CSF AND BLOOD BIOMARKERS IN ATYPICAL PARKINSONISM

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Abstract

Atypical Parkinsonism is a collective term used to describe three rare neurodegenerative disorders which manifest with diverse phenotypes. It includes progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and corticobasal degeneration (CBD). Despite the presence of specific clinical features in typical cases, many oligosymptomatic or atypical presentations are difficult to diagnose based on established clinical diagnostic criteria. To this end, one or more biomarkers, preferably with molecular specificity is paramount for the ***in vivo*** recognition of the underlying pathology in these patients. In this descriptive review we present the most important studies on cerebrospinal fluid (CSF) and plasma biomarkers in these disorders, with a particular focus on established Alzheimer's disease CSF biomarkers as well as alpha-synuclein.

Keywords: biomarkers; CSF; plasma; atypical parkinsonism; progressive supranuclear palsy; corticobasal degeneration; multiple system atrophy

ΒΙΟΔΕΙΚΤΕΣ ΕΝΥ ΚΑΙ ΠΛΑΣΜΑΤΟΣ ΣΕ ΑΤΥΠΑ ΠΑΡΚΙΝΣΟΝΙΚΑ ΣΥΝΔΡΟΜΑ

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Περίληψη

Ο όρος άτυπος παρκινσονισμός χρησιμοποιείται για να περιγράψει τρία σπάνια νευροεκφυλιστικά νοσήματα που χαρακτηρίζονται από ειδικούς φαινοτύπους. Περιλαμβάνει την προοδευτική υπερπυρηνική παράλυση (ΠΥΠ), ατροφία πολλαπλών συστημάτω (ΑΠΣ) και φλοιοβασική εκφύλιση (ΦΒΕ). Παρά την παρουσία ειδικών κλινικών χαρακτηριστικών σε τυπικές περιπτώσεις, πολλές ολιγοσυμπτωματικές ή άτυπες εκδηλώσεις των νοσημάτων αυτών δεν μπορούν να διαγνωσθούν αξιόπιστα βάσει των υπαρχόντων κλινικών διαγνωστικών κριτηρίων. Για το σκοπό αυτό, η ανάπτυξη βιοδεικτών με μοριακή ειδικότητα είναι υψίστης σημασίας για την αναγνώριση in vivo της υποκείμενης παθολογίας στους ασθενείς αυτούς. Στην παρούσα ανασκόπηση παρουσιάζουμε την υπάρχουσα βιβλιογραφία σε σχέση με τους βιοδείκτες στο εγκεφαλονωτιαίο υγρό και πλάσμα στα ανωτέρω νοσήματα, με ιδιαίτερη έμφαση στους καθιερωμένους βιοδείκτες της νόσου Alzheimer και στην α-συνουκλεΐνη

Λέξεις κλειδιά: βιοδείκτες; ΕΝΥ; πλάσμα; άτυπος παρκινσονισμός; προοδευτική υπερπυρηνική παράλυση; φλοιοβασική εκφύλιση; ατροφία πολλαπλών συστημάτων

Introduction

Atypical Parkinsonism is a termed used to include three distinct neurodegenerative disorders, with both movement and cognitive manifestations. These include progressive supranuclear palsy (PSP), a 4R-tauopathy characterized neuropathologically by tufted astrocytes, consisting of aggregated hyperphosphorylated tau protein. PSP presents with great phenotypical variability, with Richardson's syndrome being the most common manifestation. Corticobasal degeneration is an exceedingly rare 4R-tauopathy, with distinct neuropathological lesions, termed astrocytic plaques, which are also formed by aggregation of hyperphosphorylated tau protein. As is the case with PSP, CBD also presents with great clinical variability, most commonly manifesting as

ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ

corticobasal syndrome (CBS), primary progressive aphasia (PPA), Richardson's syndrome or a predominantly frontal-behavioural-visuospatial syndrome. Multiple system atrophy (MSA) is a synucleinopathy, characterized by glial cytoplasmic inclusions, which contain aggregates of misfolded, hyperphosphorylated alpha-synuclein protein (a-syn). Depending on the topographical distribution of neuropathological lesions (olivo-ponto-cerebellar vs. striato-nigral), MSA is divided into MSA-cerebellar and MSA-parkinsonian variant respectively. Despite the presence of distinct clinical and imaging features, these three disorders are often difficult to diagnose clinically, particularly in atypical cases and in oligosymptomatic patients. In an effort to enhance the accuracy of clinical diagnosis, various biomarkers have been tested. In this overview, we present the most relevant studies on biofluid markers in atypical Parkinonism, with a particular focus on established AD biomarkers and a-syn in cerebrospinal fluid (CSF) and plasma.

