state of tau, showed an even greater performance by detecting the pathology at earlier stages (Braak 0 and 1 stadium)(Ashton et al., 2021). To top up this, a third phosphorylation state of pTau with a phosphorylation at threonine-217 is emerging as promising blood biomarker (Mattsson-Carlgren et al., 2020; Palmqvist et al., 2020). Hence, pTau meritedly gained the stage by being a blood biomarker with an diagnostic specificity comparable to the accepted CSF AD-biomarkers. Moreover, promising evidence shows that the quantification of a N-terminal segment of tau may also serve as AD specific blood biomarker (Mengel et al., 2020) and already at the early disease stages (Chhatwal et al., 2020).

N. Neurodegeneration can be quantified by the levels of neurofilaments or tau. The CSF levels of tau and NfL showed an association with both neurocognitive impairment and imaging measures of disease progression (Olsson et al., 2016). The stability of neurofilaments enabled the translation of these findings to blood measurements. The deposition of Aβ and neurofibrillary tangles starts decades prior symptom onset with the prodromal phase of the disease (Masters et al., 2015). Consistently with this, an increase in NfL was detected years prior to symptom onset in retrospective studies involving patients with a familial form of the

disease (Preische et al., 2019; Quiroz et al., 2020; Weston et al., 2019, 2017). Blood NfL entails the ability of monitor disease activity over time (Lewczuk et al., 2018; Lin et al., 2018; Mattsson et al., 2017; Pereira et al., 2017; Preische et al., 2019; Sánchez-Valle et al., 2018; Weston et al., 2017); however it lacks diagnostic specificity as shown in the comparison with pTau181 (Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) investigators et al., 2020).

Moreover, the presence of ongoing brain damage is reflected by increased activation of microglia cells in AD patients (Butovsky and Weiner, 2018): the levels of a soluble fragment of TREM2 (sTREM2, that is a membrane protein present on microglia) were increased in AD patients (Bekris et al., 2018; Heslegrave et al., 2016; Ohara et al., 2019; Piccio et al., 2016) and proportional to the level of tau pathology as measured by CSF t-tau and pTau181 (Suárez-Calvet et al., 2016). However, the presence of sTREM2 also in peripheral cells (outside the CNS) from the myeloid compartment hampers its translation into a blood measurement (Zetterberg, 2017b). Further novel biomarkers are being explored, such as flotillin, an exosomal protein found by western blot to be decreased in the CSF and blood of AD patients (Abdullah et al., 2019).

Interestingly, it was unclear whether the Amyloid-Tau-Neurodegeneration (ATN) temporal profile seen on PET scans were mirrored by the same temporal sequence of Aβ followed by pTau and by NfL in the blood. A recent study shed some light on this, the NfL levels of 198 patients from the ADNI study and 116 patients from the TRIAD study with different grades of cognitive impairment were analyzed and associated with PET measures of amyloid and tau (Benedet et al., 2020). Interestingly, in pre-symptomatic individuals elevated blood NfL was associated with the presence of Aβ plaques (^{18}F-florbetapir), whereas at the symptomatic stage NfL was associated with the load of tau (^{18}F-MK6240) (Benedet et al., 2020). Hence, the combination of these biomarkers may allow both a correct diagnosis and disease staging. A practical application can be the stratification of patients for clinical trials of drugs that may be targeting the Aβ deposition (A) or the neurofibrillary tangles (T) or the prevention of

neurodegeneration (N). In fact, there are undergoing clinical trials exploring monoclonal antibodies able to remove Aβ (Selkoe, 2020) and other targeting tau are under early phase of development. Depending on the targeted disease stage and the targeted protein each blood biomarker could play a significant role. This was explored in an animal model of APP/PS1 mice where the decrease of Aβ corresponded to a decrease in NfL levels (Bacioglu et al., 2016). Finally, the clinical implementation of these biomarkers may succeed by a two-step approach with an initial screening with a blood test, that if positive will be followed by deeper investigations through CSF biomarkers and PET scan.

4. Multiple sclerosis (MS)

MS is a chronic autoimmune disease where an inflammatory and neurodegenerative process leads to an accrual in disability (Trapp and Nave, 2008). Most of the patients undergo an initial inflammatory phase with acute bouts of disease activity that is followed by a subtle phase of slow accrual in disability (Baecher-Allan et al., 2018).

Well-established clinical and MRI criteria enabled an accurate diagnosis (A. J. Thompson et al., 2018). On the other hand, the monitoring of the disease activity is challenged by the heterogeneity of the disease. In order to address this need and to reach an even earlier diagnosis, several fluid biomarkers have been explored with the most promising being structural components of the brain parenchyma that are disrupted and released upon disease activity (*e.g.*, NfL, glial fibrillary acidic protein - GFAP) or cytokines (*e.g.*, CXCL13) and enzymes (*e.g.*, metalloproteinases or chitinases) released by the activated immune system (Huang et al., 2020; Pinteac et al., 2021).

Neurodegeneration. Back in 1998 in Sweden Jan Lycke showed that CSF NfL levels in relapsing remitting (RRMS) patients were increased compared to controls, they were associated with disability and increased with higher relapse rate during the previous 2 years (Lycke et al., 1998). Further studies from independent laboratories confirmed and broaden

these findings supporting CSF NfL as marker of disease activity in MS (Burman et al., 2014; Khalil et al., 2013; Kuhle et al., 2013; Malmestrom et al., 2003; Martinez et al., 2015; Modvig et al., 2015; Norgren et al., 2004; Novakova et al., 2017; Romme et al., 2013; Teunissen et al., 2009; Trentini et al., 2014; Villar et al., 2014) and as prognostic marker (Arrambide et al., 2016). Increased CSF NfL reflected suboptimal treatment response in patients treated with first versus second line treatment (Novakova et al., 2017). Moreover, patients beginning treatment with rituximab, natalizumab and fingolimod showed a decrease in CSF NfL levels associated with less clinical and MRI disease activity over one year (de Flon et al., 2016; Gunnarsson et al., 2011; Kuhle et al., 2015). A translation of the NfL assay in blood created an unprecedented situation: tens of thousands of frozen blood samples collected over decades of observational cohorts and clinical trials became a Rosetta stone that gave the field an immense knowledge over the time course, causes and preventive measures of neurodegeneration in MS. Blood NfL measurements supported the idea of a prodromal stage of MS with neurodegeneration occurring years prior to symptom onset (Bjornevik et al., 2020). Moreover, it demonstrated that a blood measurement does not only reflect clinical and MRI disease activity (Disanto et al., 2017; Barro et al., 2018; Cantó et al., 2019; Chitnis et al., 2018; Håkansson et al., 2018; Jens Kuhle et al., 2019; J. Kuhle et al., 2019a; Kuhle et al., 2017; Manouchehrinia et al., 2020; Siller et al., 2019; Varhaug et al., 2018; Bittner et al., 2020) but it also reflects response to treatment (Calabresi et al., 2020; Delcoigne et al., 2020; Kuhle et al., 2018; J. Kuhle et al., 2019a; Sejbaek et al., 2019) or presence of subclinical disease activity (Yaldizli et al., 2018). Notably, an unexpected lack of decrease in NfL levels was seen after treatment with ibudilast in progressive MS patients (Fox et al., 2021) despite the treatment successfully improved the disease outcome in the respective clinical trial (Fox et al., 2018). The inclusion of progressive MS patients, that are more affected by comorbidities than RRMS, may have reduced the sensitivity of NfL in detecting a treatment effect. There is compelling evidence that blood NfL may be used for monitoring disease activity in patients with MS. Nevertheless, its application relies on a careful consideration of biological confounders (extensively reviewed in (Barro et al., 2020)).

Astrocytic dysfunction and damage. The early stages of the disease – RRMS – present acute inflammatory events characterized by disruption of the blood brain barrier (BBB) and migration of peripheral immune cells in the CNS (Chitnis and Weiner, 2017). The natural history of the disease results in a transition to a more subtle process compartmentalized to the CNS where plasma cells and innate immune systems are believed to be the main players (Baecher-Allan et al., 2018). At this later stage, the neurodegeneration is the result of a chronic continuous damage rather than pulses of peripheral immune activation. Consistently, this biological change was reflected by a lower magnitude of blood NfL increase upon acute disease activity (Rosso et al., 2021). Resident cells as astrocytes are also affected by this process. Astrocytes are not only damaged but they also increase their expression of glial fibrillary acidic protein (GFAP), an astrocyte specific cytoskeletal protein (Liddelow et al., 2017; Sofroniew and Vinters, 2010). Like most of the biomarkers, GFAP was initially studied in CSF and only recently translated to blood. Astrocytes are integral components of the BBB (Sofroniew and Vinters, 2010), it would therefore be expected that a disruption of the BBB seen in acute episodes of disease activity would result in an increased release of GFAP. Astonishingly, there is not such clear increase in GFAP levels with contrasting findings showing increased (Malmeström et al., 2003; Noppe et al., 1986; Watanabe et al., 2019) or normal (Ayrignac et al., 2020; Mane-Martinez et al., 2016; Norgren et al., 2004; Novakova et al., 2017; Rosengren et al., 1995) GFAP levels during an MS relapse. One speculation is that the GFAP released with a damage of the BBB is released in the blood where the half-life of the protein may be too short for remaining detectable. This can actually be a good thing. The relative stability of GFAP levels upon relapse constitute a unique property of this biomarker contrasting with the more dynamic profile of NfL. Thus, the combination of NfL and GFAP results in increased and not overlapping information. The load of astrocytic activation seen in the progressive stage of the disease was reflected by increased GFAP levels in progressive MS patients (PMS) compared to RRMS and healthy individuals (Abdelhak et al., 2018; Ayrignac et al., 2020; Gunnarsson et al., 2011; Högel et al., 2020; Huss et al., 2020; Mane-Martinez et al., 2016; Novakova et al., 2018). Moreover, in contrast to neurofilament levels, patients treated

with natalizumab or rituximab/mitoxantrone did not show a decrease in GFAP levels (Axelsson et al., 2014; Gunnarsson et al., 2011), hence this biomarker appear to be consistently influenced only by the underlying astrocytic pathology that is not targeted by any of the currently available treatments. Nonetheless, based on the data of the EXPAND study of Siponimod (J. Kuhle et al., 2019b), it might be fair to speculate that the lack of increase in GFAP levels seen in the active arm indicates that effective treatment may prevent pathologic astrocytic activation, thus disease progression.

Inflammation. Several cytokines or enzymes are secreted during an inflammatory process, the main hurdle in validating secreted molecules as CNS biomarkers is the confounding effect of a secretion from peripheral sources, hence the vast majority of the studies has focused on CSF samples. The effectivity of B-Cell therapies in MS has attracted attention to the role of the B-cells compartment with the most investigated cytokine being the chemokine ligand 13 (CXCL13) that targets B-cells (Alvarez et al., 2013). Indeed, CSF CXCL13 was found to be elevated in MS patients compared to healthy controls in a great number of studies, for instance (Alvarez et al., 2013; Axelsson et al., 2014; Brettschneider et al., 2010; Hakansson et al., 2017; Puthenparampil et al., 2017; Sellebjerg et al., 2017, 2009), although this difference was not detectable in blood (Alvarez et al., 2013). A decrease of CSF CXCL13 levels after 12-24 months of rituximab or mitoxantrone treatment suggested a possible role of CXCL13 in detecting treatment efficacy (Axelsson et al., 2014). Of note, increased levels of CSF CXCL13 appeared to be prognostic of conversion from CIS to clinically definite multiple sclerosis (Brettschneider et al., 2010; Khademi et al., 2011). It is technically important to mention that the studies around this cytokine faced an unsatisfactory sensitivity of the ELISA assay used with only a part of the samples in the detectable range (Axelsson et al., 2014; Hakansson et al., 2017). In a different category we find the chitinases, hydrolytic enzymes with the peculiarity of being more specific for the myeloid compartment of immune cells with their levels reflecting the activation of macrophages and microglia (Pinteac et al., 2021). In this family the most promising biomarker candidate is chitinase 3 like 1 (CHI3L1). Its CSF levels were shown to be increased in 48 clinically isolated syndrome (CIS) patients that converted to clinically defined

MS compared to 36 CIS that did not convert and CHI3L1 levels were associated with the presence of active and chronic MRI lesions (Comabella et al., 2010). This was confirmed in two larger studies involving more than 800 CIS patients (Canto et al., 2015; Martinez et al., 2015). Further studies found consistently that CSF CHI3L1 levels were increased in patients with MS (pwMS) compared to healthy individuals (Cantó et al., 2012; Gil-Perotin et al., 2019; Hakansson et al., 2017; Hinsinger et al., 2015; Huss et al., 2020; Malmeström et al., 2014; Mane-Martinez et al., 2016; Novakova et al., 2017; Sellebjerg et al., 2017; Thouvenot et al., 2019), although the association with MRI and clinical disease activity was less clear (Canto et al., 2015; Cantó et al., 2012; Comabella et al., 2010; Hakansson et al., 2017, p. 201; Novakova et al., 2017; Oldoni et al., 2020; Quintana et al., 2018; Thouvenot et al., 2019). The blood levels of CHI3L1 despite not being prognostic for CIS patients (Comabella et al., 2010), held promising results by being elevated in pwMS (Cantó et al., 2012; Hinsinger et al., 2015) and associated with the expanded disability status score (Cantó et al., 2012; Huss et al., 2020). The potential role of chitinases as biomarkers was reviewed to a great extend in (Pinteac et al., 2021). Of interest is also the family of metalloproteinases, catalytic enzymes, of which matrix metalloproteinase 9 (MMP9) has been the most studied for its possible role as MS biomarker of inflammation (Leppert et al., 2001). Its levels were in fact increased in the presence of either clinical or MRI inflammatory activity (Avolio et al., 2005, 2003; Fainardi et al., 2006; Leppert et al., 1996; Sellebjerg et al., 2017, 2003; Waubant et al., 2003).

There are further undergoing efforts to identify additional biomarkers for MS. For instance, Huang and colleagues applied a highly sensitive proteomic approach to identify biomarkers in the CSF of a discovery cohort of 136 pwMS and 49 healthy controls (HCs) and found 11 CSF proteins that were significantly more represented in pwMS than HC, 10 of which were validated on a replication cohort of 95 pwMS and 47 HCs (Huang et al., 2020). Nevertheless, these findings were not confirmed in plasma samples of the same patients, and this might be because of insufficient sensitivity of the methodic, hence it might be worth to investigate those proteins with highly sensitive immunoassays in blood.

The clinical utility of blood biomarkers at the individual patient level is currently unclear. Nonetheless, a prospective observational multicentre study was initiated in July 2018 by a net of German university hospitals with the goal of investigating the utility of blood biomarkers in MS, hopefully this and more prospective studies will shed a light on this aspect (Abdelhak et al., 2020). We reference also further interesting reviews on fluid biomarkers in MS (Barro et al., 2017; Varhaug et al., 2019; Kapoor et al., 2020; Williams et al., 2020; Thebault et al., 2021).

5. Amyotrophic lateral sclerosis (ALS)

ALS is a neurodegenerative disease of the upper and lower motoneurons with a poor prognosis (Hardiman et al., 2011). A precise diagnosis for patients with ALS is of utmost importance for evaluating potential treatments (Turner et al., 2013). Thus, biomarkers may have a fundamental role in correctly stratifying the patients for clinical trials and to monitor treatment efficacy and disease progression (Bakkar et al., 2015).

