Neurotoxicity Research (2019) 36:627–644
https://doi.org/10.1007/s12640-019-00080-4

REVIEW

Unequivocal Biomarker for Parkinson's Disease: A Hunt that Remains a Pester

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Received: 12 April 2019 / Revised: 17 June 2019 / Accepted: 19 June 2019 / Published online: 2 July 2019

Abstract

Devastating motor features, lack of early prognostic tools, and absence of undeviating therapies call for an endeavor to develop biomarkers for Parkinson's disease (PD). A biomarker is anticipated to help in timely and selective diagnosis as well as to hunt for an appropriate treatment option. Peripheral fingerprints can be used to assess the progression, distinguish PD from other related disorders, and monitor the efficacy of therapeutic options. From the last two decades, peripheral blood is constantly targeted in search of an appropriate marker owing to minimal invasive procedure for collection, highly dynamic nature, and insignificant ethical concern. Besides, cerebrospinal fluid (CSF) is also preferred because of its close proximity to the brain. Employing conventional and contemporary sophisticated devices, a number of protein and non-protein entities, mainly metallic elements, have been shown to hold adequate potential to be used as biomarkers for monitoring progression and assessing treatment options for such a distressing neurodegenerative disorder. Classical strategies and relatively newer sophisticated tools, such as proteomics, deciphered the presence of an altered level of highly specific blood- and CSF-specific proteins, free metals, metal-binding proteins, common inflammatory proteins, and overexpressed/modified α-synuclein in PD patients. While several chemical entities are shown to be associated, not even a single protein or metal is converted into unambiguous disease fingerprint. The article provides an update on proteins and metals that are shown to possess enormous potential in the course of biomarker exploration but are unable to deliver a reliable indicator. The review also sheds light on the reasons of ineffective hit to hunt for an authentic fingerprint and proposes the doable ways to translate the output into reality.

Keywords Parkinson's disease · Biomarkers · Proteins · Metals

Introduction

Parkinson's disease (PD) is one of the highly prevalent hypokinetic, basal ganglionic, and movement disorders, characterized by mystifying motor anomalies, such as progressive delay in performing the intended tasks, muscular inflexibility, presence of resting tremulous in the hands and several other parts of the body, and loss of control over the balance and coordination (Singh et al. 2006; Srivastava et al. 2010). Biochemical and anatomical changes at the early stage, i.e., depletion of the striatal dopamine and progressive degeneration of the nigrostriatal tyrosine hydroxylase positive cells, lead to sensory and psychiatric impairments associated with smell, behavior, mood, thought, sleep, and cognition in the later stage of disease (Tripathi et al. 2019). Nigrostriatal dopaminergic neurodegeneration usually occurs quite a few years before the appearance of motor, extra-pyramidal, sensory, and psychiatric symptoms. Despite very old record of scientific investigations on the subject, endogenous and exogenous factors influencing disease onset/progression, explicit prognosis, timely diagnosis, and ever-lasting therapy are yet to be deciphered (Srivastava et al. 2010). A reliable fingerprint is expected to predict PD at an early stage of onset and help in developing the relevant, restorative, or even permanent treatment strategy. While hair and urine are seldom used, dynamism of peripheral blood and close connectivity of cerebrospinal

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fluid (CSF) with the brain compel the investigators to take advantage of body fluids for candidate fingerprint discovery (Bocca et al. 2006; Chahine et al. 2014; Sinha et al. 2007a, 2009). Undeniably, noteworthy clues have surfaced from the studies but tangible benefits are yet to emerge.

After the arrival of "omics" era, the prospect for the development and validation of biomarkers reached a peak owing to predicted power and possible usefulness of sophisticated tools (Kiebish and Narain 2019; Singh et al. 2006; Sinha et al. 2009; Srivastava et al. 2010). The contemplation for development is boosted when technology-driven mechanistic studies led to the identification of specific fingerprints, which are anticipated to be valuable in PD diagnosis (Miller and O'Callaghan 2015; Sinha et al. 2007b). Such candidates are shown to be helpful in evaluating the impact of personalized medicines (Delenclos et al. 2016). Categorically, a few unique PD-related protein and non-protein fingerprints have been developed (Chahine et al. 2014). However, none could embrace the stage of an unambiguous marker, which paves the way for disease diagnosis, differentiation from other syndromes, progression monitoring, or treatment outcome assessment (Delenclos et al. 2016). Several non-protein and non-metal stable entities from diverse biological fluids are identified albeit with a fewer hit for candidate biomarkers. Combinational use of tiny non-coding micro RNAs, involved in the post-transcriptional regulation of proteins, is shown to differentiate PD from controls and other α-synucleinopathies (Santiago et al. 2018; Starhof et al. 2018a). Although candid efforts have been made, biomarker development is still at the same stage where it was two decades ago. Despite all endeavors, including the usage of an amalgamation of the state-of-the-art techniques for the search of a biomarker, not even a single noteworthy and indisputable fingerprint is clinched in clinics and unanimously accepted.

The present article provides an update on the potential candidate markers, mainly proteins and metals, identified from the peripheral blood and CSF. The review explores the apparent reasons accountable for unsuccessful translation of potential candidates to unambiguous biomarkers. Furthermore, an attempt is made to suggest the feasible ways to gear up the paradigm shift from basic research to application-oriented discovery and also for translating the candidate fingerprints to genuine biomarkers. The literature search was done employing one or many key words, such as biomarker, protein marker, metal, metal marker, proteomics-based biomarker, and proteomics, in combination with PD. Redundant and unrelated citations were omitted out. Although several important citations are included, a few might have missed from inclusion either due to inadvertent failure to notice or owing to restrict the number of citations.

Potential Protein and Non-protein Candidate Markers

Biological fluids are shown to be useful for developing diagnostic markers for PD (Sanyal et al. 2016). In this direction, several proteins and trace elements are found to be directly or indirectly associated with PD progression/treatment outcomes. Although a number of other promising molecules are also found to be linked, the current article does not cover them. For example, the level of 8-hydroxy-2′-deoxyguanosine in the CSF and serum of PD patients is found to be notably high in comparison with controls (Kikuchi et al. 2002). Besides, noteworthy changes in the concentration of metabolites, such as lactate and reduction in the ratio of *N*-acetylaspartate/creatine, are also found that possess potential to act as PD and PD progression markers, respectively (Henchcliffe et al. 2008; Seraji-Bozorgzad et al. 2015). The level of 3-methoxy-4-hydroxyphenylglycol in the serum and CSF is found to differentiate various stages of PD and PD with controls, PD plus syndromes, and other neurodegenerative diseases (van der Zee et al. 2018; Vermeiren and De Deyn 2017). Only prospective protein and metal candidate markers identified from the peripheral blood and CSF are elaborated in the following sections.

Promising Protein Candidate Markers

Protein markers are identified either by the conventional approaches, such as polyacrylamide gel electrophoresis (PAGE), two-dimensional PAGE (2-D PAGE), western blot analysis, enzyme-linked assays, and specific protein analysis tools or by high throughput and sophisticated instrumentations, such as mass spectrometry with or without 2-D PAGE. Protein markers are explored mainly in the serum (comparable to plasma without clotting factors and anticoagulant), plasma (corresponding to serum containing clotting factors and anticoagulant), whole blood, blood cells, and CSF.