Established AD biomarkers

Amyloid beta with 42 amino-acids ($A\beta_{42}$), total tau (t-tau) and phosphorylated tau protein (p-tau), most commonly at threonine 181 are considered classical AD biomarkers. A typical AD CSF profile includes a decrease in $A\beta_{42}$ with a concomitant elevation in t-tau and p-tau levels. CSF t-tau is considered a non-specific marker of neurodegeneration and neuronal death. $A\beta_{42}$ is a marker of amyloidosis, whereas p-tau a marker of neurofibrillary tangle pathology. The primary use of these biomarkers lies in the in vivo recognition of patients with underlying AD pathology, either with typical (amnestic) or atypical (non-amnestic) presentations. Due to their availability, and the fact that the CBS phenotype in particular may have underlying AD pathology in a significant proportion of cases, these biomarkers have also been applied in patients with atypical Parkinsonism.

a) Total tau protein

Regarding CSF t-tau in PSP, the majority of relevant studies could not establish any differences between PSP patients and control subjects[1-5]. Along the same lines, no difference in t-tau was evident between PSP and other parkinsonian disorders, including CBS [1, 3-5], MSA [1, 3, 4] and PD [3, 4]. Two studies have reported decreased t-tau levels in PSP patients compared to controls [6, 7], and another study compared to CBS (with increased t-tau levels compared to PD) [8].

Regarding CBS, several initial studies have supported that CBS exhibits inherently elevated levels of CSF t-tau protein compared to controls [4, 8-11], PSP [8, 10, 11] and PD [1, 8]. These differences in some studies reached clinically meaningful significance: CSF t-tau could differentiate CBS from PSP with high (~80%) specificity and sensitivity in one study [11], and CBS from PD in another study (sensitivity 75%; specificity 90%) [8]. In contrast to these positive studies, several other studies have been negative in establishing differences in t-tau between CBS and controls [2, 3, 5], PSP [1, 3, 4, 12], MSA[3, 4] and PD [3, 4]. The initial positive relevant studies could be attributed to the admixture of AD patients in the CBS cohorts (see "Classical CSB biomarker profiling" for a detailed discussion on the subject).

Results regarding t-tau in MSA are conflicting, with most studies showing an increase in CSF t-tau in MSA compared either to controls [12-14] or other parkinsonian disorders [1, 12, 14-16]. However, several other studies did not establish any difference among MSA and other parkinsonian disorders [4, 17, 18] with a single study supporting that MSA patients present with decreased CSF t-tau levels compared to control subjects [18].

b) Phosphorylated tau protein

Most studies have focused on tau protein phosphorylated at threonine 181 (p-tau), as a surrogate marker of tau pathology in AD. CSF p-tau does not seem to be useful in the differentiation of PSP from other parkinsonian disorders or controls [2-6, 8], with two studies reporting decreased p-tau levels compared to controls [1, 7].

Likewise, in CBS most studies could not establish any meaningful difference between CBS patients and other parkinsonian disorders or controls with regards to CSF p-tau levels[1-5]. In a single study, CSF p-tau was elevated in CBS compared to PD [8], and in another study to MSA patients [1].

Accordingly, most relevant studies on p-tau did not find any meaningful difference between MSA and other study groups [3, 4, 12, 18], with the exception of a single study that identified decreased levels of CSF p-tau in MSA compared to PD and controls [1].

c) Amyloid beta with 42 amino acids

Most studies do not report any differences in CSF amyloid beta with 42 amino acids ($A\beta_{42}$) between PSP and other parkinsonian disorders or controls [1-4, 8, 19, 20]. There have been some reports indicating lower $A\beta_{42}$ values in PSP compared to controls [5-7]. A single study reported lower $A\beta_{42}$ levels in PSP vs. PD [7].