Neurodegeneration. The majority of ALS patients present cytoplasmic aggregates of transactive response DNA-binding protein 43 (TDP-43) (De Marco et al., 2017; Emily Feneberg et al., 2018). As extensively reviewed by Feneberg and colleagues the investigation of TDP-43 revealed to be technically challenging by the lack of specific antibodies and the presence in blood of TDP-43 derived from peripheral tissues (Emily Feneberg et al., 2018). Very recent data suggest that seeds of misfolded TDP-43 can be detected in lumbar CSF from patients with ALS and FTD using a real-time quaking-induced conversion (RT-QuIC) reaction (Scialò et al., 2020). This is a very promising technology that potentially could make an *in vivo* biomarker for TDP-43 pathology come real. Neurofilaments are an established biomarker of neurodegeneration, in ALS both the light (NfL) and the heavy isoform (NfH) have been explored. NfH was studied in both its native and hyperphosphorylated isoform as NfH and pNfH, respectively (Lu et al., 2012). Because NfH formed aggregates in the blood matrix (hook effect), the translation of the NfH assay from CSF to blood was challenging, however this was elegantly solved by the group of Petzold by pre-incubating the samples in a urea-based buffer

(Lu et al., 2011). The levels of both NfL and NfH in CSF and blood were prognostic for survival (Brettschneider et al., 2006; De Schaepdryver et al., 2020; Gille et al., 2019; Lu et al., 2015; Rosengren et al., 1996; Thouvenot et al., 2020; Verde et al., 2019b; Weydt et al., 2016; Zetterberg et al., 2007) and shown a diagnostic value when discriminating ALS from other motoneuron diseases (De Schaepdryver et al., 2018; E. Feneberg et al., 2018; Gille et al., 2019; Verde et al., 2019b). Interestingly, the concentration of neurofilaments appears stable over the course of the disease (Benatar et al., 2018; Gille et al., 2019; Lu et al., 2015), hence NfL/NfH may not be suitable for monitoring ALS progression. Despite this, a recent clinical trial of tofersen found a decrease in NfL/NfH levels in the treated arm compared to the placebo arm (Miller et al., 2020). A technical note - similarly to NfL, the quantification of NfH in blood benefited from the higher sensitivity of fourth generation assays as shown in a work from Wilke and colleagues (Wilke et al., 2019) and a second work from Gray and colleagues (Gray et al., 2020a). Interestingly, the performances of both Simoa assays were excellent, however the quality of the antibodies kept playing a role in achieving a better sensitivity in a homebrew compared to the commercial assay (Wilke et al., 2019).

Inflammation. The immune system appear to have a role in the pathology (Butovsky et al., 2012), even if it is unclear whether its activation is a mere consequence or a contributor to the disease (Béland et al., 2020). Biomarkers as cytokines may be helpful in quantifying the level of immune system activation and possibly help in disease staging. Notably, the production of these molecules occurs also in the periphery, hence their use is limited to CSF samples. Recent studies in CSF found that interleukin-6 (IL-6) and tumor necrosis factor alfa (TNF-α) were particularly elevated in ALS patients (Hu et al., 2017; Tortelli et al., 2020). Chitinases were also explored as CSF biomarkers of inflammation and their levels were found increased in ALS patients compared to healthy controls (Oeckl et al., 2019; Thompson et al., 2019), prognostic for survival (A. G. Thompson et al., 2018) and stable over the course of the disease (Gray et al., 2020b; Thompson et al., 2019), similarly to what was observed with neurofilaments. However, the levels were not increased in the pre-symptomatic phase of the disease as observed in patient with the familial form of the disease (Oeckl et al., 2019). Further

interesting reviews that focused at biomarkers in ALS are for example (Bakkar et al., 2015; Ryberg and Bowser, 2008; Turner et al., 2009; Verde et al., 2019a).

6. Frontotemporal dementia (FTD)

FTD is a neurodegenerative disease with both a sporadic and genetic origin (Bang et al., 2015). Forty percent of patients suffer from a genetic form of the disease caused in 60% of cases by *GRN* or *MAPT* mutations or *C9orf72* expansion (Bang et al., 2015).

Neurodegeneration. Patients with a *MAPT* mutation present, similarly to other neurodegenerative diseases, tau aggregates in neurons (Bang et al., 2015). The recent development of assays against phosphorylated states of tau enabled the discrimination of FTD from AD (Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) investigators et al., 2020; Karikari et al., 2020). The levels of the protein progranulin (encoded by the *GRN* gene) was found to be lower in FTD patients carrying the *GRN* mutation linked to FTD compared to healthy controls (Meeter et al., 2016). This had set the basis to the use of progranulin levels for detecting treatment efficacy – increase in progranulin levels may be interpreted as therapeutical success (Meeter et al., 2016; Swift et al., 2021). Most tau-negative FTD patients have TDP-43 inclusions. As described above (in an ALS context), this pathology is hard to detect using biomarkers, but a recent RT-QuIC assay has given promising results in both ALS and FTD (Scialò et al., 2020). Neurofilaments, a more universal marker of neuronal damage, were increased in FTD patients in the pre-symptomatic (van der Ende et al., 2019) and symptomatic stage (Al Shweiki et al., 2019; Katisko et al., 2020; Wilke et al., 2016). Thus, offering a measure on the progression of the disease.

Astrocytic dysfunction and damage Notably, the presence of astrogliosis in patients harboring the GRN mutation was reflected by an increase in blood GFAP levels compared to the other forms of FTD (Heller et al., 2020).

Inflammation. Increased levels of various cytokines, most notably the highly pro-inflammatory IL-6, TNF-α has been detected in CSF and blood (Bossù et al., 2011; Sjogren et al., 2004). The presence of pro-inflammatory cytokines was matched by elevated chitinases in the CSF reflecting activated microglia, astrocytes and macrophages in the CNS (Abu-Rumeileh et al., 2019; Woollacott et al., 2020).

In such a heterogeneous disease, it is critical to achieve methods that enable a precise stratification of patients for clinical trials and fluid biomarkers may play a prominent role in this. Further interesting reviews on fluid biomarkers in FTD are (Zetterberg et al., 2019; Swift et al., 2021).

7. Parkinson's disease (PD)

The loss of dopaminergic neurons in the substantia nigra associated with the presence of α-synuclein aggregates constitutes a hallmark of this pathology (Poewe et al., 2017). It was a natural step to explore α-synuclein as PD biomarker. The levels of α-synuclein in CSF were found to be consistently lower in PD patients compared to healthy controls (Mollenhauer et al., 2017; Parnetti et al., 2008), nevertheless this was overlapping with other α-synucleinopathies. The presence of high levels of α-synuclein in red blood cells poses a technical challenge to the use of this biomarker and particularly to its translation to blood measurements (Barkovits et al., 2020). On a similar fashion to what we saw in AD, a higher specificity for the biology of PD might be achieved by measuring a specific phosphorylation state of α-synuclein phosphorylated at the serine in position 129. This was detected by mass spectrometry in the CSF of PD patients (Wang et al., 2012) but could not be confirmed by using immunoassays (Cariulo et al., 2019). Hence, it is fair to speculate that technical improvements may lead to one or more biomarkers able to discriminate the biology of PD compared to other α-synucleinopathies. Similar to TDP-43, but more advanced and well-replicated, RT-QuIC-based assays to detect α-synuclein seeds in lumbar CSF have been developed (Fairfoul et al., 2016; Shahnawaz et al., 2017; Rossi et al., 2020; Iranzo et al., 2021). The assays reproducibly detect

α-synuclein pathology in PD, dementia with Lewy bodies, multiple system atrophy and PD-associated REM-sleep disorders with diagnostic accuracies exceeding 90%. Furthermore, the level of neurodegeneration occurring in PD patients can be quantified as level of NfL in the CSF or blood. The NfL levels are very mildly increased in PD compared to other neurological conditions (Bridel et al., 2019), and this increase was found associated with the severity of the motor impairment defined with the Hoehn and Yahr scale (Lin et al., 2019). Thus, blood NfL could help in monitoring disease progression, particularly at the late stages of PD when motor impairment is manifested. Moreover, the mild increase in NfL levels contrasts with the more pronounced increase detected in atypical parkinsonian disorders (disorders with a similar initial clinical presentation as PD); hence NfL may be helpful in differentiating patients with PD from those with progressive supranuclear palsy, cortical basal syndrome or multiple system atrophy (Hansson et al., 2017; Marques et al., 2019).

8. Spinal muscular atrophy (SMA)

SMA is an autosomal recessive neurodegenerative condition that affects the second motor neuron and manifest with muscular weakness (Talbot and Tizzano, 2017). Increased CSF pNfH levels were observed in the pre-symptomatic phase (De Vivo et al., 2019). Moreover, higher blood pNfH levels were associated with earlier disease onset and worse clinical presentation (Darras et al., 2019), though this association differed in the different phenotypes of the disease (the MND-Net et al., 2020; Walter et al., 2019; Wurster et al., 2019). Nusinersen, a first therapy based on an anti-sense oligonucleotide, became recently available (Finkel et al., 2017; Mercuri et al., 2018). The efficacy of this treatment was reflected by a decrease in blood pNfH (Darras et al., 2019; De Vivo et al., 2019), CSF pNfH (Winter et al., 2019) and CSF NfL levels (Olsson et al., 2019; Winter et al., 2019). Thus, the quantification of neurofilaments can be an important tool in identifying the best time to treat and monitoring the response to treatment.

9. Huntington's disease (HD)

HD is a neurodegenerative disease caused by an autosomal dominant mutation with expansion of CAG trinucleotide repeats that affect the correct production of a protein named huntingtin (Bates et al., 2015). Similarly, to other neurodegenerative diseases the neuronal damage occurs years prior to the first clinical manifestation (Tabrizi et al., 2012). Indeed, NfL in both CSF and blood was already increased in the pre-symptomatic stage of the disease and kept increasing during the course of the disease (Byrne et al., 2017; Vinther-Jensen et al., 2016; Rodrigues et al., 2020). The levels of CSF NfL were associated with the disease progression measured by clinical assessment (Constantinescu et al., 2009; Niemelä et al., 2017; Rodrigues et al., 2020; Vinther-Jensen et al., 2016). An increased level of neuronal damage - increased NfL – was prognostic for progression to the symptomatic stage (Byrne et al., 2017; Niemelä et al., 2017). In symptomatic patients, blood NfL levels were associated with measures of brain atrophy (Byrne et al., 2017; Johnson et al., 2018; Rodrigues et al., 2020), cognitive decline (Byrne et al., 2017; Rodrigues et al., 2020). These promising results underscore the potential role of NfL in both monitoring and supporting treatment trials in this disease. A new avenue for a diagnostic and monitoring tool emerged with the development of a single-molecule-count assay able to quantify huntingtin in the CSF (Wild et al., 2015). From what we learnt from NfL, the quantification of blood biomarkers may detect treatment efficacy already 3 months after treatment start (Hauser et al., 2019; Sejbaek et al., 2019). Sensitivity limitations are still challenging the detection of huntingtin in blood. Nonetheless, the translation of the huntingtin assay to blood could offer an unprecedented opportunity to quickly detect treatment efficacy in ongoing clinical trials.

10. Conclusion

The discovery of protein biomarkers able to objectively quantitate pathological processes of neurons, astrocytes and inflammation gave us an amazing tool to deepen our understanding of the disease, follow the disease progression over time and predict its course in presence or

absence of treatments. Discovery that was possible because of an improvement in the detection methods of which both the availability of high quality monoclonal antibodies and highly sensitive platforms were critical parts (Gisslen et al., 2016; Kuhle et al., 2016). A further step was achieved in the AD biomarkers by looking at post-translational modifications of a protein: tau. Different phosphorylation states of tau revealed to be an incredibly specific blood signature of AD (Teunissen et al., 2020). A universal neurological biomarker, NfL, is specifically detecting neuronal damage independently from the disease causing it. Hence, a cautious interpretation of this biomarker can have an immense impact in the clinical care of neurological patients (Barro et al., 2020; Barro and Zetterberg, 2021). The utility of neurofilaments, both NfL and pNfH, have been demonstrated also in mouse models of neurologic disease (Bacioglu et al., 2016; Wilke et al., 2020). Thus, blood biomarkers can assist preclinical studies and basic research. Moreover, the ability to quantitate each aspect of the disease at a high definition enables a more precise stratification of the patients during inclusion to clinical trials, hence reducing the noise that may hamper the detection of therapeutical efficacy and allowing for smaller but likewise powered studies. Ultimately, we are living in an amazing time in which an increasing number of biomarkers has the potential to support the best care for patients suffering from neurological conditions.

Contributions

CB, HZ did literature search, reviewed the manuscript, drafted the manuscript, and prepared the table. All authors read and approved the final version of the manuscript.

Conflict of interest

CB has nothing to disclose. HZ has served at scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio,

Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

Funding

CB is supported by a postdoctoral fellowship from the Swiss National Science Foundation (P400PM_191077). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL.

References

Abdelhak, A., Huss, A., Kassubek, J., Tumani, H., Otto, M., 2018. Serum GFAP as a biomarker for disease severity in multiple sclerosis. Sci Rep 8, 14798. https://doi.org/10.1038/s41598-018-33158-8

Abdelhak, A., Huss, A., Stahmann, A., Senel, M., Krumbholz, M., Kowarik, M.C., Havla, J., Kümpfel, T., Kleiter, I., Wüstinger, I., Zettl, U.K., Schwartz, M., Roesler, R., Friede, T., Ludolph, A.C., Ziemann, U., Tumani, H., 2020. Explorative study of emerging blood biomarkers in progressive multiple sclerosis (EmBioProMS): Design of a prospective observational multicentre pilot study. Contemporary Clinical Trials Communications 18, 100574. https://doi.org/10.1016/j.conctc.2020.100574

Abdullah, M., Kimura, N., Akatsu, H., Hashizume, Y., Ferdous, T., Tachita, T., Iida, S., Zou, K., Matsubara, E., Michikawa, M., 2019. Flotillin is a Novel Diagnostic Blood Marker of Alzheimer's Disease. Journal of Alzheimer's Disease 72, 1165–1176. https://doi.org/10.3233/JAD-190908

Abu-Rumeileh, S., Steinacker, P., Polischi, B., Mammana, A., Bartoletti-Stella, A., Oeckl, P., Baiardi, S., Zenesini, C., Huss, A., Cortelli, P., Capellari, S., Otto, M., Parchi, P., 2019. CSF biomarkers of neuroinflammation in distinct forms and subtypes of neurodegenerative dementia. Alzheimer's Research & Therapy 12, 2. https://doi.org/10.1186/s13195-019-0562-4

Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) investigators, Thijssen, E.H., La Joie, R., Wolf, A., Strom, A., Wang, P., Iaccarino, L., Bourakova, V., Cobigo, Y., Heuer, H., Spina, S., VandeVrede, L., Chai, X., Proctor, N.K., Airey, D.C., Shcherbinin, S., Duggan Evans, C., Sims, J.R., Zetterberg, H., Blennow, K., Karydas, A.M., Teunissen, C.E., Kramer, J.H., Grinberg, L.T., Seeley, W.W., Rosen, H., Boeve, B.F., Miller, B.L., Rabinovici, G.D., Dage, J.L., Rojas, J.C., Boxer, A.L., 2020. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. Nat Med 26, 387–397. https://doi.org/10.1038/s41591-020-0762-2

Al Shweiki, M.R., Steinacker, P., Oeckl, P., Hengerer, B., Danek, A., Fassbender, K., Diehl-Schmid, J., Jahn, H., Anderl-Straub, S., Ludolph, A.C., Schönfeldt-Lecuona, C., Otto, M., 2019. Neurofilament light chain as a blood biomarker to differentiate psychiatric disorders from behavioural variant frontotemporal dementia. Journal of Psychiatric Research 113, 137–140. https://doi.org/10.1016/j.jpsychires.2019.03.019

Alvarez, E., Piccio, L., Mikesell, R.J., Klawiter, E.C., Parks, B.J., Naismith, R.T., Cross, A.H., 2013. CXCL13 is a biomarker of inflammation in multiple sclerosis, neuromyelitis optica, and other neurological conditions. Mult. Scler 19, 1204–1208. https://doi.org/10.1177/1352458512473362

Arrambide, G., Espejo, C., Eixarch, H., Villar, L.M., Alvarez-Cermeno, J.C., Picon, C., Kuhle, J., Disanto, G., Kappos, L., Sastre-Garriga, J., Pareto, D., Simon, E., Comabella, M., Rio, J., Nos, C., Tur, C., Castillo, J., Vidal-Jordana, A., Galan, I., Arevalo, M.J., Auger, C., Rovira, A., Montalban, X., Tintore, M., 2016. Neurofilament light chain level is a weak risk factor for the development of MS. Neurology 87, 1076–1084. https://doi.org/10.1212/WNL.0000000000003085

Ashton, N.J., Pascoal, T.A., Karikari, T.K., Benedet, A.L., Lantero-Rodriguez, J., Brinkmalm, G., Snellman, A., Schöll, M., Troakes, C., Hye, A., Gauthier, S., Vanmechelen, E., Zetterberg, H., Rosa-Neto, P., Blennow, K., 2021. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. Acta Neuropathol. https://doi.org/10.1007/s00401-021-02275-6

Assarsson, E., Lundberg, M., Holmquist, G., Björkesten, J., Bucht Thorsen, S., Ekman, D., Eriksson, A., Rennel Dickens, E., Ohlsson, S., Edfeldt, G., Andersson, A.-C., Lindstedt, P., Stenvang, J., Gullberg, M., Fredriksson, S., 2014. Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability. PLoS ONE 9, e95192. https://doi.org/10.1371/journal.pone.0095192

Avolio, C., Filippi, M., Tortorella, C., Rocca, M.A., Ruggieri, M., Agosta, F., Tomassini, V., Pozzilli, C., Stecchi, S., Giaquinto, P., Livrea, P., Trojano, M., 2005. Serum MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios in multiple sclerosis: relationships with different

magnetic resonance imaging measures of disease activity during IFN-beta-1a treatment. Mult. Scler 11, 441–446.