Plasma/Serum

Human serum/plasma has been constantly used for developing signature fingerprints for PD. Differential regulation of a few proteins in PD highlights their usefulness as candidate markers in predicting the mechanism of pathogenesis, onset, or treatment outcomes. Most of the differentially displayed proteins are clubbed under the category of secretory proteins, intracellular transport proteins, endogenous pro- and anti-oxidants, metabolizing enzymes, immune response proteins, cell proliferation factors, mitochondrial proteins, and abnormal protein aggregates. Such proteins possess a remarkable

potential to work as PD pathology indicators (Zhang et al. 2012; Zhao et al. 2010).

An increased level (approximately fivefold) of serum amyloid P component (SAP) in the plasma sample of PD patient in relation to control is reported. It is also validated for sensitivity (94.1%) and specificity (87.5%) in an experimental scenario (Chen et al. 2011). Similarly, an increased display of eight plasma proteins, including clusterin and serum transferrin and reduced level of one and half dozen of proteins, such as α-2-antiplasmin, complement component 4B, apolipoprotein A-1, and coagulation factor V are found in PD patients. These proteins are known to regulate protein aggregation, oxidative stress, mitochondrial function and neuroinflammation. Abnormal level of such proteins leads to impairment of these events, which subsequently direct PD pathogenesis (Zhang et al. 2012). Besides, clusterin, complement C1r subcomponent and apolipoprotein A-1, and fibrinogen gamma chain are shown to possess efficacy to be considered as diagnostic markers while apolipoprotein A-1 alone is shown to track disease progression (Kitamura et al. 2018). A total of 17 discriminating PD-specific mass to charge peaks are also identified from the serum, which include 5 peaks that potentially and precisely differentiate PD patients with controls (Li et al. 2011). Depression is one of the most common non-motor symptoms in PD and is often underdiagnosed and undertreated. In a study, a total of 17 differentially expressed proteins are correlated with lipid and glucose metabolisms while neurogenic locus notch homolog protein 2 is shown to be linked with PD-related depression (Dong et al. 2018). 3-Peptoid, oligomers of N-substituted glycine, is normally used to detect PD-related antibodies in the serum. In a combinatorial peptide library approach-based study, peptoid 2 was found to possess high affinity for immunoglobulin G subtype 3 in PD patients in comparison with controls showing that it can be used as an early stage or disease severity indicator (Yazdani et al. 2016). A classical proteomic approach also identifies a sum of 15 protein spots in the serum, which are likely involved in PD (Zhao et al. 2010). Moreover, serum C-X-C motif chemokine ligand 12 (CXCL 12) is found in high quantity in PD patients in comparison with controls that could act as a potential candidate marker of inflammation (Bagheri et al. 2018).

A differential display of fibrinogen γ, haptoglobin, thrombin, transthyretin, apolipoprotein A-1 and E, SAP, complement factor H, and complement C3 proteins in exosome is also reported (Alberio et al. 2013). Reduced and 4-hydroxy-2-trans-nonenal-modified apolipoprotein A-1 are found to act as promising diagnostic markers as well as potential therapeutic targets. However, their specificity for PD is difficult to ascertain (Keeney et al. 2013). Sensitivity, specificity, and reproducibility of SAP, effectiveness of other proteins in identification and differentiation, and a link between apolipoprotein A-1 and early-stage PD offer immense potential to consider them as biological indicators (Alberio et al. 2013; Chen et al. 2011; Zhang et al. 2012; Zhao et al. 2010). Nonetheless, PD is reported to be easily differentiated from PD with dementia since the latter possesses lower serum low-density lipoprotein, such as cholesterol and apolipoprotein B, in comparison with the former (Dong et al. 2018).

Blood Cells

Peripheral blood lymphocytes possess sensitive markers that can be employed to evaluate disease progression, stage, severity, and response to therapy. Lymphocyte cofilin-1, tropomyosin, and actin are found to fluctuate while gamma-fibrinogen is linked with disease state/duration (Mila et al. 2009). On the other hand, α-synuclein-expressing cells are found to overexpress the antioxidant proteins, including DJ-1. Levodopa treatment alters the cell surface protein expression in T cells indicating that lymphocyte could be used as an indicator of dopamine toxicity (Fasano et al. 2008). Levodopa is found to differentially regulate the expression of ATP synthase subunit β and proteasome subunit β type-2 proteins (Alberio et al. 2012). Besides, second-line therapies are also shown to regulate the expression of several other proteins. Dopamine agonist therapy is found to alter the level of peroxiredoxin 6, prolidase, actin-related protein 2 (ARP 2), proteasome activator complex subunit 1 (PACS 1), tropomyosin α-3 chain, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and F-actin-capping protein subunit β (FACPS β) showing that changes in a bunch of proteins hold enormous potential to act as a candidate marker for treatment outcome assessment (Alberio et al. 2012).

Not only lymphocytes but also mononuclear cells are used for genuine fingerprint search. Higher expression of C-X-C chemokine receptor 4 (CXCR 4) in the peripheral blood mononuclear cells of patients in relation to controls is reported (Bagheri et al. 2018). Although the level of cytokines is found to be higher, the nuclear receptor-related 1 protein (NURR1) exhibited a negative link with the expression of a group of cytokines that include tumor necrosis factor (TNF)-α and a few interleukins in PD patients. Such findings indicate that combined NURR1 and cytokines in the mononuclear cells own potential to diagnose PD (Li et al. 2018).

Study for biomarker search is not restricted to lymphocytes and mononuclear cells; erythrocyte milieu is also employed. Proteomic analysis of red blood cell extracellular vesicles isolated from different disease stages has shown the presence of unique signatures distinguishing control from mild to moderate PD (Lamontagne-Proulx et al. 2019). Nevertheless, less half-life and highly dynamic nature and many other limitations take away blood cell–derived proteins from genuine biomarkers.

CSF

Molecular changes in CSF mimic the changes in the brain; thus, CSF is anticipated to provide relatively more accurate, sensitive, and reliable candidate indicators (Dos Santos et al. 2018; Sinha et al. 2009). Proteomic approaches are employed to see the differentially displayed CSF proteins similar to whole blood, serum, and plasma. Increased level of proline-rich repeat 14 (PRR 14), serum albumin precursor (SAPP), serum albumin chain-A (SAC-A), and serum transferrin N-terminal lobe (STNTL) and reduced hemoglobin β fragment and globin (mutant) contents are found to be present in PD patients and could be used as biomarkers for disease onset and progression (Sinha et al. 2009). The level of transferrin, iron, nitric oxide, and interleukin-1β is also found to be increased in probable rapid eye movement sleep behavior disorder (RBD) patients as compared with non-rapid eye movement behavior disorder (NRBD) patients (Hu et al. 2015). Since RBD possesses more motor and non-motors symptoms, such proteins could be candidate markers of severe motor and non-motor symptoms. Intracellular protein α-synuclein, which is one of the most important components of Lewy body, is also detected in CSF of PD patients (Emmanouilidou et al. 2010). Reduced α-synuclein/synuclein concentration holds efficacy for a general synaptic degeneration marker while combined β- and α-synuclein is shown to act as an indicator for PD with dementia (Oeckl et al. 2016). Such studies clearly associate neuronal dysfunction with various forms of synuclein and other CSF proteins and project their usefulness in biomarker discovery (Oeckl et al. 2016; Sinha et al. 2009). In addition to the measurement of normal and aggregated forms of α-synuclein contents, α-synuclein-based specific assays are shown to identify PD patients. Various α-synuclein seeding aggregation assays are demonstrated to differentiate PD with non-PD patients and controls. A real-time quaking-induced conversion based assay is employed to detect α-synuclein aggregation in dementia with Lewy bodies and PD patients with 92% and 95%, sensitivity, respectively, and 100% specificity (for both). This method is shown to be useful for an early diagnosis of α-synucleinopathies since absolute specificity is observed in comparison with controls and Alzheimer disease patients. Moreover, negative results are also reported in the patients suffering from Parkinson plus syndromes, such as progressive supranuclear palsy and corticobasal degeneration (Fairfoul et al. 2016). Similarly, another assay with high sensitivity and specificity is shown to detect a small quantity of α-synuclein aggregates in the CSF of PD and other related α-synucleinopathies. It is referred to as protein misfolding cyclic amplification assay. This method is shown to effectively detect misfolded α-synuclein oligomers or oligomeric seeds in the CSF as little as 0.1 pg/ml. A blinded study is performed to identify PD patients with 88.5% sensitivity and 96.9% specificity employing this method (Shahnawaz et al. 2017). Although parameters employed for α-synuclein seeding aggregation assays are not correlating with clinical indexes, including disease rigorousness or time-span, such assays are shown to be trustworthy and reproducible. Both assays are employed to detect pathogenic oligomeric α-synuclein in the CSF samples of 105 PD patients and 79 healthy controls in another study. These tools are found to possess high accuracy (sensitivity and specificity of 95.2% and 89.9% for the latter assay and 96.2% and 82.3% for the former method) (Kang et al. 2019). While such tools are shown to be worthwhile, further studies employing multiple populations are warranted to confirm the accuracy.