Along the same lines, the majority of CSF $A\beta_{42}$ studies in CBS do not report significant differences compared to other parkinsonian disorders [1, 3, 5, 8] with few studies reporting decreased CSF $A\beta_{42}$ levels in CBS patients compared to controls [2, 5] and PD [4].

As is the case with PSP and CBS, CSF $A\beta_{42}$ levels do not seem to be useful in differentiating MSA from other parkinsonian disorders or controls [1, 3, 4, 12, 17, 18, 20]. A single study reported decreased levels of CSF $A\beta_{42}$ compared to PD, PSP and controls [19]

and a different study group reported decreased $A\beta_{42}$ levels in MSA compared only to controls [18].

d) Established AD CSF biomarkers ratios

Established AD CSF biomarker ratios include the t-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$ and p-tau/t-tau ratios. These ratios are composite markers incorporating data on two of the three biomarker categories of the AT(N) classification system. These ratios have been extensively used in the literature as surrogate markers of AD, with neuropathological studies supporting high biochemical/neuropathological correlations in AD. On a practical level, using biomarker ratios decreases the importance of confounding pre-analytical factors in biomarker measurement among different sites.

Regarding PSP, in an initial study including PSP and CBS patients, the p-tau/$A\beta_{42}$ ratio values could not differentiate between PSP and CBS [5], although it was useful in differentiating PSP patients from control subjects in another study [8]. A decreased p-tau/t-tau ratio has been reported to be useful in differentiating patients with atypical Parkinsonism (PSP and MSA) from PD [12], as well as PSP from CBS [8] and PSP patients from control subjects [4].

Few studies have included relevant data in CBS. A single study has reported increased t-tau/$A\beta_{42}$ ratio in CBS compared to PD patients and controls, and decreased p-tau/t-tau ratio compared to control subjects [8]. Another study posited that CBS patients have elevated t-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ compared to PD patients [4].

Regarding MSA, a single study reported that MSA patients present with significantly lower p-tau/t-tau ratios compared to PD [12]. Another study suggested that higher values of t-tau/$A\beta_{42}$ ratio could differentiate MSA from PD with high specificity but suboptimal sensitivity [4].

e) Established AD CSF biomarker profiling

In lack of a single biomarker with molecular specificity for AD, researchers in the field have focused on establishing classification systems for categorizing biomarkers into different groups, in an effort to create biochemical profiles with data on all molecular aspects of AD (amyloidosis, tau pathology, and neurodegeneration). Initial attempts on this approach included CSF biomarkers ratios, such as t-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$ and p-tau/t-tau, as discussed previously.

A more refined approach was the introduction of classification systems such as the BIOMARKAPD/ABSI and AT(N) systems. The implementation of biomarker profiling based on these classification systems is of paramount importance in cohorts of patients with typical (amnestic) and atypical (non-amnestic) presentations of AD, including frontal-executive predominant dementia, primary progressive aphasias, posterior cortical atrophy and corticobasal syndrome. Biomarker profiling in these cases will assist in recognizing patients with an AD underlying pathology and an atypical phenotype (e.g. corticobasal syndrome), which is of pivotal importance both clinically (for individualized management of symptoms) and on a research level (for accurate patient allocation in clinical trials).

Few studies have included CSF AD biomarker profiling data in PSP. In a large cohort, including diverse neurodegenerative disorders, 10% of PSP patients had a CSF-AD profile, as defined by an index incorporating CSF $A\beta_{42}$ and p-tau values [2]. In another study, a single PSP patient (~5%) had a typical CSF AD profile, as determined by abnormal $A\beta_{42}$, t-tau and p-tau values, in a cohort of patients with Parkinsonism [4]. These cases are more likely to represent instances with dual pathology, as neuropathological-clinical correlation studies have not described AD manifesting with Richardson's syndrome.