Avolio, C., Ruggieri, M., Giuliani, F., Liuzzi, G.M., Leante, R., Riccio, P., Livrea, P., Trojano, M., 2003. Serum MMP-2 and MMP-9 are elevated in different multiple sclerosis subtypes. J. Neuroimmunol 136, 46–53.

Axelsson, M., Malmestrom, C., Gunnarsson, M., Zetterberg, H., Sundstrom, P., Lycke, J., Svenningsson, A., 2014. Immunosuppressive therapy reduces axonal damage in progressive multiple sclerosis. Mult. Scler 20, 43–50. https://doi.org/10.1177/1352458513490544

Ayrignac, X., Le Bars, E., Duflos, C., Hirtz, C., Maleska Maceski, A., Carra-Dallière, C., Charif, M., Pinna, F., Prin, P., Menjot de Champfleur, N., Deverdun, J., Kober, T., Marechal, B., Fartaria, M.J., Corredor Jerez, R., Labauge, P., Lehmann, S., 2020. Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity. Sci Rep 10, 10923. https://doi.org/10.1038/s41598-020-67934-2

Bacioglu, M., Maia, L.F., Preische, O., Schelle, J., Apel, A., Kaeser, S.A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., Shimshek, D.R., Neumann, U., Kahle, P.J., Staufenbiel, M., Neumann, M., Maetzler, W., Kuhle, J., Jucker, M., 2016. Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. Neuron 91, 56–66.

Baecher-Allan, C., Kaskow, B.J., Weiner, H.L., 2018. Multiple Sclerosis: Mechanisms and Immunotherapy. Neuron 97, 742–768. https://doi.org/10.1016/j.neuron.2018.01.021

Bakkar, N., Boehringer, A., Bowser, R., 2015. Use of biomarkers in ALS drug development and clinical trials. Brain Res 1607, 94–107. https://doi.org/10.1016/j.brainres.2014.10.031

Bang, J., Spina, S., Miller, B.L., 2015. Frontotemporal dementia. The Lancet 386, 1672–1682. https://doi.org/10.1016/S0140-6736(15)00461-4

Barkovits, K., Kruse, N., Linden, A., Tönges, L., Pfeiffer, K., Mollenhauer, B., Marcus, K., 2020. Blood Contamination in CSF and Its Impact on Quantitative Analysis of Alpha-Synuclein. Cells 9, 370. https://doi.org/10.3390/cells9020370

Barro, C., Benkert, P., Disanto, G., Tsagkas, C., Amann, M., Naegelin, Y., Leppert, D., Gobbi, C., Granziera, C., Yaldizli, O., Michalak, Z., Wuerfel, J., Kappos, L., Parmar, K., Kuhle, J., 2018. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain : a journal of neurology 141, 2382–2391. https://doi.org/10.1093/brain/awy154

Barro, C., Chitnis, T., Weiner, H.L., 2020. Blood neurofilament light: a critical review of its application to neurologic disease. Ann Clin Transl Neurol 7, 2508–2523. https://doi.org/10.1002/acn3.51234

Barro, C., Leocani, L., Leppert, D., Comi, G., Kappos, L., Kuhle, J., 2017. Fluid biomarker and electrophysiological outcome measures for progressive MS trials. Mult Scler 23, 1600–1613. https://doi.org/10.1177/1352458517732844

Barro, C., Zetterberg, H., 2021. Neurological symptoms and blood neurofilament light levels. Acta Neurologica Scandinavica n/a. https://doi.org/10.1111/ane.13415

Bates, G.P., Dorsey, R., Gusella, J.F., Hayden, M.R., Kay, C., Leavitt, B.R., Nance, M., Ross, C.A., Scahill, R.I., Wetzel, R., Wild, E.J., Tabrizi, S.J., 2015. Huntington disease. Nat Rev Dis Primers 1, 15005. https://doi.org/10.1038/nrdp.2015.5

Bekris, L.M., Khrestian, M., Dyne, E., Shao, Y., Pillai, J.A., Rao, S.M., Bemiller, S.M., Lamb, B., Fernandez, H.H., Leverenz, J.B., 2018. Soluble TREM2 and biomarkers of central and peripheral inflammation in neurodegenerative disease. Journal of Neuroimmunology 319, 19–27. https://doi.org/10.1016/j.jneuroim.2018.03.003

Béland, L.-C., Markovinovic, A., Jakovac, H., De Marchi, F., Bilic, E., Mazzini, L., Kriz, J., Munitic, I., 2020. Immunity in amyotrophic lateral sclerosis: blurred lines between excessive inflammation and inefficient immune responses. Brain Communications 2. https://doi.org/10.1093/braincomms/fcaa124

Benatar, M., Wuu, J., Andersen, P.M., Lombardi, V., Malaspina, A., 2018. Neurofilament light: A candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. Annals of Neurology 84, 130–139. https://doi.org/10.1002/ana.25276

Benedet, A.L., Leuzy, A., Pascoal, T.A., Ashton, N.J., Mathotaarachchi, S., Savard, M., Therriault, J., Kang, M.S., Chamoun, M., Schöll, M., Zimmer, E.R., Gauthier, S., Labbe, A., Zetterberg, H., Rosa-Neto, P., Blennow, K., for the Alzheimer's Disease Neuroimaging Initiative, 2020. Stage-specific links between plasma neurofilament light and imaging biomarkers of Alzheimer's disease. Brain 143, 3793–3804. https://doi.org/10.1093/brain/awaa342

Bittner, S., Steffen, F., Uphaus, T., Muthuraman, M., Fleischer, V., Salmen, A., Luessi, F., Berthele, A., Klotz, L., Meuth, S.G., Bayas, A., Paul, F., Hartung, H.-P., Linker, R., Heesen, C., Stangel, M., Wildemann, B., Then Bergh, F., Tackenberg, B., Kuempfel, T., Weber, F., Zettl, U.K., Ziemann, U., Tumani, H., Groppa, S., Mühlau, M., Lukas, C., Hemmer, B., Wiendl, H., Gold, R., Zipp, F., 2020. Clinical implications of serum neurofilament in newly diagnosed MS patients: A longitudinal multicentre cohort study. EBioMedicine 56, 102807. https://doi.org/10.1016/j.ebiom.2020.102807

Bjornevik, K., Munger, K.L., Cortese, M., Barro, C., Healy, B.C., Niebuhr, D.W., Scher, A.I., Kuhle, J., Ascherio, A., 2020. Serum Neurofilament Light Chain Levels in Patients With Presymptomatic Multiple Sclerosis. JAMA Neurol 77, 58–64. https://doi.org/10.1001/jamaneurol.2019.3238

Blennow, K., Mattsson, N., Schöll, M., Hansson, O., Zetterberg, H., 2015. Amyloid biomarkers in Alzheimer's disease. Trends in Pharmacological Sciences 36, 297–309. https://doi.org/10.1016/j.tips.2015.03.002

Blennow, K., Zetterberg, H., 2018. The Past and the Future of Alzheimer's Disease Fluid Biomarkers1. JAD 62, 1125–1140. https://doi.org/10.3233/JAD-170773

Bossù, P., Salani, F., Alberici, A., Archetti, S., Bellelli, G., Galimberti, D., Scarpini, E., Spalletta, G., Caltagirone, C., Padovani, A., Borroni, B., 2011. Loss of function

mutations in the progranulin gene are related to pro-inflammatory cytokine dysregulation in frontotemporal lobar degeneration patients. Journal of Neuroinflammation 8, 65. https://doi.org/10.1186/1742-2094-8-65

Brettschneider, J., Czerwoniak, A., Senel, M., Fang, L., Kassubek, J., Pinkhardt, E., Lauda, F., Kapfer, T., Jesse, S., Lehmensiek, V., Ludolph, A.C., Otto, M., Tumani, H., 2010. The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS). PLoS. One 5, e11986. https://doi.org/10.1371/journal.pone.0011986

Brettschneider, J., Petzold, A., Sussmuth, S.D., Ludolph, A.C., Tumani, H., 2006. Axonal damage markers in cerebrospinal fluid are increased in ALS. Neurology 66, 852–856. https://doi.org/10.1212/01.wnl.0000203120.85850.54

Bridel, C., Wieringen, W.N. van, Zetterberg, H., Tijms, B.M., Teunissen, C.E., Alvarez-Cermeño, J.C., Andreasson, U., Axelsson, M., Bäckström, D.C., Bartos, A., Bjerke, M., Blennow, K., Boxer, A., Brundin, L., Burman, J., Christensen, T., Fialová, L., Forsgren, L., Frederiksen, J.L., Gisslén, M., Gray, E., Gunnarsson, M., Hall, S., Hansson, O., Herbert, M.K., Jakobsson, J., Jessen-Krut, J., Janelidze, S., Johannsson, G., Jonsson, M., Kappos, L., Khademi, M., Khalil, M., Kuhle, J., Landén, M., Leinonen, V., Logroscino, G., Lu, C.-H., Lycke, J., Magdalinou, N.K., Malaspina, A., Mattsson, N., Meeter, L.H., Mehta, S.R., Modvig, S., Olsson, T., Paterson, R.W., Pérez-Santiago, J., Piehl, F., Pijnenburg, Y.A.L., Pyykkö, O.T., Ragnarsson, O., Rojas, J.C., Christensen, J.R., Sandberg, L., Scherling, C.S., Schott, J.M., Sellebjerg, F.T., Simone, I.L., Skillbäck, T., Stilund, M., Sundström, P., Svenningsson, A., Tortelli, R., Tortorella, C., Trentini, A., Troiano, M., Turner, M.R., Swieten, J.C. van, Vågberg, M., Verbeek, M.M., Villar, L.M., Visser, P.J., Wallin, A., Weiss, A., Wikkelsø, C., Wild, E.J., 2019. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. JAMA Neurol 76, 1035–1048. https://doi.org/10.1001/jamaneurol.2019.1534

Burman, J., Zetterberg, H., Fransson, M., Loskog, A.S., Raininko, R., Fagius, J., 2014. Assessing tissue damage in multiple sclerosis: a biomarker approach. Acta Neurol. Scand 130, 81–89. https://doi.org/10.1111/ane.12239

Butovsky, O., Siddiqui, S., Gabriely, G., Lanser, A.J., Dake, B., Murugaiyan, G., Doykan, C.E., Wu, P.M., Gali, R.R., Iyer, L.K., Lawson, R., Berry, J., Krichevsky, A.M., Cudkowicz, M.E., Weiner, H.L., 2012. Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J. Clin. Invest. 122, 3063–3087. https://doi.org/10.1172/JCI62636

Butovsky, O., Weiner, H.L., 2018. Microglial signatures and their role in health and disease. Nat Rev Neurosci 19, 622–635. https://doi.org/10.1038/s41583-018-0057-5

Byrne, L.M., Rodrigues, F.B., Blennow, K., Durr, A., Leavitt, B.R., Roos, R.A.C., Scahill, R.I., Tabrizi, S.J., Zetterberg, H., Langbehn, D., Wild, E.J., 2017. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. Lancet Neurol 16, 601–609. https://doi.org/10.1016/S1474-4422(17)30124-2

Calabresi, P.A., Arnold, D.L., Sangurdekar, D., Singh, C.M., Altincatal, A., de Moor, C., Engle, B., Goyal, J., Deykin, A., Szak, S., Kieseier, B.C., Rudick, R.A., Plavina, T., 2020. Temporal profile of serum neurofilament light in multiple sclerosis: Implications for patient monitoring. Mult Scler 1352458520972573. https://doi.org/10.1177/1352458520972573

Cantó, E., Barro, C., Zhao, C., Caillier, S.J., Michalak, Z., Bove, R., Tomic, D., Santaniello, A., Häring, D.A., Hollenbach, J., Henry, R.G., Cree, B.A.C., Kappos, L., Leppert, D., Hauser, S.L., Benkert, P., Oksenberg, J.R., Kuhle, J., 2019. Association Between Serum Neurofilament Light Chain Levels and Long-term Disease Course Among Patients With Multiple Sclerosis Followed up for 12 Years. JAMA Neurol 76, 1359–1366. https://doi.org/10.1001/jamaneurol.2019.2137

Cantó, E., Reverter, F., Morcillo-Suárez, C., Matesanz, F., Fernández, O., Izquierdo, G., Vandenbroeck, K., Rodríguez-Antigüedad, A., Urcelay, E., Arroyo, R., Otaegui, D.,

Olascoaga, J., Saiz, A., Navarro, A., Sanchez, A., Domínguez, C., Caminero, A., Horga, A., Tintoré, M., Montalban, X., Comabella, M., 2012. Chitinase 3-like 1 plasma levels are increased in patients with progressive forms of multiple sclerosis. Mult Scler 18, 983–990. https://doi.org/10.1177/1352458511433063

Canto, E., Tintore, M., Villar, L.M., Costa, C., Nurtdinov, R., Alvarez-Cermeno, J.C., Arrambide, G., Reverter, F., Deisenhammer, F., Hegen, H., Khademi, M., Olsson, T., Tumani, H., Rodriguez-Martin, E., Piehl, F., Bartos, A., Zimova, D., Kotoucova, J., Kuhle, J., Kappos, L., Garcia-Merino, J.A., Sanchez, A.J., Saiz, A., Blanco, Y., Hintzen, R., Jafari, N., Brassat, D., Lauda, F., Roesler, R., Rejdak, K., Papuc, E., de, A.C., Rauch, S., Khalil, M., Enzinger, C., Galimberti, D., Scarpini, E., Teunissen, C., Sanchez, A., Rovira, A., Montalban, X., Comabella, M., 2015. Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes. Brain 138, 918–931. https://doi.org/10.1093/brain/awv017