Ferroxidase, commonly referred to as ceruloplasmin, regulates iron homeostasis and protects against oxidative damage (Barbariga et al. 2015; Olivieri et al. 2011). Reduced (~40%) ceruloplasmin-ferroxidase activity is reported in untreated PD. The fraction of copper linked to protein in untreated patients is inversely related to clinical stage and may act as a diagnostic marker (Boll et al. 1999). Endogenous ceruloplasmin exhibits structural changes due to the presence of aberrant level of hydrogen peroxide. Exogenously added ceruloplasmin undergoes structural changes resulting in the reduction of protein activity and increase in integrin-binding ability (Barbariga et al. 2015). Its role in PD is also supported by the fact that an iron chelator, deferiprone, augments CSF ceruloplasmin-ferroxidase activity (Grolez et al. 2015).

Several investigators have shown the usefulness of CSF candidate markers not only for diagnosis but also for staging and differentiation of PD with PD plus syndrome. CSF neurofilament light chain, normal and phosphorylated microtubule-associated tau protein, α-synuclein, chitinase-3-like protein 1, glial fibrillary acidic protein, and β-amyloid 1–42 ($A\beta_{42}$) are shown to be useful for diagnosis and prognosis (Ba et al. 2018; Emamzadeh and Surguchov 2018; Hall et al. 2018; Hu et al. 2017). Measurement of CSF $A\beta_{42}$, tau, threonine-181-phosphorylated tau, and α-synuclein and their ratio are shown to be useful for short- to mid-term prediction (Kang et al. 2013; Kim et al. 2019). Lower level of $A\beta_{42}$ and phosphorylated tau could be used as diagnostic markers while decreased tau and α-synuclein could be used as the markers of increased motor severity (Kang et al. 2013). High levels of inflammatory and axonal proteins in the CSF, such as α-synuclein and tau, are linked with motor and cognitive impairments and are increased in PD with dementia and multiple system atrophy (Hall et al. 2018). CSF $A\beta_{42}$, a usual hallmark of Alzheimer's disease, is found to work as a forecaster of freezing of gait in early PD patients (Kim et al. 2019). $A\beta_{42}$ is also associated with cognitive decline in early PD with RBD. It could be used to identify early PD with high risk for cognitive decline in RBD patients (Ba et al. 2018). Augmentation in microglia-derived cytokines in multiple system atrophy or progressive supranuclear palsy in comparison with PD patients indicates its usefulness in the differential

diagnosis of PD with PD plus syndrome (Starhof et al. 2018b). Contrary to a few reports, studies also show that the flux of tau from neurons to CSF is not a suitable candidate for biomarker since it is a consequence of neurodegeneration (Björkhem et al. 2018). Similarly, a few CSF candidate protein markers are indicative of brain damage and do not essentially change with duration, progression, severity, or surgical procedure and therefore cannot be used for disease staging (Constantinescu et al. 2018). A few important protein markers, methodological details, and a number of subjects employed in the study are also listed for clarity (Table 1).

It is a well-known fact that impaired autophagy plays an imperative role in PD (Tripathi et al. 2019). Acidification of the lysosome and its fusion with autophagosome are regulated by the hydrolytic enzymes (Mishra et al. 2018). The activity of hydrolytic enzymes of the lysosome, such as β-glucocerebrosidase, cathepsin D, and β-hexosaminidase is found to be differentially regulated in PD patients in comparison with controls (Parnetti et al. 2014, 2017). A case-control study comprising 79 cases and 61 controls has shown attenuation in the activity of β-glucocerebrosidase, cathepsin D, and β-hexosaminidase in PD (Parnetti et al. 2017). Besides, an enhancement of β-glucocerebrosidase activity is found to effectively attenuate α-synuclein content and reinstate lysosome function (Mazzulli et al. 2016). Similarly, the reduction in β-glucocerebrosidase and augmentation in β-hexosaminidase activity are found in PD in another study that employed CSF sample of 71 patients and 45 neurological controls (Parnetti et al. 2014). In this study, total α-synuclein content is found to be reduced while α-synuclein oligomers is increased along with a higher oligomeric to total α-synuclein ratio in PD patients. The study found that a combination of β-glucocerebrosidase activity, oligomeric to total α-synuclein ratio, and aging altogether differentiates PD patients from neurological controls more precisely than either alone (Parnetti et al. 2014). The activity of the selected lysosomal and endosomal enzymes are also estimated in a study that employed CSF sample of 58 patients and 52 controls (van Dijk et al. 2013). In this study, the normalized activity of cathepsin E (endosomal) and β-galactosidase is found to be significantly higher in PD patients. Besides, α-fucosidase activity is found to be reduced while β-glucocerebrosidase activity is unchanged in PD patients when compared with controls. The study concluded that amalgamation of normalized α-fucosidase and β-galactosidase possesses the potential to identify PD (van Dijk et al. 2013). Although alteration in β-glucocerebrosidase activity is found to be associated with PD in some studies, a lack of association in another study (Parnetti et al. 2014, 2017; van Dijk et al. 2013) indicates that this protein also cannot be used as an unequivocal PD marker.

Potential Non-protein Candidate Markers

A number of trace elements, such as iron, manganese, copper, and zinc, are reported to act as a cofactor for biologically active enzymes/proteins. Besides, free metal accumulation is also linked with altered PD risk (Ur Rasheed et al. 2017; Willkommen et al. 2018). The level of trace elements has been constantly measured in PD patients and correlated with controls. The concentration of trace elements, including aluminum, calcium, copper, iron, magnesium, manganese, silicon, and zinc, is determined in blood components, CSF, urine, and hair employing conventional or sophisticated tools in the course of biomarker discovery (Forte et al. 2004).