CSF biomarker profiling is of pivotal importance in CBS. An initial study concluded that 20% of CBS patients harboured a CSF AD profile (as defined by abnormal t-tau, $A\beta_{42}$ and t-tau/$A\beta_{42}$ ratio values) [21]. In another study, 38% of CBS patients harboured a CSF AD profile, based on an p-tau and Aβ42 derived index [2]. Along the same lines, a third study concluded that ~30% of CBS patients had a typical CSF-AD profile (abnormal values in all three AD biomarkers [4]. This study initially reported an increase in t-tau and a decrease in $A\beta_{42}$ in the CBS group, in accordance to previous studies. When the CBS patients with an AD CSF profile were excluded, these differences disappeared, indicating that the admixture of AD patients was driving these differences. CSF profiling was implemented in a follow-up study by the same study group, to investigate possible differences between AD and non-AD pathology in a CBS cohort [22].

The problems arising from implementing different classification criteria in cohorts of atypical or mixed cases of AD [23-25] have been systematically studied in CBS [26]. Depending on the classification criterion used, classification of a CBS patient varied from 39% to 46% in a study including 40 patients with a probable CBD diagnosis based on established diagnostic criteria (28 of these patients fulfilled criteria for probable CBS).

α-synuclein

A-syn is a mainly synaptic, 140 amino acid protein, expressed by neurons. Aggregated a-syn is the main constituent of Lewy bodies and Lewy neurites, the main neuropathological features of PD, PDD and DLB, as well as of glial cytoplasmic inclusions (GCIs), the neuropathological hallmark of MSA [27]. To this end, several studies have focused on total CSF a-syn as a candidate biomarker for MSA. Researchers

initially focused on CSF total a-syn as a candidate biomarker of synucleinopathies. However, over time focus has shifted on phosphorylated and oligomeric forms of a-synuclein, as post-translational alterations in a-syn seem to be driving neurodegeneration [28]. A breakthrough in the field of biomarkers in synucleinopathies has been achieved over the past 5 years with seeding amplification assays (SAAs), a technique currently applied in Creutzfeldt-Jacob disease. These SAAs are being tested for a-syn, due to the demonstration of prion-like properties of a-syn experimentally ***in vitro*** and ***in vivo*** [29, 30]. An overview of the a-syn studies in synucleinopathies is presented in the following section.

a) Total CSF and plasma a-synuclein

Several studies have measured total CSF a-syn levels in MSA. Most of these studies have reported a mild decrease in total a-syn levels in MSA, compared to control subjects [17, 18]. However, there is significant overlap in a-syn levels between MSA and healthy subjects [4, 17, 31]. For this reason CSF a-syn is not a clinically useful biomarker for MSA identification. Likewise, a similar decrease in CSF a-syn is evident in other synucleinopathies (i.e. PD and DLB). Thus, no significant differences were reported when comparing MSA with other synucleinopathies [4, 17, 18, 31, 32] or with other atypical parkinsonian syndromes (i.e.CBS, PSP) [4, 31]. A single study reported that CSF a-syn provided high positive predictive value for synucleinopathies, and could thus be used as a means for patient stratification in clinical trials [17].

Another approach is measuring panels of multiple biomarkers, in an effort to identify composite biomarkers. In accordance to this approach, CSF total a-syn was measured alongside four other biomarkers (Aβ42, t-tau, p-tau, NFL) in a large cohort comprising heterogeneous neurodegenerative disorders [1]. In this cohort, a-syn was significantly decreased in all synucleinopathies but could not independently differentiate among different synucleinopathies. A similar approach was implemented by another study group by assessing a panel of nine CSF biomarkers (including total a-syn) in a cohort of synucleinopathies (PD, MSA), tauopathies (PSP, CBS) as well as FTD and AD patients [3]. Contrary to most studies in the field, total a-syn in this cohort was decreased in the MSA group compared to PD patients, providing suboptimal diagnostic accuracy in differentiating MSA from PD. A subsequent study applied a panel of ten biomarkers, including CSF total and phosphorylated a-syn and plasma total a-syn in a cohort with diverse neurodegenerative disorders, including MSA [33]. MSA patients exhibited a non-specific decrease in CSF and plasma total a-syn levels, whereas phosphorylated a-syn was also decreased in MSA compared to the control groups. However, these a-syn forms were not useful in the differentiation of MSA from PD.