Cariulo, C., Martufi, P., Verani, M., Azzollini, L., Bruni, G., Weiss, A., Deguire, S.M., Lashuel, H.A., Scaricamazza, E., Sancesario, G.M., Schirinzi, T., Mercuri, N.B., Sancesario, G., Caricasole, A., Petricca, L., 2019. Phospho-S129 Alpha-Synuclein Is Present in Human Plasma but Not in Cerebrospinal Fluid as Determined by an Ultrasensitive Immunoassay. Front. Neurosci. 13. https://doi.org/10.3389/fnins.2019.00889

Chhatwal, J.P., Schultz, A.P., Dang, Y., Ostaszewski, B., Liu, L., Yang, H.-S., Johnson, K.A., Sperling, R.A., Selkoe, D.J., 2020. Plasma N-terminal tau fragment levels predict future cognitive decline and neurodegeneration in healthy elderly individuals. Nat Commun 11. https://doi.org/10.1038/s41467-020-19543-w

Chitnis, T., Gonzalez, C., Healy, B.C., Saxena, S., Rosso, M., Barro, C., Michalak, Z., Paul, A., Kivisakk, P., Diaz-Cruz, C., Sattarnezhad, N., Pierre, I.V., Glanz, B.I., Tomic, D., Kropshofer, H., Haring, D., Leppert, D., Kappos, L., Bakshi, R., Weiner, H.L., Kuhle, J., 2018. Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. Annals of clinical and translational neurology 5, 1478–1491. https://doi.org/10.1002/acn3.638

Chitnis, T., Weiner, H.L., 2017. CNS inflammation and neurodegeneration. Journal of Clinical Investigation 127, 3577–3587. https://doi.org/10.1172/JCI90609

Chitnis, T., Yano, H., Saxena, S., 2019. ECTRIMS 2019 - Poster Session 3. Mult Scler 25, 581–805. https://doi.org/10.1177/1352458519868081

Citron, M., Vigo-Pelfrey, C., Teplow, D.B., Miller, C., Schenk, D., Johnston, J., Winblad, B., Venizelos, N., Lannfelt, L., Selkoe, D.J., 1994. Excessive production of amyloid beta-protein by peripheral cells of symptomatic and presymptomatic patients carrying the Swedish familial Alzheimer disease mutation. PNAS 91, 11993–11997. https://doi.org/10.1073/pnas.91.25.11993

Comabella, M., Fernandez, M., Martin, R., Rivera-Vallve, S., Borras, E., Chiva, C., Julia, E., Rovira, A., Canto, E., Alvarez-Cermeno, J.C., Villar, L.M., Tintore, M., Montalban, X., 2010. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. Brain 133, 1082–1093. https://doi.org/10.1093/brain/awq035

Constantinescu, R., Romer, M., Oakes, D., Rosengren, L., Kieburtz, K., 2009. Levels of the light subunit of neurofilament triplet protein in cerebrospinal fluid in Huntington's disease. Parkinsonism. Relat Disord 15, 245–248. https://doi.org/10.1016/j.parkreldis.2008.05.012

Cramm, M., Schmitz, M., Karch, A., Mitrova, E., Kuhn, F., Schroeder, B., Raeber, A., Varges, D., Kim, Y.-S., Satoh, K., Collins, S., Zerr, I., 2016. Stability and Reproducibility Underscore Utility of RT-QuIC for Diagnosis of Creutzfeldt-Jakob Disease. Mol Neurobiol 53, 1896–1904. https://doi.org/10.1007/s12035-015-9133-2

Darras, B.T., Crawford, T.O., Finkel, R.S., Mercuri, E., De Vivo, D.C., Oskoui, M., Tizzano, E.F., Ryan, M.M., Muntoni, F., Zhao, G., Staropoli, J., McCampbell, A., Petrillo, M., Stebbins, C., Fradette, S., Farwell, W., Sumner, C.J., 2019. Neurofilament as a potential biomarker for spinal muscular atrophy. Ann Clin Transl Neurol 6, 932–944. https://doi.org/10.1002/acn3.779

de Flon, P., Gunnarsson, M., Laurell, K., Söderström, L., Birgander, R., Lindqvist, T., Krauss, W., Dring, A., Bergman, J., Sundström, P., Svenningsson, A., 2016. Reduced

inflammation in relapsing-remitting multiple sclerosis after therapy switch to rituximab. Neurology 87, 141–147. https://doi.org/10.1212/WNL.0000000000002832

De Marco, G., Lomartire, A., Calvo, A., Risso, A., De Luca, E., Mostert, M., Mandrioli, J., Caponnetto, C., Borghero, G., Manera, U., Canosa, A., Moglia, C., Restagno, G., Fini, N., Tarella, C., Giordana, M.T., Rinaudo, M.T., Chiò, A., 2017. Monocytes of patients with amyotrophic lateral sclerosis linked to gene mutations display altered TDP-43 subcellular distribution. Neuropathol Appl Neurobiol 43, 133–153. https://doi.org/10.1111/nan.12328

De Schaepdryver, M., Jeromin, A., Gille, B., Claeys, K.G., Herbst, V., Brix, B., Van Damme, P., Poesen, K., 2018. Comparison of elevated phosphorylated neurofilament heavy chains in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 89, 367–373. https://doi.org/10.1136/jnnp-2017-316605

De Schaepdryver, M., Lunetta, C., Tarlarini, C., Mosca, L., Chio, A., Van Damme, P., Poesen, K., 2020. Neurofilament light chain and C reactive protein explored as predictors of survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 91, 436–437. https://doi.org/10.1136/jnnp-2019-322309

De Vivo, D.C., Bertini, E., Swoboda, K.J., Hwu, W.-L., Crawford, T.O., Finkel, R.S., Kirschner, J., Kuntz, N.L., Parsons, J.A., Ryan, M.M., Butterfield, R.J., Topaloglu, H., Ben-Omran, T., Sansone, V.A., Jong, Y.-J., Shu, F., Staropoli, J.F., Kerr, D., Sandrock, A.W., Stebbins, C., Petrillo, M., Braley, G., Johnson, K., Foster, R., Gheuens, S., Bhan, I., Reyna, S.P., Fradette, S., Farwell, W., 2019. Nusinersen initiated in infants during the presymptomatic stage of spinal muscular atrophy: Interim efficacy and safety results from the Phase 2 NURTURE study. Neuromuscular Disorders 29, 842–856. https://doi.org/10.1016/j.nmd.2019.09.007

Delcoigne, B., Manouchehrinia, A., Barro, C., Benkert, P., Michalak, Z., Kappos, L., Leppert, D., Tsai, J.A., Plavina, T., Kieseier, B.C., Lycke, J., Alfredsson, L., Kockum, I., Kuhle, J., Olsson, T., Piehl, F., 2020. Blood neurofilament light levels segregate treatment

effects in multiple sclerosis. Neurology 94, e1201–e1212. https://doi.org/10.1212/WNL.0000000000009097

Disanto, G., Barro, C., Benkert, P., Naegelin, Y., Schadelin, S., Giardiello, A., Zecca, C., Blennow, K., Zetterberg, H., Leppert, D., Kappos, L., Gobbi, C., Kuhle, J., Swiss Multiple Sclerosis Cohort Study, G., 2017. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol 81, 857–870. https://doi.org/10.1002/ana.24954

Dysinger, M., Marusov, G., Fraser, S., 2017. Quantitative analysis of four protein biomarkers: An automated microfluidic cartridge-based method and its comparison to colorimetric ELISA. Journal of Immunological Methods 451, 1–10. https://doi.org/10.1016/j.jim.2017.08.009

Fainardi, E., Castellazzi, M., Bellini, T., Manfrinato, M.C., Baldi, E., Casetta, I., Paolino, E., Granieri, E., Dallocchio, F., 2006. Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. Mult. Scler 12, 294–301. https://doi.org/10.1191/135248506ms1274oa

Fairfoul, G., McGuire, L.I., Pal, S., Ironside, J.W., Neumann, J., Christie, S., Joachim, C., Esiri, M., Evetts, S.G., Rolinski, M., Baig, F., Ruffmann, C., Wade-Martins, R., Hu, M.T.M., Parkkinen, L., Green, A.J.E., 2016. Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies. Annals of Clinical and Translational Neurology 3, 812–818. https://doi.org/10.1002/acn3.338

Feneberg, Emily, Gray, E., Ansorge, O., Talbot, K., Turner, M.R., 2018. Towards a TDP-43-Based Biomarker for ALS and FTLD. Mol Neurobiol 55, 7789–7801. https://doi.org/10.1007/s12035-018-0947-6

Feneberg, E., Oeckl, P., Steinacker, P., Verde, F., Barro, C., Van Damme, P., Gray, E., Grosskreutz, J., Jardel, C., Kuhle, J., Koerner, S., Lamari, F., Amador, M.D.M., Mayer, B., Morelli, C., Muckova, P., Petri, S., Poesen, K., Raaphorst, J., Salachas, F., Silani, V., Stubendorff, B., Turner, M.R., Verbeek, M.M., Weishaupt, J.H., Weydt, P.,

Ludolph, A.C., Otto, M., 2018. Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. Neurology 90, e22–e30. https://doi.org/10.1212/WNL.0000000000004761

Finkel, R.S., Mercuri, E., Darras, B.T., Connolly, A.M., Kuntz, N.L., Kirschner, J., Chiriboga, C.A., Saito, K., Servais, L., Tizzano, E., Topaloglu, H., Tulinius, M., Montes, J., Glanzman, A.M., Bishop, K., Zhong, Z.J., Gheuens, S., Bennett, C.F., Schneider, E., Farwell, W., De Vivo, D.C., 2017. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med 377, 1723–1732. https://doi.org/10.1056/NEJMoa1702752

Fischer, S.K., Joyce, A., Spengler, M., Yang, T.-Y., Zhuang, Y., Fjording, M.S., Mikulskis, A., 2015. Emerging Technologies to Increase Ligand Binding Assay Sensitivity. AAPS J 17, 93–101. https://doi.org/10.1208/s12248-014-9682-8

Fox, R.J., Coffey, C.S., Conwit, R., Cudkowicz, M.E., Gleason, T., Goodman, A., Klawiter, E.C., Matsuda, K., McGovern, M., Naismith, R.T., Ashokkumar, A., Barnes, J., Ecklund, D., Klingner, E., Koepp, M., Long, J.D., Natarajan, S., Thornell, B., Yankey, J., Bermel, R.A., Debbins, J.P., Huang, X., Jagodnik, P., Lowe, M.J., Nakamura, K., Narayanan, S., Sakaie, K.E., Thoomukuntla, B., Zhou, X., Krieger, S., Alvarez, E., Apperson, M., Bashir, K., Cohen, B.A., Coyle, P.K., Delgado, S., Dewitt, L.D., Flores, A., Giesser, B.S., Goldman, M.D., Jubelt, B., Lava, N., Lynch, S.G., Moses, H., Ontaneda, D., Perumal, J.S., Racke, M., Repovic, P., Riley, C.S., Severson, C., Shinnar, S., Suski, V., Weinstock-Guttman, B., Yadav, V., Zabeti, A., 2018. Phase 2 Trial of Ibudilast in Progressive Multiple Sclerosis. N Engl J Med 379, 846–855. https://doi.org/10.1056/NEJMoa1803583

Fox, R.J., Raska, P., Barro, C., Karafa, M., Konig, V., Bermel, R.A., Chase, M., Coffey, C.S., Goodman, A.D., Klawiter, E.C., Naismith, R.T., Kuhle, J., 2021. Neurofilament light chain in a phase 2 clinical trial of ibudilast in progressive multiple sclerosis. Mult Scler 1352458520986956. https://doi.org/10.1177/1352458520986956

Gauthier, A., Viel, S., Perret, M., Brocard, G., Casey, R., Lombard, C., Laurent-Chabalier, S., Debouverie, M., Edan, G., Vukusic, S., Lebrun-Frénay, C., Sèze, J.D., Laplaud, D.A., Castelnovo, G., Gout, O., Ruet, A., Moreau, T., Casez, O., Clavelou, P., Berger, E., Zephir, H., Trouillet-Assant, S., Thouvenot, E., 2021. Comparison of SimoaTM and EllaTM to assess serum neurofilament-light chain in multiple sclerosis. Annals of Clinical and Translational Neurology 8, 1141–1150. https://doi.org/10.1002/acn3.51355

Gille, B., De Schaepdryver, M., Goossens, J., Dedeene, L., De Vocht, J., Oldoni, E., Goris, A., Van Den Bosch, L., Depreitere, B., Claeys, K.G., Tournoy, J., Van Damme, P., Poesen, K., 2019. Serum neurofilament light chain levels as a marker of upper motor neuron degeneration in patients with Amyotrophic Lateral Sclerosis. Neuropathol Appl Neurobiol 45, 291–304. https://doi.org/10.1111/nan.12511

Gil-Perotin, S., Castillo-Villalba, J., Cubas-Nuñez, L., Gasque, R., Hervas, D., Gomez-Mateu, J., Alcala, C., Perez-Miralles, F., Gascon, F., Dominguez, J.A., Casanova, B., 2019. Combined Cerebrospinal Fluid Neurofilament Light Chain Protein and Chitinase-3 Like-1 Levels in Defining Disease Course and Prognosis in Multiple Sclerosis. Front Neurol 10. https://doi.org/10.3389/fneur.2019.01008

Gisslen, M., Price, R.W., Andreasson, U., Norgren, N., Nilsson, S., Hagberg, L., Fuchs, D., Spudich, S., Blennow, K., Zetterberg, H., 2016. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. EBioMedicine 3, 135–140. https://doi.org/10.1016/j.ebiom.2015.11.036

Gray, E., Oeckl, P., Amador, M.D.M., Andreasson, U., An, J., Blennow, K., Bowser, R., De Schaepdryver, M., Heslegrave, A., Kuhle, J., Maceski, A., Koel-Simmelink, M., Lamari, F., Lombardi, V., Malaspina, A., Nilsson, I., Poesen, K., Salachas, F., Steinacker, P., Teunissen, C.E., Van Damme, P., Zetterberg, H., Ludolph, A., Jeromin, A., Turner, M.R., Otto, M., 2020a. A multi-center study of neurofilament

assay reliability and inter-laboratory variability. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 1–7. https://doi.org/10.1080/21678421.2020.1779300

Gray, E., Thompson, A.G., Wuu, J., Pelt, J., Talbot, K., Benatar, M., Turner, M.R., 2020b. CSF chitinases before and after symptom onset in amyotrophic lateral sclerosis. Annals of Clinical and Translational Neurology 7, 1296–1306. https://doi.org/10.1002/acn3.51114

Gunnarsson, M., Malmestrom, C., Axelsson, M., Sundstrom, P., Dahle, C., Vrethem, M., Olsson, T., Piehl, F., Norgren, N., Rosengren, L., Svenningsson, A., Lycke, J., 2011. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann. Neurol 69, 83–89. https://doi.org/10.1002/ana.22247

Håkansson, I., Tisell, A., Cassel, P., Blennow, K., Zetterberg, H., Lundberg, P., Dahle, C., Vrethem, M., Ernerudh, J., 2018. Neurofilament levels, disease activity and brain volume during follow-up in multiple sclerosis. J Neuroinflammation 15, 209. https://doi.org/10.1186/s12974-018-1249-7

Hakansson, I., Tisell, A., Cassel, P., Blennow, K., Zetterberg, H., Lundberg, P., Dahle, C., Vrethem, M., Ernerudh, J., 2017. Neurofilament light chain in cerebrospinal fluid and prediction of disease activity in clinically isolated syndrome and relapsing-remitting multiple sclerosis. Eur J Neurol 24, 703–712. https://doi.org/10.1111/ene.13274

Hansson, O., Janelidze, S., Hall, S., Magdalinou, N., Lees, A.J., Andreasson, U., Norgren, N., Linder, J., Forsgren, L., Constantinescu, R., Zetterberg, H., Blennow, K., 2017. Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder. Neurology 88, 930–937. https://doi.org/10.1212/WNL.0000000000003680

Hardiman, O., Berg, L.H. van den, Kiernan, M.C., 2011. Clinical diagnosis and management of amyotrophic lateral sclerosis. Nat Rev Neurol 7, 639–649. https://doi.org/10.1038/nrneurol.2011.153

Hauser, S.L., Bar-Or, A., Cohen, J., Comi, G., Correale, J., Coyle, P.K., Cross, A.H., de Seze, J., Montalban, X., Selmaj, K., Wiendl, H., Goodyear, A., Häring, D.A.,

Kerloeguen, C., Tomic, D., Willi, R., Ramanathan, K., Merschhemke, M., Kappos, L., 2019. ECTRIMS 2019 – Late Breaking News Abstracts. Multiple Sclerosis Journal 49.