Serum/Plasma/Blood

Peripheral blood components, mainly plasma and serum, are used to estimate metal concentration for establishing an association with PD. The content of a total of 6 elements is found to be variable in the serum of PD patients. Variability in calcium, magnesium, and iron levels indicates their usefulness as candidate diagnostic markers (Sanyal et al. 2016). An iron chelator, deferiprone, which increases the serum ceruloplasmin-ferroxidase activity, substantiates the significance of iron in PD (Grolez et al. 2015). The presence of high levels of calcium, copper, iron, magnesium, and zinc in the blood and low levels of aluminum and copper in the serum of PD patients suggesting that metals are utilizable to differentiate PD with case controls (Bocca et al. 2006). While calcium and zinc levels in the serum/blood of PD patients are found to be increased, concentration of copper, aluminum, and magnesium is shown to be decreased (Forte et al. 2004). Contrary, the serum levels of iron, copper, and zinc remained unchanged in a study (Qureshi et al. 2006). Since altered metal concentration in the serum/blood, i.e., augmented the level of iron and zinc or attenuated level of copper is associated with PD pathology, measurement of metals holds a promising way in effectively identifying PD patients (Forte et al. 2004).

CSF

A total of 10 trace elements, including calcium, magnesium, and iron, are reported to be altered in PD sufferers and could act as diagnostic markers (Sanyal et al. 2016). Ceruloplasmin is found to be more acidic in PD and induces self-oxidation, reduction in ferroxidase activity thus promoting intracellular iron retention (Olivieri et al. 2011). Therefore, the presence of oxidized ceruloplasmin may act as a candidate marker (Olivieri et al. 2011). Even in CSF, deferiprone augments the ceruloplasmin-ferroxidase activity, which further authenticates the contribution of iron in PD (Grolez et al. 2015).

Table 1 Differentially expressed proteins in the serum/plasma, blood cells, and CSF, which are shown to act as candidate markers for PD diagnosis and differentiation, are listed. Methodology used and number of subjects along with statistical significance/fold change/value and sensitivity/specificity of a few are also given

S. No.	Protein	Source	Status in PD	Methodology	Subjects	Statistical significance/fold change/value	Sensitivity and specificity	Reference
1	SAP	Plasma	Increased	Two-dimensional gel electrophoresis, mass spectrometry, and enzyme-linked immunosorbent assay	PD, 36 Control, 16	PD vs. control, $p < 0.001$/fivefold/PD, 65.9 ± 18.7 and control, 35.0 ± 12.5 (in μg/ml; values in mean ± standard deviation)	94.1% and 87.5%	(Chen et al. 2011)
2	Sero-transferrin and clusterin Complement component-4B, coagulation factor V, apolipoprotein A-1, and α-2-antiplasmin	Serum	Enhanced Reduced	Two-dimensional liquid chromatography-tandem mass spectrometry coupled with isobaric tags for relative and absolute quantification labeling	PD, 16 (stage I~II, 8 and stage III~V, 8) Control, 6	– –	– –	(Zhang et al. 2012)
3	Clusterin, complement C1r sub-component, apolipoprotein A-1 and fibrinogen gamma chain	Plasma	Decreased	Two-dimensional differential gel electrophoresis and matrix-assisted laser desorption/ionization source and tandem time of flight mass spectrometry	PD, 16 (stage II, 8 and stage III, 8) Control, 8	PD vs. control, $p < 0.05$	–	(Kitamura et al. 2018)
4	CXCL 12	Serum	Increased	Enzyme-linked immunosorbent assay	PD, 30 Control, 40	PD vs. control, $p < 0.0001$/PD, 140.8 ± 14.87 and control, 59.50 ± 3.049 (in pg/ml, values in mean ± standard error of the mean)	–	(Bagheri et al. 2018)
5	Fibrinogen γ, haptoglobin, apolipoprotein E, transthyretin, complement C3, apolipoprotein A-1, SAP, complement factor H, and thrombin	Plasma	Differential expression in the form of 32 spots in PD	Two-dimensional gel electrophoresis and liquid chromatography-tandem mass spectrometry	PD, 45 Control, 45	PD vs. control, $p < 0.05$	–	(Alberio et al. 2013)
6	β-Globin and haptoglobin-related protein precursor	Plasma	Increased	Two-dimensional gel electrophoresis, matrix-assisted laser desorption time of flight, and liquid chromatography-tandem mass spectrometry	PD,52 (levodopa--untreated, 15 and levodopa-treated, 37) Control, 37	PD (levodopa-untreated) vs. control, $p < 0.001$ and $p < 0.001$, respectively	–	(Sinha et al. 2007a)
7	TNF-α and interleukins-1β, 4, 6, and 10	Mononuclear cells	Increased	Quantitative real-time-polymerase chain reaction	PD,312 (untreated, 82 and treated with anti-PD medications, 230) Control, 318 Neurological control, 332	PD vs. control, $p < 0.001$, $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.01$ and PD vs. neurological control, $p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively	–	(Li et al. 2018)
8	Mutant globin and hemoglobin β fragment	CSF	Decreased Increased	2D-PAGE and mass spectrometry	PD, 14 Control, 14	PD vs. control, $p < 0.05$ and $p < 0.01$, respectively	–	(Sinha et al. 2009)

Table 1 (continued)

S. No.	Protein	Source	Status in PD	Methodology	Subjects	Statistical significance/fold change/value	Sensitivity and specificity	Reference
	PRR 14, SAPP, SAC-A, and STNTL				Neurological control, 6	PD vs. control, $p < 0.001$, $p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively		
9	Transferrin and interleukin-1β	CSF	Increased	Enzyme-linked immunosorbent assay	PD, 67 (with RBD, 16 and NRBD, 51) Control, 31	PD with RBD vs. control, $p = 0.001$ and $p = 0.001$, PD with NRBD vs. control, $p = 0.796$ and $p = 0.125$ and PD with RBD vs. PD with NRBD, $p = 0.003$ and $p = 0.028$, respectively/PD with RBD, 0.262 (0.155~0.357), PD with NRBD, 0.145 (0.081~0.191) and control, 0.153 (0.117~0.172) for transferrin (in μg/ml; values in median (quartile)) PD with RBD, 67.032 (10.438~146.820), PD with NRBD, 11.122 (8.862~69.511) and control, 9.409 (8.535~36.169) for interleukin-1β (in pg/ml; values in median (quartile))	–	(Hu et al. 2015)
10	Ceruloplasmin-ferroxidase	CSF	Reduced	Atomic absorption spectrometry	PD, 49 (levodopa--untreated, 35 and levodopa-treated, 14) Control, 26	PD (levodopa-untreated) vs. control, $p < 0.001$/40% reduction in PD (levodopa-untreated)	–	(Boll et al. 1999)
11	$A\beta_{42}$	CSF	Reduced	Multiplexed immunoassay	PD, 350 (with RBD, 136 and with NRBD, 214)	PD with RBD vs. PD with no RBD, $p = 0.005$/PD with RBD, 354.70 ± 103.45 and PD with no RBD, 384.73 ± 90.77 (in pg/ml; values in mean ± standard deviation)	–	(Ba et al. 2018)
12	Chitinase-3-like protein 1	CSF	Attenuated	Enzyme-linked immunosorbent assay	PD, 158 (non-demented, 131 and with dementia, 27) Multiple system atrophy, 24 Progressive supranuclear palsy, 14 Control, 50	PD (non-demented) vs. control, $p = 0.029$, PD (non-demented) vs. Multiple system atrophy, $p = 0.010$ and PD (non-demented) vs. Progressive supranuclear palsy, $p = 0.037$	–	(Hall et al. 2018)
13	Oxidized ceruloplasmin	CSF	Increased	Two-dimensional gel electrophoresis	PD, 14 Control, 15 Amyotrophic lateral sclerosis, 16 Peripheral neuropathies, 13	PD vs. control, $p < 0.001$	71.4% and 93.3%	(Olivieri et al. 2011)