Several studies have measured plasma a-syn by ELISA. Most of these studies report a non-significant increase in plasma total a-syn in MSA compared to control groups, with significant between-group overlap [34, 35]. Another study reported significant plasma a-syn elevation in MSA patients compared to control subjects [36]. This increase was particularly pronounced in the MSA-P compared to the MSA-C group. Scatterplots of individual values of plasma a-syn indicated a large variability within the MSA group, with only a subset of MSA patients exhibiting significant a-syn elevation.

b) Phosphorylated and oligomeric CSF a-synuclein

Wang et al. measured total and phosphorylated at serine 129 CSF a-syn in a cohort of MSA, PD, PSP, AD patients and control subjects[37]. Total a-syn was decreased in the PD and MSA groups compared to controls. Phosphorylated a-syn was exclusively increased in the PD group, while MSA patients exhibited a decrease in phosphorylated a-syn compared to the control group. The phosphorylated/total a-syn ratio was significantly increased in both the PD and MSA groups compared to other study groups in both the discovery and validation cohorts of this study. These a-syn forms were not useful in differentiating PD from MSA.

Another study measured total, phosphorylated and oligomeric CSF a-syn in a cohort of 135 patients with diverse neurodegenerative disorders. Although numerical differences among differences emerged, synucleinopathies as a group (PD and MSA) presented with lower total a-syn and higher phosphorylated to total a-syn ratios compared to tauopathies (PSP and CBS)[38].

Foulds et al. measured total, oligomeric, phosphorylated and phosphorylated oligomeric a-syn in post-mortem ventricular CSF of a cohort of synucleinopathies [39]. MSA presented with numerically higher mean values of total, oligomeric and phosphorylated a-syn levels, whereas phosphorylated oligomeric a-syn levels were significantly higher (~20fold) compared to PD, DLB and PSP groups, indicating that this a-syn form may be a candidate biomarker for MSA. This finding has not been validated to date by follow-up studies.

c) A-synuclein in erythrocytes, exosomes

Zhang et al. measured haemoglobin-binding a-syn (Hb-a-syn) in erythrocytes in a large cohort of MSA patients (n=149), compared to healthy controls (n=149) [40]. Hb-a-syn could be considered a good surrogate marker of brain a-syn accumulation, but this requires further study. By use of ELISA, the authors concluded that Hb-a-syn in erythrocytes is

significantly increased in MSA compared to healthy subjects, with adequate specificity (~80%) but suboptimal sensitivity (~70%).

Another approach on a-synuclein quantification is isolation of exosomes from blood via immunoprecipitation. By using neuronal and oligodendroglial markers, Dutta et al. measured total a-syn in neuronal and oligodendroglial exosomes [41]. MSA patients exhibited significantly increased a-syn, particularly in oligodendroglial exosomes, compared to PD patients and control subjects. An elevated oligodendroglial / neuronal exosome a-syn ratio was highly indicative of MSA. This marker, established in a discovery cohort, was validated in a validation cohort.

A novel approach is measuring a-syn in erythrocyte membranes. A-synuclein is abundant in both erythrocyte membrane and cytoplasm. Liu et al. quantified total and oligomeric a-syn in erythrocytes membrane and cytoplasm through electrochemiluminescence immunoassays [42]. Both total and oligomeric, as well as the ratio of oligomeric/total a-syn were elevated in erythrocyte membranes in MSA patients compared to controls. These differences were not evident for cytoplasmic a-syn. The ratio provided suboptimal combined sensitivity and specificity for the differentiation of MSA from controls.

A study by Li et al. focused on erythrocyte phosphorylated a-syn (at serine 129) as a candidate biomarker for MSA[43]. In this study, the MSA group (n=107) exhibited significantly higher values of p-a-syn compared to control subjects (n=220), producing a~70% sensitivity and ~90% specificity for an MSA diagnosis. MSA-P patients presented elevated p-a-syn values compared to MSA-C.