Heller, C., Foiani, M.S., Moore, K., Convery, R., Bocchetta, M., Neason, M., Cash, D.M., Thomas, D., Greaves, C.V., Woollacott, I.O., Shafei, R., Van Swieten, J.C., Moreno, F., Sanchez-Valle, R., Borroni, B., Laforce Jr, R., Masellis, M., Tartaglia, M.C., Graff, C., Galimberti, D., Rowe, J.B., Finger, E., Synofzik, M., Vandenberghe, R., de Mendonca, A., Tagliavini, F., Santana, I., Ducharme, S., Butler, C.R., Gerhard, A., Levin, J., Danek, A., Frisoni, G., Sorbi, S., Otto, M., Heslegrave, A.J., Zetterberg, H., Rohrer, J.D., 2020. Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia. J Neurol Neurosurg Psychiatry 91, 263–270. https://doi.org/10.1136/jnnp-2019-321954

Heslegrave, A., Heywood, W., Paterson, R., Magdalinou, N., Svensson, J., Johansson, P., Öhrfelt, A., Blennow, K., Hardy, J., Schott, J., Mills, K., Zetterberg, H., 2016. Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. Molecular Neurodegeneration 11, 3. https://doi.org/10.1186/s13024-016-0071-x

Hinsinger, G., Galeotti, N., Nabholz, N., Urbach, S., Rigau, V., Demattei, C., Lehmann, S., Camu, W., Labauge, P., Castelnovo, G., Brassat, D., Loussouarn, D., Salou, M., Laplaud, D., Casez, O., Bockaert, J., Marin, P., Thouvenot, E., 2015. Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. Mult. Scler 21, 1251–1261. https://doi.org/10.1177/1352458514561906

Högel, H., Rissanen, E., Barro, C., Matilainen, M., Nylund, M., Kuhle, J., Airas, L., 2020. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. Mult Scler 26, 210–219. https://doi.org/10.1177/1352458518819380

Hu, Y., Cao, C., Qin, X.-Y., Yu, Y., Yuan, J., Zhao, Y., Cheng, Y., 2017. Increased peripheral blood inflammatory cytokine levels in amyotrophic lateral sclerosis: a meta-analysis study. Sci Rep 7, 9094. https://doi.org/10.1038/s41598-017-09097-1

Huang, J., Khademi, M., Fugger, L., Lindhe, Ö., Novakova, L., Axelsson, M., Malmeström, C., Constantinescu, C., Lycke, J., Piehl, F., Olsson, T., Kockum, I., 2020.

Inflammation-related plasma and CSF biomarkers for multiple sclerosis. Proc Natl Acad Sci USA 201912839. https://doi.org/10.1073/pnas.1912839117

Huss, A., Otto, M., Senel, M., Ludolph, A.C., Abdelhak, A., Tumani, H., 2020. A Score Based on NfL and Glial Markers May Differentiate Between Relapsing–Remitting and Progressive MS Course. Front. Neurol. 11. https://doi.org/10.3389/fneur.2020.00608

Iranzo, A., Fairfoul, G., Ayudhaya, A.C.N., Serradell, M., Gelpi, E., Vilaseca, I., Sanchez-Valle, R., Gaig, C., Santamaria, J., Tolosa, E., Riha, R.L., Green, A.J.E., 2021. Detection of α-synuclein in CSF by RT-QuIC in patients with isolated rapid-eye-movement sleep behaviour disorder: a longitudinal observational study. The Lancet Neurology 20, 203–212. https://doi.org/10.1016/S1474-4422(20)30449-X

Jack, C.R., Bennett, D.A., Blennow, K., Carrillo, M.C., Feldman, H.H., Frisoni, G.B., Hampel, H., Jagust, W.J., Johnson, K.A., Knopman, D.S., Petersen, R.C., Scheltens, P., Sperling, R.A., Dubois, B., 2016. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology 87, 539–547. https://doi.org/10.1212/WNL.0000000000002923

Janelidze, S., Mattsson, N., Palmqvist, S., Smith, R., Beach, T.G., Serrano, G.E., Chai, X., Proctor, N.K., Eichenlaub, U., Zetterberg, H., Blennow, K., Reiman, E.M., Stomrud, E., Dage, J.L., Hansson, O., 2020. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. Nat Med 26, 379–386. https://doi.org/10.1038/s41591-020-0755-1

Janelidze, S., Stomrud, E., Palmqvist, S., Zetterberg, H., van Westen, D., Jeromin, A., Song, L., Hanlon, D., Tan Hehir, C.A., Baker, D., Blennow, K., Hansson, O., 2016. Plasma β-amyloid in Alzheimer's disease and vascular disease. Sci Rep 6. https://doi.org/10.1038/srep26801

Johnson, E.B., Byrne, L.M., Gregory, S., Rodrigues, F.B., Blennow, K., Durr, A., Leavitt, B.R., Roos, R.A., Zetterberg, H., Tabrizi, S.J., Scahill, R.I., Wild, E.J., for the TRACK-HD Study Group, 2018. Neurofilament light protein in blood predicts regional atrophy

in Huntington disease. Neurology 90, e717–e723. https://doi.org/10.1212/WNL.0000000000005005

Kanmert, D., Cantlon, A., Muratore, C.R., Jin, M., O'Malley, T.T., Lee, G., Young-Pearse, T.L., Selkoe, D.J., Walsh, D.M., 2015. C-Terminally Truncated Forms of Tau, But Not Full-Length Tau or Its C-Terminal Fragments, Are Released from Neurons Independently of Cell Death. J Neurosci 35, 10851–10865. https://doi.org/10.1523/JNEUROSCI.0387-15.2015

Kapoor, R., Smith, K.E., Allegretta, M., Arnold, D.L., Carroll, W., Comabella, M., Furlan, R., Harp, C., Kuhle, J., Leppert, D., Plavina, T., Sellebjerg, F., Sincock, C., Teunissen, C.E., Topalli, I., von Raison, F., Walker, E., Fox, R.J., 2020. Serum neurofilament light as a biomarker in progressive multiple sclerosis. Neurology 95, 436–444. https://doi.org/10.1212/WNL.0000000000010346

Karikari, T.K., Pascoal, T.A., Ashton, N.J., Janelidze, S., Benedet, A.L., Rodriguez, J.L., Chamoun, M., Savard, M., Kang, M.S., Therriault, J., Schöll, M., Massarweh, G., Soucy, J.-P., Höglund, K., Brinkmalm, G., Mattsson, N., Palmqvist, S., Gauthier, S., Stomrud, E., Zetterberg, H., Hansson, O., Rosa-Neto, P., Blennow, K., 2020. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. The Lancet Neurology 19, 422–433. https://doi.org/10.1016/S1474-4422(20)30071-5

Katisko, K., Cajanus, A., Jääskeläinen, O., Kontkanen, A., Hartikainen, P., Korhonen, V.E., Helisalmi, S., Haapasalo, A., Koivumaa-Honkanen, H., Herukka, S.-K., Remes, A.M., Solje, E., 2020. Serum neurofilament light chain is a discriminative biomarker between frontotemporal lobar degeneration and primary psychiatric disorders. J Neurol 267, 162–167. https://doi.org/10.1007/s00415-019-09567-8

Khademi, M., Kockum, I., Andersson, M.L., Iacobaeus, E., Brundin, L., Sellebjerg, F., Hillert, J., Piehl, F., Olsson, T., 2011. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult. Scler 17, 335–343. https://doi.org/10.1177/1352458510389102

Khalil, M., Enzinger, C., Langkammer, C., Ropele, S., Mader, A., Trentini, A., Vane, M.L., Wallner-Blazek, M., Bachmaier, G., Archelos, J.J., Koel-Simmelink, M.J., Blankenstein, M.A., Fuchs, S., Fazekas, F., Teunissen, C.E., 2013. CSF neurofilament and N-acetylaspartate related brain changes in clinically isolated syndrome. Mult. Scler 19, 436–442. https://doi.org/10.1177/1352458512458010

Khalil, M., Teunissen, C.E., Otto, M., Piehl, F., Sormani, M.P., Gattringer, T., Barro, C., Kappos, L., Comabella, M., Fazekas, F., Petzold, A., Blennow, K., Zetterberg, H., Kuhle, J., 2018. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 14, 577–589. https://doi.org/10.1038/s41582-018-0058-z

Kuhle, J., Barro, C., Andreasson, U., Derfuss, T., Lindberg, R., Sandelius, A., Liman, V., Norgren, N., Blennow, K., Zetterberg, H., 2016. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med 54, 1655–61. https://doi.org/10.1515/cclm-2015-1195

Kuhle, J., Disanto, G., Lorscheider, J., Stites, T., Chen, Y., Dahlke, F., Francis, G., Shrinivasan, A., Radue, E.W., Giovannoni, G., Kappos, L., 2015. Fingolimod and CSF neurofilament light chain levels in relapsing-remitting multiple sclerosis. Neurology 84, 1639–1643. https://doi.org/10.1212/WNL.0000000000001491

Kuhle, J., Kropshofer, H., Barro, C., Meinert, R., Häring, D.A., Leppert, D., Tomic, D., Dahlke, F., Kappos, L., 2018. Siponimod Reduces Neurofilament Light Chain Blood Levels in Secondary Progressive Multiple Sclerosis Patients (S8.006). Neurology 90, S8.006.

Kuhle, J., Kropshofer, H., Haering, D.A., Kundu, U., Meinert, R., Barro, C., Dahlke, F., Tomic, D., Leppert, D., Kappos, L., 2019a. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology 92, e1007–e1015. https://doi.org/10.1212/WNL.0000000000007032

Kuhle, J., Kropshofer, H., Meinert, R., Maceski, A.M., Dahlke, F., Hach, T., Tomic, D., Kappos, L., Leppert, D., 2019b. ECTRIMS 2019 – Late Breaking News Abstracts. Mult Scler P1630, 25, 890–938. https://doi.org/10.1177/1352458519869496

Kuhle, J., Nourbakhsh, B., Grant, D., Morant, S., Barro, C., Yaldizli, Ö., Pelletier, D., Giovannoni, G., Waubant, E., Gnanapavan, S., 2017. Serum neurofilament is associated with progression of brain atrophy and disability in early MS. Neurology 88, 826–831. https://doi.org/10.1212/WNL.0000000000003653

Kuhle, J., Plattner, K., Bestwick, J.P., Lindberg, R.L., Ramagopalan, S.V., Norgren, N., Nissim, A., Malaspina, A., Leppert, D., Giovannoni, G., Kappos, L., 2013. A comparative study of CSF neurofilament light and heavy chain protein in MS. Mult. Scler 19, 1597–1603. https://doi.org/10.1177/1352458513482374

Kuhle, Jens, Plavina, T., Barro, C., Disanto, G., Sangurdekar, D., Singh, C.M., de Moor, C., Engle, B., Kieseier, B.C., Fisher, E., Kappos, L., Rudick, R.A., Goyal, J., 2019. Neurofilament light levels are associated with long-term outcomes in multiple sclerosis. Mult Scler 135245851988561. https://doi.org/10.1177/1352458519885613

Leppert, D., Lindberg, R.L., Kappos, L., Leib, S.L., 2001. Matrix metalloproteinases: multifunctional effectors of inflammation in multiple sclerosis and bacterial meningitis. Brain Res. Brain Res. Rev 36, 249–257.

Leppert, D., Waubant, E., Burk, M.R., Oksenberg, J.R., Hauser, S.L., 1996. Interferon beta-1b inhibits gelatinase secretion and in vitro migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis. Ann. Neurol 40, 846–852. https://doi.org/10.1002/ana.410400606

Lewczuk, P., Ermann, N., Andreasson, U., Schultheis, C., Podhorna, J., Spitzer, P., Maler, J.M., Kornhuber, J., Blennow, K., Zetterberg, H., 2018. Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. Alz Res Therapy 10, 71. https://doi.org/10.1186/s13195-018-0404-9

Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.-S., Peterson, T.C., Wilton, D.K., Frouin, A., Napier, B.A., Panicker, N., Kumar, M., Buckwalter, M.S., Rowitch, D.H., Dawson, V.L., Dawson, T.M., Stevens, B., Barres, B.A., 2017. Neurotoxic reactive astrocytes

are induced by activated microglia. Nature 541, 481–487. https://doi.org/10.1038/nature21029

Lin, C.-H., Li, C.-H., Yang, K.-C., Lin, F.-J., Wu, C.-C., Chieh, J.-J., Chiu, M.-J., 2019. Blood NfL: A biomarker for disease severity and progression in Parkinson disease. Neurology 93, e1104–e1111. https://doi.org/10.1212/WNL.0000000000008088

Lin, Y.-S., Lee, W.-J., Wang, S.-J., Fuh, J.-L., 2018. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. Sci Rep 8, 17368. https://doi.org/10.1038/s41598-018-35766-w

Lu, C.H., Kalmar, B., Malaspina, A., Greensmith, L., Petzold, A., 2011. A method to solubilise protein aggregates for immunoassay quantification which overcomes the neurofilament “hook” effect. J. Neurosci. Methods 195, 143–150. https://doi.org/10.1016/j.jneumeth.2010.11.026

Lu, C.H., Macdonald-Wallis, C., Gray, E., Pearce, N., Petzold, A., Norgren, N., Giovannoni, G., Fratta, P., Sidle, K., Fish, M., Orrell, R., Howard, R., Talbot, K., Greensmith, L., Kuhle, J., Turner, M.R., Malaspina, A., 2015. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. Neurology 84, 2247–57. https://doi.org/10.1212/WNL.0000000000001642

Lu, C.-H., Petzold, A., Kalmar, B., Dick, J., Malaspina, A., Greensmith, L., 2012. Plasma Neurofilament Heavy Chain Levels Correlate to Markers of Late Stage Disease Progression and Treatment Response in SOD1G93A Mice that Model ALS. PLoS One 7. https://doi.org/10.1371/journal.pone.0040998

Lue, L.-F., Kuo, Y.-M., Sabbagh, M., 2019. Advance in Plasma AD Core Biomarker Development: Current Findings from Immunomagnetic Reduction-Based SQUID Technology. Neurol Ther 8, 95–111. https://doi.org/10.1007/s40120-019-00167-2

Lycke, J.N., Karlsson, J.E., Andersen, O., Rosengren, L.E., 1998. Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 64, 402–404.

Maia, L.F., Kaeser, S.A., Reichwald, J., Hruscha, M., Martus, P., Staufenbiel, M., Jucker, M., 2013. Changes in Amyloid-β and Tau in the Cerebrospinal Fluid of Transgenic Mice Overexpressing Amyloid Precursor Protein. Science Translational Medicine 5, 194re2-194re2. https://doi.org/10.1126/scitranslmed.3006446

Malmeström, C., Axelsson, M., Lycke, J., Zetterberg, H., Blennow, K., Olsson, B., 2014. CSF levels of YKL-40 are increased in MS and decrease with immunosuppressive treatment. Journal of Neuroimmunology 269, 87–89. https://doi.org/10.1016/j.jneuroim.2014.02.004

Malmestrom, C., Haghighi, S., Rosengren, L., Andersen, O., Lycke, J., 2003. Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS. Neurology 61, 1720–1725.