Table 1 (continued)

S. No.	Protein	Source	Status in PD	Methodology	Subjects	Statistical significance/fold change/value	Sensitivity and specificity	Reference
					Alzheimer's disease, 14			

Although the level of iron is attenuated in PD, a positive correlation is shown between iron and nitric oxide contents and interleukin-1β concentration in PD with RBD (Forte et al. 2004; Hu et al. 2015). Reduced ceruloplasmin or ferroxidase activity also augments iron or free copper accumulation and could be employed as diagnostic PD markers (Barbariga et al. 2015; Boll et al. 2008). Moreover, the level of copper, zinc, and manganese is increased in PD (Hozumi et al. 2011). Alteration in manganese, iron, copper, and zinc due to different permeation behavior at the blood-CSF-barrier is also observed (Nischwitz et al. 2008). Even a small amount of copper is associated with ferroxidase activity and inversely affects PD risk (Boll et al. 1999). While combined metal concentration and speciation are found to exhibit minor variation, a strong alteration in the ratio of total iron and amino acid fraction of copper is observed (Willkommen et al. 2018). A significant reduction in cobalt, chromium, iron, lead, silicon, and tin in the CSF of PD patients suggests that lead, chromium, and iron can differentiate PD with control (Alimonti et al. 2007). Some of the important non-protein markers, methodological details, and the number of cases and controls are also tabulated for lucidity (Table 2).

Significant decrease in zinc, increase in iron and selenium, and no change in copper are shown to be associated with on and on/off PD patients (Qureshi et al. 2006). The deficiency of zinc deteriorates the clinical condition of off patients while an increase in iron and selenium is correlated with reduced dopamine content and augmented oxidative stress (Qureshi et al. 2006). On the other hand, magnesium concentration is reduced with the duration and severity of PD (Bocca et al. 2006). Increase in selenium is albeit shown to be associated with protection, chromium is found to be unchanged in PD (Aguilar et al. 1998). Similar to proteins, altered metal concentration is also associated with treatment outcome and can be used as an indicator of specific treatment paradigm (Table 3). Although anti-Parkinsonian therapy does not influence the selenium content, PD patients not treated with levodopa possess higher selenium (Aguilar et al. 1998). Results of such studies indicate that metal or metal-associated proteins can serve as candidate markers to identify and differentiate various disease stages as well as to infer treatment results.

Undoubtedly, investigations highlighted the presence of one or the other metals in the blood components and CSF; conflicting result mainly in the CSF is also reported (Cicero et al. 2017). Results related to CSF metal biomarkers have been found to be consistently inconsistent. Furthermore, the concentration of metals in the biological fluid of PD patients is found to be influenced by anti-Parkinsonian therapy in a few studies; even then, the lack of significant association cannot be completely overlooked (Aguilar et al. 1998; Bocca et al. 2006). Inconsistent observations severely hamper the notion of the usefulness of metals as PD biomarkers.

Urine and Hair

Urine and hair samples are obtained employing non-invasive procedure and apt for candidate marker discovery. Studies measuring the level of trace elements in hair and urine are limited and inconclusive. A few studies have shown that metal level in such samples helps in identifying the gender specificity and age-specific effects and also in distinguishing PD with control (Bocca et al. 2006). The level of calcium and iron in the urine of PD patients is shown to be higher in comparison with controls. Similarly, urinary excretion of tin is found to be exceedingly high in PD subjects (Forte et al. 2004). Owing to the strong relationship between food habit and urine composition or environmental exposure and hair composition, urine and hair could not indisputably back PD biomarker discovery.

Translational Obstacles in the Conversion of Potential Candidate Fingerprints to Authentic Biomarkers

Majority of candidate proteins carrying ample potential for biomarkers do not possess robustness and reproducibility in identifying PD at an early stage (Constantinescu and Mondello 2013; Dos Santos et al. 2018; Miller and O'Callaghan 2015). Owing to this reason, the potential fingerprints could not be converted into genuine biomarkers in spite of extensive efforts. It has been an important enigma in PD research so far and thus needs a permanent solution. The major obstacle in developing a specific fingerprint of this central nervous system disorder from the blood/CSF is the lack of appropriate tissue for correlation with disease progression and its subsequent validation (Dunckley et al. 2005; Miller and O'Callaghan 2015). Human brain tissue cannot be taken like peripheral tissue for subsequent clarity. Moreover, processing of the post-mortem brain for correlation, even if available, has many ethical and non-ethical issues (Ravid and Park 2014). The alternative, which is left, is the reproducibility assessment of a candidate fingerprint obtained from a peripheral tissue/CSF in analogous tissue/fluid derived from other patients of genetically the same or distinct population. In such condition, inter-individual variability that arises from age, physical status, genetics, and exogenous and endogenous factors is difficult to fix (Mayeux 2004; Willemse and Teunissen 2015). Besides, obtaining CSF is not only tricky, cumbersome, and invasive but also anxious to patients. The collection of healthy control CSF is ethically not possible since its withdrawal can adversely affect the fitness of a healthy volunteer (Teunissen et al. 2014; Willemse and Teunissen 2015). Studies are mostly performed with the leftover CSF collected for the assessment of infections in doubtful cases and persons found infection free after the test (Sinha et al. 2009).

Incontrovertibly, the least invasive tool and less ethical concern make the peripheral blood one of the most attractive choices for PD fingerprint search (Chahine et al. 2014) (Fig. 1). Regardless of general attempts, inconsistent finding across various studies owing to multiple reasons raises a concern (Mayeux 2004). Since blood is one of the most highly dynamic systems of the body, sudden and even minor changes, such as dietary, environmental, personal, and lifestyle factors, could alter the blood protein/trace element profile (Willemse and Teunissen 2015). Second, it has been a common practice to search a biomarker for PD from the fingerprints already identified/tested for other diseases (Chahine et al. 2014). Although the strategy is appreciated since a bit is better than zilch, specificity is not guaranteed. Sometimes, candidate fingerprint projected for PD is found to be associated with other diseases as well since most of the case-control studies do not consider both non-neurological and neurological controls to rule out the likelihood (Chen et al. 2011; Nafee et al. 2012; Polimeno et al. 2018; Shabayek et al. 2014). Blood often does not reflect the change occurring in the brain thus limits the biomarker discovery (Sinha et al. 2009). Basically, the absence of appropriate tissue/body fluid, which actively mimics the changes in the brain, is one of the major hurdles in biomarker discovery.

A search for specific candidate fingerprint is often restricted to specific laboratory/group. Altered level of a unique protein/metal or bunch of any of these two reported by a group is often not validated by other laboratories/groups rather a fresh study from different angles is initiated, which restricts the wide acceptability of prospective marker(s) (Lewczuk et al. 2018; Willemse and Teunissen 2015). Sometimes, a fingerprint identified by a person in the laboratory is not seen by other researchers even employing similar sample and in the same laboratory. Suitable biomarker could not be developed owing to the lack of reproducibility of a candidate fingerprint, which is shown to possess immense potential in a study, in a separate investigation (Lewczuk et al. 2018). Concerns over reproducibility lead to a considerable dispute (Dos Santos et al. 2018). Besides, slightly different tools are employed for investigating similar samples across different studies. Even if similar tools are employed, erratic results could be expected due to inconsistency in disease symptoms owing to variable ethnicity, environmental exposure, endogenous environment, variable instrumentation, age, sex, race, religion, geographical region, personal habits, lifestyle factors, and experimental and post-experimental errors (Mayeux 2004; Willemse and Teunissen 2015).