Along the same lines, Wang et al. focused on the oligomeric a-syn quantification in red blood cells (RBC), as a candidate marker of synucleinopathies [44]. The oligomeric a-syn to total RBC protein ratio differentiated PD from control subjects, with suboptimal specificity. This ratio was also elevated in the MSA group compared to the control subjects, but did not produce adequate diagnostic accuracy for the differentiation of MSA from PD or the control group.

Folke et al. studied possible differences in CSF and plasma anti-a-syn IGM and IgG naturally occurring antibodies (nAbs) in MSA vs. PD [45]. This study reported an elevation of total CSF IgG nAbs, as well as IgG subclasses in MSA and PD compared to controls, with MSA presenting with increased CSF anti-a-syn IgG1, IgG3 and IgG4 nAbs levels compared to PD. The same pattern was evident for plasma IgG subgroups, with PD and MSA exhibiting lower levels of anti-a-syn IgM nAbs compared to controls in CSF and plasma. The utility of nAbs quantification as a surrogate biomarker of synucleinopathies need further validation.

A small study by Cao et al. measured total, phosphorylated and oligomeric a-syn in extracellular vesicles from saliva of MSA (n=16) and PD patients (n=26)[46]. The two groups did not exhibit significant differences in any of the aforementioned a-syn forms.

d) Seeding assays of a-synuclein

Shahnawaz et al. implemented a seeding assay (protein misfolding cyclic amplification – PMCA) in CSF of MSA and PD patients[47]. Using different amyloid-conformation-specific dyes, the authors concluded that a-syn PMCA in CSF samples can readily differentiate between PD and MSA, due to differences in a-syn conformational strains in these disorders. The overall sensitivity of this methodology approached 95%.

Likewise, Rossi et al. applied RT-QuIC in a large cohort (n=439) of CSF samples of diverse neurodegenerative disorders [48]. Only two of the 31 MSA patients exhibited seeding activity with this assay, indicating inherent differences in the conformational strains underlying MSA compared to Lewy body disease (LBD).

Poggiolini et al. applied an RT-QuIC assay in a cohort of synucleinopathies, in an effort to look into the possible value of this assay in predicting disease progression of synucleinopathies [49]. Sensitivity for MSA was 75%, with differences in reaction kinetics compared to PD (longer T_{50} and lower V_{max}). Reaction kinetics correlated with disease progression only in the MSA group.

Another approach in identifying biomarkers is the use of composite markers, which include >1 biomarkers. Using this approach, Singer et al. implemented an a-syn PMCA alongside CSF NFL in an effort to differentiate MSA patients from control subjects and PD/DLB [50]. CSF NFL was markedly increased compared to control subjects, whereas a-syn PMCA was reactive in almost all MSA, but with distinct reaction kinetics (MSA exhibited earlier but significantly lower fluorescence compared to LBD). This dual approach differentiated MSA from controls (NFL) as well as LBD (PMCA).

The same approach was followed by another study group, by combining a-syn RT-QuIC with CSF/plasma NFL [51]. RT-QuIC produced a positive seeding reaction in 3/65 MSA patients. The kinetic curves of RT-QuIC in MSA patients differed significantly from the respective curves in PD (significantly lower relative fluorescent units). Combined use of a-syn RT-QuIC and NFL further optimized the differentiation of MSA from PD.

Okuzumi et al. combined immunoprecipitation (IP), in a attempt to concentrate a-syn seeds from serum, followed by real-time quaking-induced conversion (RT-QuIC) assay (IP/RT-QuIC), in a cohort of synucleinopathies [52]. This method provided moderate diagnostic performance for differentiation of MSA

from control subjects, in two discovery cohorts and an external blinded validation cohort (AUCs: 0.64 to 0.80). Interestingly, amplified seeds from different synucleinopathies, maintained their morphological features of fibrils, as evidenced by transmission electron microscopy.