Malmeström, C., Haghighi, S., Rosengren, L., Andersen, O., Lycke, J., 2003. Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS 7.

Mane-Martinez, M.A., Olsson, B., Bau, L., Matas, E., Cobo-Calvo, A., Andreasson, U., Blennow, K., Romero-Pinel, L., Martinez-Yelamos, S., Zetterberg, H., 2016. Glial and neuronal markers in cerebrospinal fluid in different types of multiple sclerosis. J. Neuroimmunol 299, 112–117. https://doi.org/10.1016/j.jneuroim.2016.08.004

Manouchehrinia, A., Stridh, P., Khademi, M., Leppert, D., Barro, C., Michalak, Z., Benkert, P., Lycke, J., Alfredsson, L., Kappos, L., Piehl, F., Olsson, T., Kuhle, J., Kockum, I., 2020. Plasma neurofilament light levels are associated with the risk of disability in multiple sclerosis. Neurology 10.1212/WNL.0000000000009571. https://doi.org/10.1212/WNL.0000000000009571

Marques, T.M., van Rumund, A., Oeckl, P., Kuiperij, H.B., Esselink, R.A.J., Bloem, B.R., Otto, M., Verbeek, M.M., 2019. Serum NFL discriminates Parkinson disease from atypical parkinsonisms. Neurology 92, e1479–e1486. https://doi.org/10.1212/WNL.0000000000007179

Martinez, M.A., Olsson, B., Bau, L., Matas, E., Cobo, C.A., Andreasson, U., Blennow, K., Romero-Pinel, L., Martinez-Yelamos, S., Zetterberg, H., 2015. Glial and neuronal

markers in cerebrospinal fluid predict progression in multiple sclerosis. Mult. Scler 21, 550–561. https://doi.org/10.1177/1352458514549397

Masters, C.L., Bateman, R., Blennow, K., Rowe, C.C., Sperling, R.A., Cummings, J.L., 2015. Alzheimer's disease. Nat Rev Dis Primers 1, 15056. https://doi.org/10.1038/nrdp.2015.56

Mattsson, N., Andreasson, U., Zetterberg, H., Blennow, K., Alzheimer's Disease Neuroimaging, I., 2017. Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. JAMA Neurol 74, 557–566. https://doi.org/10.1001/jamaneurol.2016.6117

Mattsson-Carlgren, N., Janelidze, S., Palmqvist, S., Cullen, N., Svenningsson, A.L., Strandberg, O., Mengel, D., Walsh, D.M., Stomrud, E., Dage, J.L., Hansson, O., 2020. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. Brain 143, 3234–3241. https://doi.org/10.1093/brain/awaa286

Meeter, L.H.H., Patzke, H., Loewen, G., Dopper, E.G.P., Pijnenburg, Y.A.L., van Minkelen, R., van Swieten, J.C., 2016. Progranulin Levels in Plasma and Cerebrospinal Fluid in Granulin Mutation Carriers. Dement Geriatr Cogn Disord Extra 6, 330–340. https://doi.org/10.1159/000447738

Mengel, D., Janelidze, S., Glynn, R.J., Liu, W., Hansson, O., Walsh, D.M., 2020. Plasma NT1 Tau is a Specific and Early Marker of Alzheimer's Disease. Annals of Neurology 88, 878–892. https://doi.org/10.1002/ana.25885

Mercuri, E., Darras, B.T., Chiriboga, C.A., Day, J.W., Campbell, C., Connolly, A.M., Iannaccone, S.T., Kirschner, J., Kuntz, N.L., Saito, K., Shieh, P.B., Tulinius, M., Mazzone, E.S., Montes, J., Bishop, K.M., Yang, Q., Foster, R., Gheuens, S., Bennett, C.F., Farwell, W., Schneider, E., De Vivo, D.C., Finkel, R.S., 2018. Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. N Engl J Med 378, 625–635. https://doi.org/10.1056/NEJMoa1710504

Miller, T., Cudkowicz, M., Shaw, P.J., Andersen, P.M., Atassi, N., Bucelli, R.C., Genge, A., Glass, J., Ladha, S., Ludolph, A.L., Maragakis, N.J., McDermott, C.J., Pestronk, A.,

Ravits, J., Salachas, F., Trudell, R., Van Damme, P., Zinman, L., Bennett, C.F., Lane, R., Sandrock, A., Runz, H., Graham, D., Houshyar, H., McCampbell, A., Nestorov, I., Chang, I., McNeill, M., Fanning, L., Fradette, S., Ferguson, T.A., 2020. Phase 1–2 Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS. N Engl J Med 383, 109–119. https://doi.org/10.1056/NEJMoa2003715

Modvig, S., Degn, M., Roed, H., Sorensen, T.L., Larsson, H.B., Langkilde, A.R., Frederiksen, J.L., Sellebjerg, F., 2015. Cerebrospinal fluid levels of chitinase 3-like 1 and neurofilament light chain predict multiple sclerosis development and disability after optic neuritis. Mult. Scler 21, 1761–1770. https://doi.org/10.1177/1352458515574148

Mollenhauer, B., Caspell-Garcia, C.J., Coffey, C.S., Taylor, P., Shaw, L.M., Trojanowski, J.Q., Singleton, A., Frasier, M., Marek, K., Galasko, D., 2017. Longitudinal CSF biomarkers in patients with early Parkinson disease and healthy controls. Neurology 89, 1959–1969. https://doi.org/10.1212/WNL.0000000000004609

Nakamura, A., Kaneko, N., Villemagne, V.L., Kato, T., Doecke, J., Doré, V., Fowler, C., Li, Q.-X., Martins, R., Rowe, C., Tomita, T., Matsuzaki, K., Ishii, Kenji, Ishii, Kazunari, Arahata, Y., Iwamoto, S., Ito, K., Tanaka, K., Masters, C.L., Yanagisawa, K., 2018. High performance plasma amyloid-β biomarkers for Alzheimer's disease. Nature 554, 249–254. https://doi.org/10.1038/nature25456

Niemelä, V., Landtblom, A.-M., Blennow, K., Sundblom, J., 2017. Tau or neurofilament light—Which is the more suitable biomarker for Huntington's disease? PLOS ONE 12, e0172762. https://doi.org/10.1371/journal.pone.0172762

Noppe, M., Crols, R., Andries, D., Lowenthal, A., 1986. Determination in human cerebrospinal fluid of glial fibrillary acidic protein, S-100 and myelin basic protein as indices of non-specific or specific central nervous tissue pathology. Clinica Chimica Acta 155, 143–150. https://doi.org/10.1016/0009-8981(86)90275-5

Norgren, N., Sundstrom, P., Svenningsson, A., Rosengren, L., Stigbrand, T., Gunnarsson, M., 2004. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. Neurology 63, 1586–1590.

Novakova, L., Axelsson, M., Khademi, M., Zetterberg, H., Blennow, K., Malmestrom, C., Piehl, F., Olsson, T., Lycke, J., 2017. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. J. Neurochem 141, 296–304. https://doi.org/10.1111/jnc.13881

Novakova, L., Axelsson, M., Malmestrom, C., Imberg, H., Elias, O., Zetterberg, H., Nerman, O., Lycke, J., 2018. Searching for neurodegeneration in multiple sclerosis at clinical onset: Diagnostic value of biomarkers. PLoS One 13, e0194828. https://doi.org/10.1371/journal.pone.0194828

Oeckl, P., Weydt, P., Steinacker, P., Anderl-Straub, S., Nordin, F., Volk, A.E., Diehl-Schmid, J., Andersen, P.M., Kornhuber, J., Danek, A., Fassbender, K., Fliessbach, K., German Consortium for Frontotemporal Lobar Degeneration, Jahn, H., Lauer, M., Müller, K., Knehr, A., Prudlo, J., Schneider, A., Thal, D.R., Yilmazer-Hanke, D., Weishaupt, J.H., Ludolph, A.C., Otto, M., 2019. Different neuroinflammatory profile in amyotrophic lateral sclerosis and frontotemporal dementia is linked to the clinical phase. J Neurol Neurosurg Psychiatry 90, 4–10. https://doi.org/10.1136/jnnp-2018-318868

Ohara, T., Hata, J., Tanaka, M., Honda, T., Yamakage, H., Yoshida, D., Inoue, T., Hirakawa, Y., Kusakabe, T., Shibata, M., Teraoka, T., Kitazono, T., Kanba, S., Satoh-Asahara, N., Ninomiya, T., 2019. Serum Soluble Triggering Receptor Expressed on Myeloid Cells 2 as a Biomarker for Incident Dementia: The Hisayama Study: TREM2 and Risk of Dementia. Ann Neurol. 85, 47–58. https://doi.org/10.1002/ana.25385

Oldoni, E., Smets, I., Mallants, K., Vandebergh, M., Van Horebeek, L., Poesen, K., Dupont, P., Dubois, B., Goris, A., 2020. CHIT1 at Diagnosis Reflects Long-Term Multiple Sclerosis Disease Activity. Ann Neurol 87, 633–645. https://doi.org/10.1002/ana.25691

Olsson, B., Alberg, L., Cullen, N.C., Michael, E., Wahlgren, L., Kroksmark, A.-K., Rostasy, K., Blennow, K., Zetterberg, H., Tulinius, M., 2019. NFL is a marker of treatment

response in children with SMA treated with nusinersen. J Neurol 266, 2129–2136. https://doi.org/10.1007/s00415-019-09389-8

Olsson, B., Lautner, R., Andreasson, U., Ohrfelt, A., Portelius, E., Bjerke, M., Holtta, M., Rosen, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., Zetterberg, H., 2016. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 15, 673–684. https://doi.org/10.1016/S1474-4422(16)00070-3

Ovod, V., Ramsey, K.N., Mawuenyega, K.G., Bollinger, J.G., Hicks, T., Schneider, T., Sullivan, M., Paumier, K., Holtzman, D.M., Morris, J.C., Benzinger, T., Fagan, A.M., Patterson, B.W., Bateman, R.J., 2017. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimer's & Dementia 13, 841–849. https://doi.org/10.1016/j.jalz.2017.06.2266

Palmqvist, S., Janelidze, S., Quiroz, Y.T., Zetterberg, H., Lopera, F., Stomrud, E., Su, Y., Chen, Y., Serrano, G.E., Leuzy, A., Mattsson-Carlgren, N., Strandberg, O., Smith, R., Villegas, A., Sepulveda-Falla, D., Chai, X., Proctor, N.K., Beach, T.G., Blennow, K., Dage, J.L., Reiman, E.M., Hansson, O., 2020. Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. JAMA 324, 1–11. https://doi.org/10.1001/jama.2020.12134

Palmqvist, S., Janelidze, S., Stomrud, E., Zetterberg, H., Karl, J., Zink, K., Bittner, T., Mattsson, N., Eichenlaub, U., Blennow, K., Hansson, O., 2019. Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease–Related β-Amyloid Status. JAMA Neurol 76, 1060–1069. https://doi.org/10.1001/jamaneurol.2019.1632

Palmqvist, S., Zetterberg, H., Blennow, K., Vestberg, S., Andreasson, U., Brooks, D.J., Owenius, R., Hägerström, D., Wollmer, P., Minthon, L., Hansson, O., 2014. Accuracy of Brain Amyloid Detection in Clinical Practice Using Cerebrospinal Fluid β-Amyloid 42: A Cross-Validation Study Against Amyloid Positron Emission Tomography. JAMA Neurol 71, 1282–1289. https://doi.org/10.1001/jamaneurol.2014.1358

Parnetti, L., Tiraboschi, P., Lanari, A., Peducci, M., Padiglioni, C., D'Amore, C., Pierguidi, L., Tambasco, N., Rossi, A., Calabresi, P., 2008. Cerebrospinal fluid biomarkers in Parkinson's disease with dementia and dementia with Lewy bodies. Biol. Psychiatry 64, 850–855. https://doi.org/10.1016/j.biopsych.2008.02.016

Pereira, J.B., Westman, E., Hansson, O., 2017. Association between cerebrospinal fluid and plasma neurodegeneration biomarkers with brain atrophy in Alzheimer's disease. Neurobiology of Aging 58, 14–29. https://doi.org/10.1016/j.neurobiolaging.2017.06.002

Piccio, L., Deming, Y., Del-Águila, J.L., Ghezzi, L., Holtzman, D.M., Fagan, A.M., Fenoglio, C., Galimberti, D., Borroni, B., Cruchaga, C., 2016. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. Acta Neuropathol 131, 925–933. https://doi.org/10.1007/s00401-016-1533-5

Pinteac, R., Montalban, X., Comabella, M., 2021. Chitinases and chitinase-like proteins as biomarkers in neurologic disorders. Neurol Neuroimmunol Neuroinflamm 8, e921. https://doi.org/10.1212/NXI.0000000000000921

Plavina, T., Rudick, R.A., Calabresi, P.A., Singh, C.M., Stevenson, L., Lee, S., Green, C., Matias, M., Uzgiris, A.J., 2019. ECTRIMS 2019 - Poster Session 1. Mult Scler P588, 25, 131–356. https://doi.org/10.1177/1352458519868078

Poewe, W., Seppi, K., Tanner, C.M., Halliday, G.M., Brundin, P., Volkmann, J., Schrag, A.-E., Lang, A.E., 2017. Parkinson disease. Nat Rev Dis Primers 3, 17013. https://doi.org/10.1038/nrdp.2017.13

Preische, O., Schultz, S.A., Apel, A., Kuhle, J., Kaeser, S.A., Barro, C., Graber, S., Kuder-Buletta, E., LaFougere, C., Laske, C., Voglein, J., Levin, J., Masters, C.L., Martins, R., Schofield, P.R., Rossor, M.N., Graff-Radford, N.R., Salloway, S., Ghetti, B., Ringman, J.M., Noble, J.M., Chhatwal, J., Goate, A.M., Benzinger, T.L.S., Morris, J.C., Bateman, R.J., Wang, G., Fagan, A.M., McDade, E.M., Gordon, B.A., Jucker, M., Dominantly Inherited Alzheimer, N., 2019. Serum neurofilament dynamics

predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nature medicine 25, 277–283. https://doi.org/10.1038/s41591-018-0304-3

Puthenparampil, M., Federle, L., Miante, S., Zito, A., Toffanin, E., Ruggero, S., Ermani, M., Pravato, S., Poggiali, D., Perini, P., Rinaldi, F., Gallo, P., 2017. BAFF Index and CXCL13 levels in the cerebrospinal fluid associate respectively with intrathecal IgG synthesis and cortical atrophy in multiple sclerosis at clinical onset. Journal of Neuroinflammation 14, 11. https://doi.org/10.1186/s12974-016-0785-2

Quintana, E., Coll, C., Salavedra-Pont, J., Muñoz-San Martín, M., Robles-Cedeño, R., Tomàs-Roig, J., Buxó, M., Matute-Blanch, C., Villar, L.M., Montalban, X., Comabella, M., Perkal, H., Gich, J., Ramió-Torrentà, L., 2018. Cognitive impairment in early stages of multiple sclerosis is associated with high cerebrospinal fluid levels of chitinase 3-like 1 and neurofilament light chain. Eur J Neurol 25, 1189–1191. https://doi.org/10.1111/ene.13687

Quiroz, Y.T., Zetterberg, H., Reiman, E.M., Chen, Y., Su, Y., Fox-Fuller, J.T., Garcia, G., Villegas, A., Sepulveda-Falla, D., Villada, M., Arboleda-Velasquez, J.F., Guzmán-Vélez, E., Vila-Castelar, C., Gordon, B.A., Schultz, S.A., Protas, H.D., Ghisays, V., Giraldo, M., Tirado, V., Baena, A., Munoz, C., Rios-Romenets, S., Tariot, P.N., Blennow, K., Lopera, F., 2020. Plasma neurofilament light chain in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional and longitudinal cohort study. The Lancet Neurology 19, 513–521. https://doi.org/10.1016/S1474-4422(20)30137-X

Rissin, D.M., Kan, C.W., Campbell, T.G., Howes, S.C., Fournier, D.R., Song, L., Piech, T., Patel, P.P., Chang, L., Rivnak, A.J., Ferrell, E.P., Randall, J.D., Provuncher, G.K., Walt, D.R., Duffy, D.C., 2010. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol 28, 595–9. https://doi.org/10.1038/nbt.1641

Rodrigues, F.B., Byrne, L.M., Tortelli, R., Johnson, E.B., Wijeratne, P.A., Arridge, M., De Vita, E., Ghazaleh, N., Houghton, R., Furby, H., Alexander, D.C., Tabrizi, S.J.,

Schobel, S., Scahill, R.I., Heslegrave, A., Zetterberg, H., Wild, E.J., 2020. Mutant huntingtin and neurofilament light have distinct longitudinal dynamics in Huntington's disease. Sci. Transl. Med. 12, eabc2888. https://doi.org/10.1126/scitranslmed.abc2888

Romme, C.J., Bornsen, L., Khademi, M., Olsson, T., Jensen, P.E., Sorensen, P.S., Sellebjerg, F., 2013. CSF inflammation and axonal damage are increased and correlate in progressive multiple sclerosis. Mult. Scler 19, 877–884. https://doi.org/10.1177/1352458512466929

Rosengren, L.E., Karlsson, J.E., Karlsson, J.O., Persson, L.I., Wikkelso, C., 1996. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J. Neurochem 67, 2013–2018.