Protein biomarker discovery approaches are often roaming around the complex tools and techniques, such as proteomics (Barkovits et al. 2015; Caudle et al. 2010; Chahine et al. 2014; Licker and Burkhard 2014). Identical sophisticated techniques, such as 2-D PAGE and mass spectrometry combo, are albeit used across many studies; variable results are found

Table 2 List of a few differentially displayed metals in the body fluids of patients, which are shown to possess promising prospective to act as biomarkers, is given. Methodology used and number of subjects along with statistical significance/fold change/value and sensitivity/specificity of a few are also listed

S. No	Metals	Source	Status in PD	Methodology	Subjects	Statistical significance/fold change/value	Sensitivity and specificity	Reference
1.	Calcium, magnesium, iron, and zinc	Blood	Increased	Inductively coupled plasma atomic emission spectrometry and sector field inductively coupled plasma mass spectrometry	PD, 91 Control, 18	PD vs. control, $p \leq 0.01$, $p \leq 0.001$, $p < 0.001$, and $p = 0.002$, respectively/ PD, 50.2 ± 9.7, 31.7 ± 4.7, 433 ± 72, and 5342 ± 943; and control, 40.1 ± 9.1, 26.5 ± 4.8, 348 ± 50, and 4469 ± 972, respectively (in μg/ml for calcium, magnesium, and iron, and ng/ml for zinc; values in mean ± standard deviation)	83% and 71%, 80% and 70%, 83% and 70%, and 81% and 75%, respectively	(Bocca et al. 2006)
	Aluminum and copper	Serum	Decreased			PD vs. control, $p \leq 0.01$ and $p = 0.04$, respectively/ PD, 3 ± 1.8 and 981 ± 461; and control, 5.3 ± 3.4 and 1168 ± 285, respectively (in ng/ml; values in mean ± standard deviation)		
2.	Calcium and magnesium	Serum	Increased	Atomic absorption spectrophotometry and flame atomic absorption spectrophotometry	PD, 250 Control, 280	PD vs. control, $p < 0.00001$ and $p = 0.0002269$, respectively/ PD, 710950 ± 2177 and 20,403.71 ± 1244.19; and control, 65,392.97 ± 1755 and 19,883.8 ± 1480.82, respectively (in μg/l; values in mean ± standard deviation)	–	(Sanyal et al. 2016)
	Iron		Decreased			PD vs. control, $p = 0.01231$/ PD, 1155.55 ± 264.94; and control, 1205.49 ± 316.09 (in μg/l; values in mean ± standard deviation)		
3.	Calcium, zinc, and magnesium	Blood/ serum	Increased	Inductively coupled plasma atomic emission spectrometry, sector field inductively coupled plasma spectrometry	PD, 26 Control, 13	PD vs. control, $p = 0.037$, $p = 0.021$, and $p = 0.015$, respectively/ PD, 71926 ± 6962, 4711 ± 892, and 19,163 ± 1788; and control, 66416 ± 2242, 4022 ± 652, and 17,491 ± 1296, respectively (in μg/l; values in mean ± standard deviation)	–	(Forte et al. 2004)
	Copper and aluminum		Decreased			PD vs. control, $p = 0.013$ and $p \leq 0.002$, respectively/ PD, 994 ± 171 and 3.43 ± 2.23; and control, 1216 ± 326 and 6.65 ± 3.13, respectively (in μg/l; values in mean ± standard deviation)		
4.	Copper, zinc, and manganese	CSF	Increased	Inductively coupled plasma mass spectrometry	PD, 20 Control, 15	PD vs. control, $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively/ PD, 18.8, 14.5, and 3.3; and control, 10.2, 5.3, and 1.9, respectively (in ng/ml)	–	(Hozumi et al. 2011)
5.	Iron	CSF	Reduced	Enzyme-linked immunosorbent assay	PD (with probable RBD), 16 PD (with NRBD), 51 Control, 31	PD with RBD vs. control, $p = 0.000$; PD with NRBD vs. control, $p = 0.853$; and PD with RBD vs. PD with NRBD, $p = 0.002$/ PD with RBD, 0.699 ± 0.369; PD with NRBD, 0.458 ± 0.197; and control, 0.495 ± 0.173 (in nmol/ml; values in mean ± standard deviation)	–	(Hu et al. 2015)
				Inductively coupled plasma atomic emission spectrometry, sector field inductively coupled plasma spectrometry	PD, 26 Control, 13	PD vs. control, $p = 0.052$/ PD, 33.0 ± 29.4; and control, 73.3 ± 72.7 (in μg/l; values in mean ± standard deviation)	–	(Forte et al. 2004)
6.		CSF	Reduced		PD, 42			

Table 2 (continued)

S. No	Metals	Source	Status in PD	Methodology	Subjects	Statistical significance/fold change/value	Sensitivity and specificity	Reference
	Cobalt, chromium, iron, lead, silicon and tin			Inductively coupled plasma atomic emission spectrometry and sector field inductively coupled plasma spectrometry	Control, 20	PD vs. control, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.01$, and $p \leq 0.05$, respectively/ PD, 0.09 ± 0.09, 0.65 ± 0.46, 28.2 ± 14.6, 0.46 ± 0.24, 58.4 ± 44.8, and 0.26 ± 0.11; and control, 0.13 ± 0.05, 1.28 ± 0.59, 35.5 ± 5.03, 0.91 ± 0.36, 95 ± 38.3, and 0.32 ± 0.07, respectively (in ng/ml; values in mean ± standard deviation)	83% and 67%, 75% and 74%, 83% and 79%, 92% and 75%, 92% and 64%, and 75% and 64%, respectively	(Alimonti et al. 2007)
7.	Free copper	CSF	Increased	Atomic absorption spectrophotometry	PD patients, 22 Control, 11 Huntington's disease, 23 Amyotrophic lateral sclerosis, 27 Alzheimer's disease, 8	PD vs. control, $p = 0.007$; Huntington's disease vs. control, $p = 0.153$; Amyotrophic lateral sclerosis vs. control, $p = 1$; and Alzheimer's disease vs. control, $p = 0.405$	–	(Boll et al. 2008)
8.	Zinc	CSF	Attenuated	Atomic absorption spectrophotometry	PD (on), 17 PD (on/off), 19 Control, 21	PD (on) vs. control, $p < 0.01$; PD (off) vs. control, $p < 0.001$/PD (on), 117 ± 19; PD (on/off), 96 ± 11; and control, 161 ± 31 (in μg/l; values in mean ± standard deviation)	–	(Qureshi et al. 2006)
	Iron and selenium		Augmented			PD (on) vs. control, $p < 0.01$ and $p < 0.01$; PD (off) vs. control, $p < 0.001$ and $p < 0.01$; respectively/ PD (on), 345 ± 47 and 19.7 ± 1.9; PD (on/off), 397 ± 50 and 22.7 ± 2.1; and control, 237 ± 37 and 14.2 ± 1.8, respectively (in μg/l; values in mean ± standard deviation)	–	
9.	Magnesium	CSF	Reduced with disease duration	Inductively coupled plasma atomic emission spectrometry	PD, 91 Control, 18	PD vs. control, $p \leq 0.001$/ late (more than 8 years of disease), 20.9 ± 1.7; and early (less than 1 year of disease), 26.9 ± 3.0 (in μg/ml; values in mean ± standard deviation)	–	(Bocca et al. 2006)
10.	Selenium	CSF	Increased	Atomic absorption spectrophotometry	PD, 28 Control, 43	PD, 17.9 ± 12.3 and control, 13.5 ± 8.2 (in ng/ml; values in mean ± standard deviation)	–	(Aguilar et al. 1998)