Other biomarkers

Neurofilament light chain (NFL) is a non-specific marker of neuroaxonal damage. CSF NFL was quantified by ELISA in an study comparing 19 PD patients, 12 PSP and 10 MSA patients. Mean NFL levels were significantly elevated in the MSA and PSP groups compared to PD patients, with some overlap between PSP/MSA and PD. NFL levels correlated with disease progression in atypical parkinsonian groups [53]. A subsequent study including a CBS group validated these results, further establishing NFL as a useful marker in the differentiation of PD from atypical Parkinsonism. Moreover, NFL remained unaltered in consecutive analyses, indicating a stable rate of axonal damage within the atypical parkinsonian disorders [54]. These findings were supported by follow-up studies comparing MSA with PD [14, 55] and PSP with synucleinopathies [56]. Baseline CSF and plasma NFL has been used as a predictor of disease progression in atypical parkinsonian disorders [55, 57, 58]. Plasma NFL has also been reported to assist in the differential diagnosis of PD from atypical Parkinsonism [55, 59].

MicroRNAs (miRNAs) participate in protein translation and are present in CSF. Several studies have focused on miRNAs profiles in CSF, as candidate biomarkers of neurodegenerative disorders. To this end, Marques et al. reported differences in miRNAs between PD and MSA patients from control subjects. Combinations of miRNAs could discriminate both MSA and PD from healthy subjects [60]. The same approach was implemented in plasma miRNA profiles in cohorts of PD and MSA, with differences emerging between groups regarding the expression of various miRNAs[61]. This concept was extended in CSF samples in two PSP cohorts, identifying an upregulation of multiple miRNAs in this disease group[62, 63], as well as in plasma samples in PSP[64].

Glial fibrillary acidic protein (GFAP) is a monomeric protein located in the astroglial cytoskeleton. It has been tested as a candidate biomarker for the differentiation of MSA from spinocerebellar ataxias and in the differentiation of PD from DLB and MSA [65-67]. Although mild elevations of CSF GFAP as measured by ELISA have been reported, GFAP did not assist in the differential diagnosis among synucleinopathies.

Coenzyme Q10 is a key component of the mitochondrial respiratory chain, and has been tried in clinical trials of PSP [68]. To this end, CSF and plasma Q10 levels have been measured in cohorts of synucleinopathies, particularly MSA. In these studies, a non-significant decrease in Q10 levels was reported, with significant overlap between PD and MSA groups [69-72].

A multitude of diverse candidate biomarkers in CSF and plasma, including YKL-40 [73, 74], myelin basic protein – MBP [65], various neurotransmitters [75-78] have been tested in the past. These studies have largely yielded negative results. The exhaustive review of all these studies is beyond the scope of the present overview.

Conclusions

Over the past three decades, studies on candidate biomarkers for atypical parkinsonian disorders have increased exponentially, thus providing us with valuable insight into the pathophysiological mechanisms underlying their complex neurodegenerative disorders. Despite these efforts, a clinically applicable CSF or plasma biomarker for neurodegenerative parkinsonian disorders is currently lacking.

However, from a practical standpoint, established AD CSF biomarker profiling is recommended in all instances of typical or atypical manifestations of AD, including corticobasal syndrome. This should be incorporated into everyday clinical practice where available, since the recognition of CBS-AD patients ***in vivo*** greatly enhances their individualized pharmacological management and assists in providing more accurate information regarding prognosis. Additionally, studies on CSF and plasma NFL levels have supported its use as a biomarker for the differentiation of PD from atypical Parkinsonism. Additional studies are needed to further validate the use of NFL in parkinsonian neurodegenerative disorders.

CSF AD biomarkers are routinely used in the setting of clinical trials of candidate disease-modifying, protein-targeting treatments in AD, and are starting to be implemented in a clinical setting. We are hopeful that the paradigm of AD will be followed in other proteinopathies, such as tauopathies, TDP-43 proteinopathies and synucleinopathies. The emergence of a-syn seeding assays has provided us with encouraging results regarding the development of a clinically relevant biomarker for synucleinopathies in the near future. If similar assays were developed also for Tauopathies, this would greatly aid in the differential diagnosis of atypical Tauopathies leading to Parkinsonism, such as PSP and CBS.

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