Rosengren, L.E., Lycke, J., Andersen, O., 1995. Glial fibrillary acidic protein in CSF of multiple sclerosis patients: relation to neurological deficit. J. Neurol. Sci 133, 61–65.

Rossi, M., Candelise, N., Baiardi, S., Capellari, S., Giannini, G., Orrù, C.D., Antelmi, E., Mammana, A., Hughson, A.G., Calandra-Buonaura, G., Ladogana, A., Plazzi, G., Cortelli, P., Caughey, B., Parchi, P., 2020. Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. Acta Neuropathol 140, 49–62. https://doi.org/10.1007/s00401-020-02160-8

Rosso, M., Healy, B.C., Saxena, S., Paul, A., Bjornevik, K., Kuhle, J., Benkert, P., Leppert, D., Guttmann, C., Bakshi, R., Weiner, H.L., Chitnis, T., 2021. MRI Lesion State Modulates the Relationship Between Serum Neurofilament Light and Age in Multiple Sclerosis. Journal of Neuroimaging n/a. https://doi.org/10.1111/jon.12826

Ryberg, H., Bowser, R., 2008. Protein biomarkers for amyotrophic lateral sclerosis. Expert Review of Proteomics 5, 249–262. https://doi.org/10.1586/14789450.5.2.249

Sánchez-Valle, R., Heslegrave, A., Foiani, M.S., Bosch, B., Antonell, A., Balasa, M., Lladó, A., Zetterberg, H., Fox, N.C., 2018. Serum neurofilament light levels correlate with severity measures and neurodegeneration markers in autosomal dominant

Alzheimer's disease. Alz Res Therapy 10, 113. https://doi.org/10.1186/s13195-018-0439-y

Sato, C., Barthélemy, N.R., Mawuenyega, K.G., Patterson, B.W., Gordon, B.A., Jockel-Balsarotti, J., Sullivan, M., Crisp, M.J., Kasten, T., Kirmess, K.M., Kanaan, N.M., Yarasheski, K.E., Baker-Nigh, A., Benzinger, T.L.S., Miller, T.M., Karch, C.M., Bateman, R.J., 2018. Tau Kinetics in Neurons and the Human Central Nervous System. Neuron 97, 1284-1298.e7. https://doi.org/10.1016/j.neuron.2018.02.015

Schindler, S.E., Bollinger, J.G., Ovod, V., Mawuenyega, K.G., Li, Y., Gordon, B.A., Holtzman, D.M., Morris, J.C., Benzinger, T.L.S., Xiong, C., Fagan, A.M., Bateman, R.J., 2019. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. Neurology 10.1212/WNL.0000000000008081. https://doi.org/10.1212/WNL.0000000000008081

Scialò, C., Tran, T.H., Salzano, G., Novi, G., Caponnetto, C., Chiò, A., Calvo, A., Canosa, A., Moda, F., Caroppo, P., Silani, V., Ticozzi, N., Ratti, A., Borroni, B., Benussi, L., Ghidoni, R., Furlanis, G., Manganotti, P., Senigagliesi, B., Parisse, P., Brasselet, R., Buratti, E., Legname, G., 2020. TDP-43 real-time quaking induced conversion reaction optimization and detection of seeding activity in CSF of amyotrophic lateral sclerosis and frontotemporal dementia patients. Brain Communications 2. https://doi.org/10.1093/braincomms/fcaa142

Sejbaek, T., Nielsen, H.H., Penner, N., Plavina, T., Mendoza, J.P., Martin, N.A., Elkjaer, M.L., Ravnborg, M.H., Illes, Z., 2019. Dimethyl fumarate decreases neurofilament light chain in CSF and blood of treatment naïve relapsing MS patients. J Neurol Neurosurg Psychiatry jnnp-2019-321321. https://doi.org/10.1136/jnnp-2019-321321

Selkoe, D.J., 2020. A Is for Amyloid. J Prev Alzheimers Dis 1–2. https://doi.org/10.14283/jpad.2020.27

Sellebjerg, F., Börnsen, L., Ammitzbøll, C., Nielsen, J.E., Vinther-Jensen, T., Hjermind, L.E., von Essen, M., Ratzer, R.L., Soelberg Sørensen, P., Romme Christensen, J., 2017.

Defining active progressive multiple sclerosis. Mult Scler 23, 1727–1735. https://doi.org/10.1177/1352458517726592

Sellebjerg, F., Bornsen, L., Khademi, M., Krakauer, M., Olsson, T., Frederiksen, J.L., Sorensen, P.S., 2009. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. Neurology 73, 2003–2010. https://doi.org/10.1212/WNL.0b013e3181c5b457

Sellebjerg, F., Jensen, C.V., Larsson, H.B., Frederiksen, J.L., 2003. Gadolinium-enhanced magnetic resonance imaging predicts response to methylprednisolone in multiple sclerosis. Mult. Scler 9, 102–107. https://doi.org/10.1191/1352458503ms880sr

Shahnawaz, M., Tokuda, T., Waragai, M., Mendez, N., Ishii, R., Trenkwalder, C., Mollenhauer, B., Soto, C., 2017. Development of a Biochemical Diagnosis of Parkinson Disease by Detection of α-Synuclein Misfolded Aggregates in Cerebrospinal Fluid. JAMA Neurol 74, 163. https://doi.org/10.1001/jamaneurol.2016.4547

Siller, N., Kuhle, J., Muthuraman, M., Barro, C., Uphaus, T., Groppa, S., Kappos, L., Zipp, F., Bittner, S., 2019. Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler 25, 678–686. https://doi.org/10.1177/1352458518765666

Sjogren, M., Folkesson, S., Blennow, K., Tarkowski, E., 2004. Increased intrathecal inflammatory activity in frontotemporal dementia: pathophysiological implications. J Neurol Neurosurg Psychiatry 75, 1107–1111. https://doi.org/10.1136/jnnp.2003.019422

Skillbäck, T., Farahmand, B.Y., Rosén, C., Mattsson, N., Nägga, K., Kilander, L., Religa, D., Wimo, A., Winblad, B., Schott, J.M., Blennow, K., Eriksdotter, M., Zetterberg, H., 2015. Cerebrospinal fluid tau and amyloid-β_{1-42} in patients with dementia. Brain 138, 2716–2731. https://doi.org/10.1093/brain/awv181

Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol 119, 7–35. https://doi.org/10.1007/s00401-009-0619-8

Suárez-Calvet, M., Kleinberger, G., Araque Caballero, M.Á., Brendel, M., Rominger, A., Alcolea, D., Fortea, J., Lleó, A., Blesa, R., Gispert, J.D., Sánchez-Valle, R., Antonell, A., Rami, L., Molinuevo, J.L., Brosseron, F., Traschütz, A., Heneka, M.T., Struyfs, H., Engelborghs, S., Sleegers, K., Van Broeckhoven, C., Zetterberg, H., Nellgård, B., Blennow, K., Crispin, A., Ewers, M., Haass, C., 2016. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. EMBO Mol Med 8, 466–476. https://doi.org/10.15252/emmm.201506123

Swift, I.J., Sogorb-Esteve, A., Heller, C., Synofzik, M., Otto, M., Graff, C., Galimberti, D., Todd, E., Heslegrave, A.J., van der Ende, E.L., Van Swieten, J.C., Zetterberg, H., Rohrer, J.D., 2021. Fluid biomarkers in frontotemporal dementia: past, present and future. J Neurol Neurosurg Psychiatry 92, 204–215. https://doi.org/10.1136/jnnp-2020-323520

Tabrizi, S.J., Reilmann, R., Roos, R.A., Durr, A., Leavitt, B., Owen, G., Jones, R., Johnson, H., Craufurd, D., Hicks, S.L., Kennard, C., Landwehrmeyer, B., Stout, J.C., Borowsky, B., Scahill, R.I., Frost, C., Langbehn, D.R., 2012. Potential endpoints for clinical trials in premanifest and early Huntington's disease in the TRACK-HD study: analysis of 24 month observational data. The Lancet Neurology 11, 42–53. https://doi.org/10.1016/S1474-4422(11)70263-0

Talbot, K., Tizzano, E.F., 2017. The clinical landscape for SMA in a new therapeutic era. Gene Ther 24, 529–533. https://doi.org/10.1038/gt.2017.52

Teunissen, C.E., Iacobaeus, E., Khademi, M., Brundin, L., Norgren, N., Koel-Simmelink, M.J., Schepens, M., Bouwman, F., Twaalfhoven, H.A., Blom, H.J., Jakobs, C., Dijkstra, C.D., 2009. Combination of CSF N-acetylaspartate and neurofilaments in multiple sclerosis. Neurology 72, 1322–1329.

Teunissen, C.E., Thijssen, E.H., Verberk, I.M.W., 2020. Plasma p-tau217: from 'new kid' to most promising candidate for Alzheimer's disease blood test. Brain 143, 3170–3172. https://doi.org/10.1093/brain/awaa329

the MND-Net, Wurster, C.D., Steinacker, P., Günther, R., Koch, J.C., Lingor, P., Uzelac, Z., Witzel, S., Wollinsky, K., Winter, B., Osmanovic, A., Schreiber-Katz, O., Al Shweiki, R., Ludolph, A.C., Petri, S., Hermann, A., Otto, M., 2020. Neurofilament light chain in serum of adolescent and adult SMA patients under treatment with nusinersen. J Neurol 267, 36–44. https://doi.org/10.1007/s00415-019-09547-y

Thebault, S., Bose, G., Booth, R., Freedman, M.S., 2021. Serum neurofilament light in MS: The first true blood-based biomarker? Mult Scler 1352458521993066. https://doi.org/10.1177/1352458521993066

Thompson, A.G., Gray, E., Bampton, A., Raciborska, D., Talbot, K., Turner, M.R., 2019. CSF chitinase proteins in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 90, 1215–1220. https://doi.org/10.1136/jnnp-2019-320442

Thompson, A.G., Gray, E., Thézénas, M.-L., Charles, P.D., Evetts, S., Hu, M.T., Talbot, K., Fischer, R., Kessler, B.M., Turner, M.R., 2018. Cerebrospinal fluid macrophage biomarkers in amyotrophic lateral sclerosis. Annals of Neurology 83, 258–268. https://doi.org/10.1002/ana.25143

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., Correale, J., Fazekas, F., Filippi, M., Freedman, M.S., Fujihara, K., Galetta, S.L., Hartung, H.P., Kappos, L., Lublin, F.D., Marrie, R.A., Miller, A.E., Miller, D.H., Montalban, X., Mowry, E.M., Sorensen, P.S., Tintore, M., Traboulsee, A.L., Trojano, M., Uitdehaag, B.M.J., Vukusic, S., Waubant, E., Weinshenker, B.G., Reingold, S.C., Cohen, J.A., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17, 162–173.

Thouvenot, E., Demattei, C., Lehmann, S., Maceski-Maleska, A., Hirtz, C., Juntas-Morales, R., Pageot, N., Esselin, F., Alphandéry, S., Vincent, T., Camu, W., 2020. Serum neurofilament light chain at time of diagnosis is an independent prognostic factor of survival in amyotrophic lateral sclerosis. Eur J Neurol 27, 251–257. https://doi.org/10.1111/ene.14063

Thouvenot, E., Hinsinger, G., Demattei, C., Uygunoglu, U., Castelnovo, G., Pittion-Vouyovitch, S., Okuda, D., Kantarci, O., Pelletier, D., Lehmann, S., Marin, P., Siva, A., Lebrun, C., 2019. Cerebrospinal fluid chitinase-3-like protein 1 level is not an independent predictive factor for the risk of clinical conversion in radiologically isolated syndrome. Mult Scler 25, 669–677. https://doi.org/10.1177/1352458518767043

Todd, J., Freese, B., Lu, A., Held, D., Morey, J., Livingston, R., Goix, P., 2007. Ultrasensitive Flow-based Immunoassays Using Single-Molecule Counting. Clinical Chemistry 53, 1990–1995. https://doi.org/10.1373/clinchem.2007.091181

Tortelli, R., Zecca, C., Piccininni, M., Benmahamed, S., Dell'Abate, M.T., Barulli, M.R., Capozzo, R., Battista, P., Logroscino, G., 2020. Plasma Inflammatory Cytokines Are Elevated in ALS. Front. Neurol. 11. https://doi.org/10.3389/fneur.2020.552295

Trapp, B., Nave, K.-A., 2008. Multiple Sclerosis: An Immune or Neurodegenerative Disorder? | Annual Review of Neuroscience [WWW Document]. URL https://www-annualreviews-org.ezp-prod1.hul.harvard.edu/doi/10.1146/annurev.neuro.30.051606.094313 (accessed 2.19.21).