owing to dissimilar methods employed for processing of tissue/proteins, such as extraction, precipitation, isolation, purification, reconstitution, solubilization, and validation (Licker and Burkhard 2014; Mollenhauer and Zhang 2012; Sinha et al. 2007b). Inconsistency always raises serious concerns over the veracity of biomarkers (Mayeux 2004; Dos Santos et al. 2018). Another reason for the discrepancy is the inescapable disadvantage of simple proteomic approaches, i.e., low accuracy and sensitivity to identify the less abundant and overexpressed proteins (Barkovits et al. 2015; Caudle et al. 2010; Constantinescu and Mondello 2013; Srivastava et al. 2010). Most of the potential fingerprints are present in very minute quantity and failure to identify such proteins limits the authentic biomarker discovery (Mollenhauer and Zhang 2012). Another limitation is an inaccurate detection of fingerprints employing proteomic tools due to the nature of protein per se. Several proteins, which have been identified to possess a potential for candidate biomarkers, have multiple forms owing to posttranslational processing (Junqueira et al. 2018; Srivastava et al. 2010). A constraint in clear-cut identification of a specific form of a protein confines the use of appropriate form as a biomarker. Appropriateness of confirmatory techniques also limits precise protein biomarker discovery (Farotti et al. 2017; Solier and Langen 2014). The most commonly used substantiation technique is the western blot, which relies mainly on the protein-antibody interface, i.e., an interaction between the binding capacity of a specific stretch of protein containing a few amino acids with an antibody developed using the similar criteria. Although the concentration of a candidate protein marker is decisive for disease association and progression, in essence, only the interaction between two proteins matters. Similarly, inconsistency in the processing of sample, instrumentation, and analysis leads to variable results in metal biomarker discovery.

In addition to uneven tools for processing, identification, and characterization of fingerprints, several other factors are also accountable for the nonexistence of an appropriate fingerprint. Multi-factorial etiology, complex pathology, heterogeneous origin, genetic predisposition, environmental impact, effect of gender, ethnicity, lifestyle and age of disease onset, and inconsistency in sample collection are a few perplexing factors that influence the authentic biomarker discovery approach (Caudle et al. 2010; Constantinescu and Mondello 2013; Frasier and Kang 2014; Mayeux 2004; Miller and O'Callaghan 2015; Singh et al. 2006; Srivastava et al. 2010). Contribution of sporadic or inconsistent environmental and genetic factors could lead to variability in candidate proteins/metals since they can change the body fluid composition for a while. Isolation of blood and CSF also matters since it contributes to sample variability even in the absence of an obvious inter-individual variability (Frasier et al. 2010; Miller and O'Callaghan 2015; Teunissen et al. 2014; Willemse and Teunissen 2015). Sometimes, pure form of plasma, serum, and CSF is not separated because of hemolysis of red blood cells or chemical contamination (Atik et al. 2016; Constantinescu and Mondello 2013; Lewczuk et al. 2018; Willemse and Teunissen 2015). The number and gauze of needle, size of syringe, sample quantity, cleanliness during collection, method and route of sample withdrawal, and transportation of sample could also contribute to inconsistent findings (Farotti et al. 2017; Frasier et al. 2010; Lewczuk et al. 2018; Miller and O'Callaghan 2015; Willemse and Teunissen 2015). Such factors are not as critical in metal biomarker search as in protein marker discovery. The lifestyle of volunteers, endogenous and exogenous factors, minor infections, temperature, pressure, humidity, fasting, and several other variables during collection and storage; experimental methods; and isolation and fractionation of blood/CSF and variable statistical tools used for data analysis and interpretation could also be responsible for lack of reproducibility (Kiebish and Narain 2019; Mayeux 2004; Miller and O'Callaghan 2015; Teunissen et al. 2009; Weckle et al. 2015; Willemse and Teunissen 2015). As all such factors influence the final outcome, proper subtraction of confounding factors during interpretation needs to be performed. Most of the times, if not always, these factors are ignored in biomarker discovery programs.

Despite specific changes in the level of proteins and metals, such as α-synuclein, globin, transferrin, apolipoprotein A-1, iron, copper, etc., the lack of comparable trends in identical studies created complexity in ascertaining consistency (Chahine et al. 2014, 2018; Cicero et al. 2017; Qureshi et al. 2006; Sinha et al. 2009). While hundreds of publications on protein and metal candidate indicators could be seen in the last two decades and many potential fingerprints have been proposed, the outcomes are precisely imprecise since none could reach to the destination. The search for sensitive, unique, and unfailing fingerprint for the onset, progression, timely diagnosis, prognosis, and personalized therapy is still on the way. Unbiased validation and subsequent use of a candidate fingerprint are still missing in clinical trials (Delenclos et al. 2016).

Authors' Perspective

The area of biomarker discovery has been an inconclusive topic as of now and not even a single potential protein or metal fingerprint is converted to reality. Theoretically, one indicator is shown to be better than others. Such reports are albeit available from case-control studies, investigations correlating the presence/absence of protein/metal fingerprints with increasing age, genetics, and environmental exposure are not known. Most PD patients if presented, a defined value of a unique candidate marker, occasionally the same level is observed even in a few controls or vice versa. It can be observed by looking at the error bars of the differentially expressed

Table 3 Changes in the level of a few metals and proteins in response to therapy may act as treatment response biomarkers. The table also contains the methodology used, the number of subjects, and statistical significance/value/change in the level

S. No.	Protein/metal	Source	Methods	Subjects	Status in PD	After treatment	Statistical significance/fold change/value	Reference
1	Selenium	CSF	Atomic absorption spectrophotometry	PD, 28 (levodopa-treated, 18 and levodopa--untreated,10) Control,43	Increased	No change after levodopa treatment	Levodopa-untreated vs. control, $p < 0.01$/ levodopa-treated, 15.5 ± 11.6; untreated, 22.2 ± 12.9; and control, 13.5 ± 8.2 (in ng/ml; values in mean ± standard deviation)	(Aguilar et al. 1998)
2	Ceruloplasmin-ferroxidase	CSF	Ceruloplasmin-ferroxidase activity measurement	PD, 40 (delayed start, 19 and early start, 21)	Reduced	Increased after metal chelator	Early start vs. delayed start, $p = 0.04$/ early start group, 0.28 to 0.30; and delayed start group, 0.27 to 0.27 (in g/l) before and after treatment, respectively	(Grolez et al. 2015)
3	ATP synthase subunit β and proteasome subunit β type-2	Lymphocytes	Two-dimensional electrophoresis and liquid chromatography-mass spectrometry/mass spectrometry	PD, 17 (levodopa-treated, 13 and dopamine agonist-treated, 10)	Altered	ATP synthase subunit β increased and proteasome subunit β type-2 decreased after levodopa	Treated vs. untreated, $p = 0.029$, and $p = 0.003$, respectively	(Alberio et al. 2012)
	Peroxiredoxin 6, prolidase, ARP 2, PACS 1, FACPS β, tropomyosin α-3 chain, and GAPDH					Peroxiredoxin 6, prolidase, ARP 2, PACS 1, and FACPS β- increased and tropomyosin α-3 chain and GAPDH- decreased after dopamine agonist	Treated vs. untreated, $p = 0.025$, $p = 0.014$, $p = 0.025$, $p = 0.033$, $p = 0.042$, $p = 0.004$, and $p = 0.014$, respectively	