Trentini, A., Comabella, M., Tintore, M., Koel-Simmelink, M.J., Killestein, J., Roos, B., Rovira, A., Korth, C., Ottis, P., Blankenstein, M.A., Montalban, X., Bellini, T., Teunissen, C.E., 2014. N-acetylaspartate and neurofilaments as biomarkers of axonal damage in patients with progressive forms of multiple sclerosis. J. Neurol 261, 2338–2343. https://doi.org/10.1007/s00415-014-7507-4

Turner, M.R., Hardiman, O., Benatar, M., Brooks, B.R., Chio, A., de Carvalho, M., Ince, P.G., Lin, C., Miller, R.G., Mitsumoto, H., Nicholson, G., Ravits, J., Shaw, P.J., Swash, M., Talbot, K., Traynor, B.J., Van den Berg, L.H., Veldink, J.H., Vucic, S., Kiernan, M.C., 2013. Controversies and priorities in amyotrophic lateral sclerosis. The Lancet Neurology 12, 310–322. https://doi.org/10.1016/S1474-4422(13)70036-X

Turner, M.R., Kiernan, M.C., Leigh, P.N., Talbot, K., 2009. Biomarkers in amyotrophic lateral sclerosis. The Lancet Neurology 8, 94–109. https://doi.org/10.1016/S1474-4422(08)70293-X

van der Ende, E.L., Meeter, L.H., Poos, J.M., Panman, J.L., Jiskoot, L.C., Dopper, E.G.P., Papma, J.M., de Jong, F.J., Verberk, I.M.W., Teunissen, C., Rizopoulos, D., Heller, C., Convery, R.S., Moore, K.M., Bocchetta, M., Neason, M., Cash, D.M., Borroni, B., Galimberti, D., Sanchez-Valle, R., Laforce, R., Moreno, F., Synofzik, M., Graff, C., Masellis, M., Carmela Tartaglia, M., Rowe, J.B., Vandenberghe, R., Finger, E., Tagliavini, F., de Mendonça, A., Santana, I., Butler, C., Ducharme, S., Gerhard, A., Danek, A., Levin, J., Otto, M., Frisoni, G.B., Cappa, S., Pijnenburg, Y.A.L., Rohrer, J.D., van Swieten, J.C., 2019. Serum neurofilament light chain in genetic frontotemporal dementia: a longitudinal, multicentre cohort study. The Lancet Neurology 18, 1103–1111. https://doi.org/10.1016/S1474-4422(19)30354-0

Varhaug, K.N., Barro, C., Bjornevik, K., Myhr, K.M., Torkildsen, O., Wergeland, S., Bindoff, L.A., Kuhle, J., Vedeler, C., 2018. Neurofilament light chain predicts disease activity in relapsing-remitting MS. Neurol Neuroimmunol Neuroinflamm 5, e422. https://doi.org/10.1212/NXI.0000000000000422

Varhaug, K.N., Torkildsen, Ø., Myhr, K.-M., Vedeler, C.A., 2019. Neurofilament Light Chain as a Biomarker in Multiple Sclerosis. Front. Neurol. 10, 338. https://doi.org/10.3389/fneur.2019.00338

Verde, F., Silani, V., Otto, M., 2019a. Neurochemical biomarkers in amyotrophic lateral sclerosis. Current Opinion in Neurology 32, 747–757. https://doi.org/10.1097/WCO.0000000000000744

Verde, F., Steinacker, P., Weishaupt, J.H., Kassubek, J., Oeckl, P., Halbgebauer, S., Tumani, H., von Arnim, C.A.F., Dorst, J., Feneberg, E., Mayer, B., Müller, H.-P., Gorges, M., Rosenbohm, A., Volk, A.E., Silani, V., Ludolph, A.C., Otto, M., 2019b. Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 90, 157–164. https://doi.org/10.1136/jnnp-2018-318704

Villar, L.M., Picon, C., Costa-Frossard, L., Alenda, R., Garcia-Caldentey, J., Espino, M., Muriel, A., Alvarez-Cermeno, J.C., 2014. Cerebrospinal fluid immunological biomarkers associated with axonal damage in multiple sclerosis. Eur. J. Neurol. https://doi.org/10.1111/ene.12579

Vinther-Jensen, T., Börnsen, L., Budtz-Jørgensen, E., Ammitzbøll, C., Larsen, I.U., Hjermind, L.E., Sellebjerg, F., Nielsen, J.E., 2016. Selected CSF biomarkers indicate no evidence of early neuroinflammation in Huntington disease. Neurology - Neuroimmunology Neuroinflammation 3. https://doi.org/10.1212/NXI.0000000000000287

Walter, M.C., Wenninger, S., Thiele, S., Stauber, J., Hiebeler, M., Greckl, E., Stahl, K., Pechmann, A., Lochmüller, H., Kirschner, J., Schoser, B., 2019. Safety and Treatment Effects of Nusinersen in Longstanding Adult 5q-SMA Type 3 – A Prospective Observational Study. JND 6, 453–465. https://doi.org/10.3233/JND-190416

Wang, Y., Shi, M., Chung, K.A., Zabetian, C.P., Leverenz, J.B., Berg, D., Srulijes, K., Trojanowski, J.Q., Lee, V.M.-Y., Siderowf, A.D., Hurtig, H., Litvan, I., Schiess, M.C., Peskind, E.R., Masuda, M., Hasegawa, M., Lin, X., Pan, C., Galasko, D., Goldstein, D.S., Jensen, P.H., Yang, H., Cain, K.C., Zhang, J., 2012. Phosphorylated α-Synuclein in Parkinson's Disease. Sci Transl Med 4, 121ra20. https://doi.org/10.1126/scitranslmed.3002566

Watanabe, M., Nakamura, Y., Michalak, Z., Isobe, N., Barro, C., Leppert, D., Matsushita, T., Hayashi, F., Yamasaki, R., Kuhle, J., Kira, J., 2019. Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD. Neurology 93, e1299–e1311. https://doi.org/10.1212/WNL.0000000000008160

Waubant, E., Goodkin, D., Bostrom, A., Bacchetti, P., Hietpas, J., Lindberg, R., Leppert, D., 2003. IFNbeta lowers MMP-9/TIMP-1 ratio, which predicts new enhancing lesions in patients with SPMS. Neurology 60, 52–57.

Weston, P.S.J., Poole, T., O'Connor, A., Heslegrave, A., Ryan, N.S., Liang, Y., Druyeh, R., Mead, S., Blennow, K., Schott, J.M., Frost, C., Zetterberg, H., Fox, N.C., 2019. Longitudinal measurement of serum neurofilament light in presymptomatic familial Alzheimer's disease. Alz Res Therapy 11, 19. https://doi.org/10.1186/s13195-019-0472-5

Weston, P.S.J., Poole, T., Ryan, N.S., Nair, A., Liang, Y., Macpherson, K., Druyeh, R., Malone, I.B., Ahsan, R.L., Pemberton, H., Klimova, J., Mead, S., Blennow, K., Rossor, M.N., Schott, J.M., Zetterberg, H., Fox, N.C., 2017. Serum neurofilament light in familial Alzheimer disease: A marker of early neurodegeneration. Neurology 89, 2167–2175. https://doi.org/10.1212/WNL.0000000000004667

Weydt, P., Oeckl, P., Huss, A., Muller, K., Volk, A.E., Kuhle, J., Knehr, A., Andersen, P.M., Prudlo, J., Steinacker, P., Weishaupt, J.H., Ludolph, A.C., Otto, M., 2016. Neurofilament levels as biomarkers in asymptomatic and symptomatic familial amyotrophic lateral sclerosis. Ann. Neurol 79, 152–158. https://doi.org/10.1002/ana.24552

Wild, E.J., Boggio, R., Langbehn, D., Robertson, N., Haider, S., Miller, J.R.C., Zetterberg, H., Leavitt, B.R., Kuhn, R., Tabrizi, S.J., Macdonald, D., Weiss, A., 2015. Quantification of mutant huntingtin protein in cerebrospinal fluid from Huntington's disease patients. J Clin Invest 125, 1979–1986. https://doi.org/10.1172/JCI80743

Wilke, C., Haas, E., Reetz, K., Faber, J., Garcia-Moreno, H., Santana, M.M., Warrenburg, B., Hengel, H., Lima, M., Filla, A., Durr, A., Melegh, B., Masciullo, M., Infante, J., Giunti, P., Neumann, M., Vries, J., Pereira de Almeida, L., Rakowicz, M., Jacobi, H., Schüle, R., Kaeser, S.A., Kuhle, J., Klockgether, T., Schöls, L., SCA3 neurofilament study group, Barro, C., Hübener-Schmid, J., Synofzik, M., Deuschle, C., Stransky, E., Brockmann, K., Schulz, J.B., Baliko, L., Gaalen, J., Raposo, M., Jeromin, A., 2020. Neurofilaments in spinocerebellar ataxia type 3: blood biomarkers at the preataxic and ataxic stage in humans and mice. EMBO Mol Med. https://doi.org/10.15252/emmm.201911803

Wilke, C., Preische, O., Deuschle, C., Roeben, B., Apel, A., Barro, C., Maia, L., Maetzler, W., Kuhle, J., Synofzik, M., 2016. Neurofilament light chain in FTD is elevated not only in cerebrospinal fluid, but also in serum. J Neurol Neurosurg Psychiatry 87, 1270–1272. https://doi.org/10.1136/jnnp-2015-312972

Wilke, C., Pujol-Calderón, F., Barro, C., Stransky, E., Blennow, K., Michalak, Z., Deuschle, C., Jeromin, A., Zetterberg, H., Schüle, R., Höglund, K., Kuhle, J., Synofzik, M., 2019. Correlations between serum and CSF pNfH levels in ALS, FTD and controls: a comparison of three analytical approaches. Clinical Chemistry and Laboratory Medicine (CCLM) 57, 1556–1564. https://doi.org/10.1515/cclm-2019-0015

Williams, T., Zetterberg, H., Chataway, J., 2020. Neurofilaments in progressive multiple sclerosis: a systematic review. J Neurol. https://doi.org/10.1007/s00415-020-09917-x

Winter, B., Guenther, R., Ludolph, A.C., Hermann, A., Otto, M., Wurster, C.D., 2019. Neurofilaments and tau in CSF in an infant with SMA type 1 treated with nusinersen. J Neurol Neurosurg Psychiatry 90, 1068.2-1069. https://doi.org/10.1136/jnnp-2018-320033

Woollacott, I.O.C., Nicholas, J.M., Heller, C., Foiani, M.S., Moore, K.M., Russell, L.L., Paterson, R.W., Keshavan, A., Schott, J.M., Warren, J.D., Heslegrave, A., Zetterberg, H., Rohrer, J.D., 2020. Cerebrospinal Fluid YKL-40 and Chitotriosidase Levels in Frontotemporal Dementia Vary by Clinical, Genetic and Pathological Subtype. Dement Geriatr Cogn Disord 49, 56–76. https://doi.org/10.1159/000506282

Wurster, C.D., Günther, R., Steinacker, P., Dreyhaupt, J., Wollinsky, K., Uzelac, Z., Witzel, S., Kocak, T., Winter, B., Koch, J.C., Lingor, P., Petri, S., Ludolph, A.C., Hermann, A., Otto, M., 2019. Neurochemical markers in CSF of adolescent and adult SMA patients undergoing nusinersen treatment. Ther Adv Neurol Disord 12, 175628641984605. https://doi.org/10.1177/1756286419846058

Yaldizli, O., Barro, C., Michalak, Z., Vehoff, J., Lascano, A.M., Disanto, G., Salmen, A., Derfuss, T., Granziera, C., Achtnichts, L., Findling, O., Nedeltchev, K., Kamm, C.P., Pot, C., Schluep, M., Du Pasquier, R., von Wyl, V., Wuerfel, J., Vallejo, V., Chan, A.,

Zecca, C., Gobbi, C., Lalive, P.H., Muller, S., Kappos, L., Benkert, P., Kuhle, J., Study, S.M.C., 2018. Value of serum neurofilament light chain levels as a biomarker of suboptimal treatment response in MS clinical practice. Mult Scler J ECTRIMS Late Breaking Oral presentation, 24, 97–98.

Zetterberg, H., 2017a. Review: Tau in biofluids – relation to pathology, imaging and clinical features. Neuropathology and Applied Neurobiology 43, 194–199. https://doi.org/10.1111/nan.12378

Zetterberg, H., 2017b. Fluid biomarkers for microglial activation and axonal injury in multiple sclerosis. Acta Neurologica Scandinavica 136, 15–17. https://doi.org/10.1111/ane.12845

Zetterberg, H., Jacobsson, J., Rosengren, L., Blennow, K., Andersen, P.M., 2007. Cerebrospinal fluid neurofilament light levels in amyotrophic lateral sclerosis: impact of SOD1 genotype. Eur. J. Neurol 14, 1329–1333. https://doi.org/10.1111/j.1468-1331.2007.01972.x

Zetterberg, H., Swieten, J.C. van, Boxer, A.L., Rohrer, J.D., 2019. Review: Fluid biomarkers for frontotemporal dementias. Neuropathology and Applied Neurobiology 45, 81–87. https://doi.org/10.1111/nan.12530

Figure 1. Overview of available and perspective blood biomarkers with a potential clinical utility for neurodegenerative diseases. In brackets perspective biomarkers.

Table 1. Blood biomarkers with their pathological interpretation and detection methods.

Blood biomarkers	**Pathological process**	**Potential application**	**Detection methods**	**References**
A*β42;* A*β42/* A*β40*	Amyloid plaques	AD	ECL, IP-MS, Simoa	(Nakamura et al., 2018; Ovod et al., 2017; Palmqvist et al., 2019)
N-terminal segment of tau	Tau tangles	AD	Simoa	(Mengel et al., 2020)
pTau181	Phosphorylation in tau tangles	AD	ECL Simoa	(Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) investigators et al., 2020; Janelidze et al., 2020) (Karikari et al., 2020)
pTau231			Simoa	(Ashton et al., 2021)
pTau217			ECL	(Mattsson-Carlgren et al., 2020; Palmqvist et al., 2020)
NfL	Neurodegeneration	All neurodegenerative conditions	Simoa, Ella, ECL, PEA, ADVIA Centaur and Atellica	(Barro et al., 2021; Chitnis et al., 2019; Gauthier et al., 2021; Kuhle et al., 2016; Plavina et al., 2019)
pNfH			ELISA	(Lu et al., 2011)

			Simoa	(Wilke et al., 2019)
GFAP	Astrogliosis and astrocytic activation	MS, FTD	Simoa	(Heller et al., 2020; Högel et al., 2020)
TDP-43	Formation of protein aggregates	ALS, FTD	RT-QuIC	(Scialò et al., 2020)
CHI3L1	Activation of the innate immune system	All neurodegenerative conditions	ELISA	(Cantó et al., 2012; Huss et al., 2020)
IL-6	Inflammation	All neurodegenerative conditions	Bio-Plex	(Tortelli et al., 2020)
TNF-α				

A*β*: beta-amyloid; AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; ECL: electrochemiluminescence; FTD: frontotemporal dementia; GFAP: glial fibrillary acidic protein; IL-6: interleukin 6; IP-MS: immunoprecipitation mass spectrometry; MS: multiple sclerosis; NfL: neurofilament light chain; pNfH: phosphorylated neurofilament heavy chain; PEA: Proximity Extension Assay; pTau: phosphorylated tau; RT-QuIC: Real-time quaking-induced conversion; Simoa: single molecule array; SMC: Single Molecule Counting; TDP-43: transactive response DNA-binding protein 43; TNF- α: tumor necrosis factor alfa.

Table 2. Highly sensitive immunoassay platforms used for quantifying protein biomarkers in the blood.

Technology	**Assay generation**	**Automated**	**Sample processing**	**Core characteristic**	**Advantages**	**Disadvantages**	**Ref**
ECL	3rd	No	Simultaneous	Electrochemical	- Cost - High flexibility for in-house development	- Lower sensitivity than 4th generation technologies - Not automated - Long processing time	(Kuhle et al., 2016; Limberg et al., 2016)
Simoa	4th	Yes	Consecutive	Bead-based	- High flexibility for in-house development - High throughput	- Cost - Consecutive sample processing	(Rissin et al., 2010)
Ella		Yes	Simultaneous	Microfluidic	- Cost - Minimal hands-on time - Rapid measurement - Integrated calibrator curves	- Limited flexibility for in-house development	(Dysinger et al., 2017)
Proximity extension assay		Yes	Simultaneous	DNA barcode	- Low sample volume - High level of multiplexing - High throughput	- Long processing time	(Assarsson et al., 2014)

Single Molecule Counting		Yes	?	Focal excitation	?	?	(Todd et al., 2007)
Immunomagnetic Reduction		Yes	Simultaneous	Magnetic stimulus	?	?	(Lue et al., 2019)
Real-time quaking-induced conversion	-	No	Simultaneous	Quake of the sample	- Measures proteins that are otherwise aggregated	- Long processing time - May require an ultracentrifuge	(Scialò et al., 2020)

*range volume needed for a duplicate determination. ECL: electrochemiluminescence; CSF: cerebrospinal fluid; LLOQ: lowest limit of quantification;

Simoa: Single molecule array.