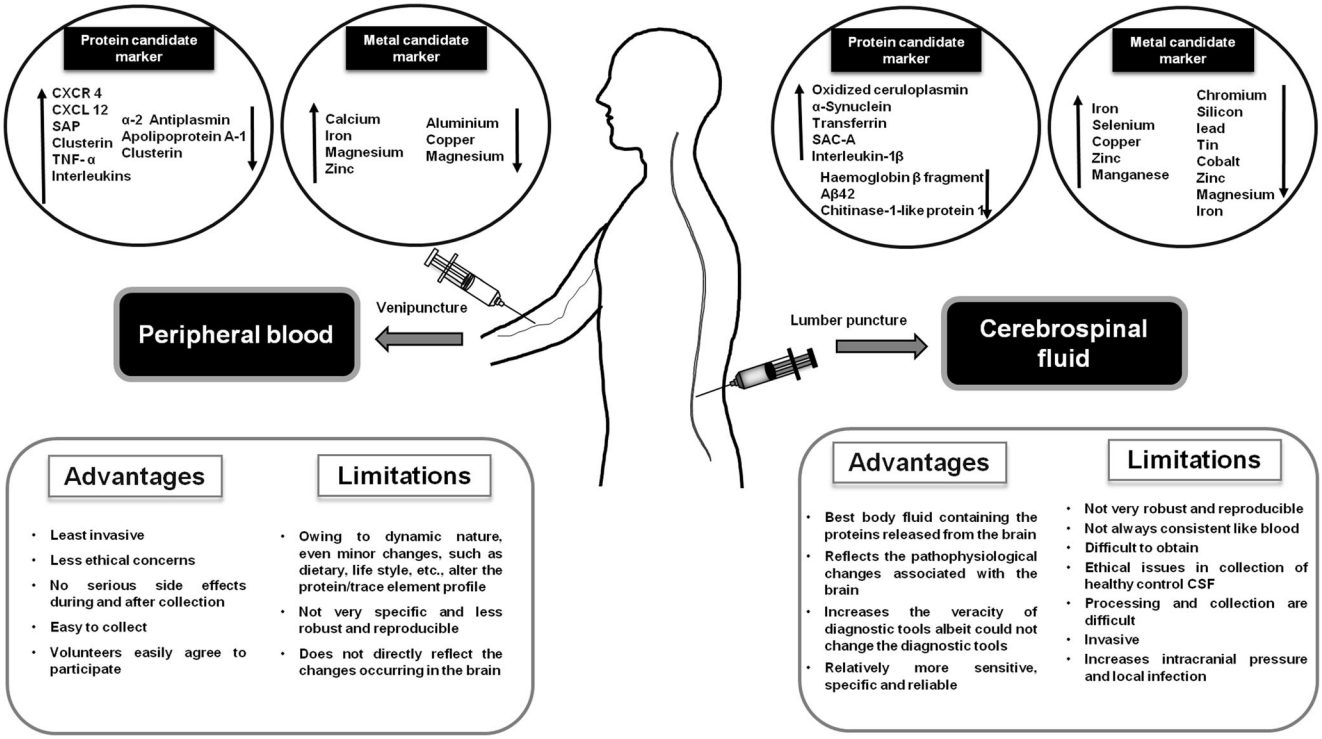

Fig. 1 A diagrammatic representation of the collection of peripheral blood and CSF from a human volunteer along with major protein and metal candidate markers. Advantages and limitations of markers are also summed up at the base of the diagram

proteins reported in the research publications. As a result, such studies offer a lack of confidence among medical experts for clinical usage of a peripheral/CSF biomarker. Nonetheless, the development of a combinational approach involving various techniques is expected to enhance the reliability of biomarker discovery.

Biomarkers offer a better understanding of disease progression, role of modifying factors, and effect of therapy. Predictive relationship of a biomarker with any aspects of PD is still bleak. The literature contains many case-control studies showing several fingerprints. In the first appearance, a fingerprint seems to possess enough potential. However, several aspects need to be seen before drawing a conclusion. Most of the studies performed to date are restricted to an investigator, an investigating team, or an institute with fixed and time-bound-defined objectives and goals. Limited financial and human resources are diverted by the investigator, group, or institute for the proposal. The outcome albeit helps the investigator, group, or institution to accomplish the professional goal, tangible achievement in biomarker discovery is hampered. Sometimes, inadequate intramural or extramural funds are sanctioned by the parent organization or national/international public/private supporting agency with the hope for quality research publications under the newly launched limited budget thematic schemes. Such themes albeit attain the basic aim, i.e., generation of fundamental knowledge to understand neurodegeneration and translational neuroscience, the actual conversion of output into reality is not achieved. Consequently, such studies are often not pursued further either by the same investigator, group, organization, or funding agency. Various other issues could also be responsible for the hitch, such as a change in the thirst area, institute and personal priority of researcher or his group, or lack of adequate funds in the same institute or at the new place to pursue the discovery further. Most importantly, several such fingerprints are not tested by others for accuracy, sensitivity, reproducibility, and veracity. Even if studies are performed by a group or combination of a few groups, detailed information about the selection of subjects, inclusion and exclusion criteria, statistics used, and follow-ups are not available to the scientific masses. The lack of success in this direction could also be due to insufficient attempts made to build an international collaborative team to amicably address the issue and to provide an agreeable solution. Therefore, in addition to technological limitations, biological heterogeneity, variable experimental design, and lack of collaborative international approach are also responsible for dearth of a success story (Frasier and Kang 2014).

International collaboration is required to minimize technological errors, control the quality of samples, and obtain samples from multiple populations. For the accomplishment of the aim, trivial and promising candidate markers should be re-analyzed in a multi-centric study in an unbiased way. Such study should include a large

sample size by considering the details of clinical, environmental, habit, habitat, and lifestyle factors. The study needs to be performed in multiple populations employing similar tools/techniques. The impact of all confounding factors, such as age, ethnicity, gender, geographical area, race, religion, food habit, lifestyle factors, environmental variables, and other clinical conditions, should be correctly subtracted. A precise conclusion should be drawn to clinch an onerous path of biomarker discovery. Easy accessibility of complex statistical analysis, raw-data, and analytical approaches employed in the study to the general researchers and public should be insured. Else, such information should be stored in the public domain. If something near to reality is observed, the same can be validated by any other parties. Hopefully, it would provide a better approach to embrace the desired destination.

Conclusions

Technological innovations in biomarker discovery albeit offered useful results, multi-centric study with clinically defined cases, controls, cohorts, environmental exposures, contributory factors, methodology, and statistics for association and risk analysis are still required for unambiguous translation of biomarkers in clinical practice (Maass et al. 2018). Therefore, an earnest effort needs to be made for a multi-centric and multination study encompassing all potential candidate fingerprints described in previous studies. The newer approach needs to employ larger sample size, fix methods, detailed personal, professional and occupation history of subjects, multiple clinical and non-clinical investigators across the globe, and defined statistics. Subsequently, an unbiased validation needs to be done. The strategy can possibly pave the way for developing an unambiguous PD biomarker.

Acknowledgments The Department of Biotechnology, India, provided junior research fellowship to Alika Sarkar and Council of Scientific and Industrial Research, India, to Neeraj Rawat. Authors sincerely acknowledged the abovementioned agencies. The CSIR-IITR communication number of the article is 3587.

Authors' Contribution The idea was conceived by Mahendra Pratap Singh Alika Sarkar, Neeraj Rawat and Nidhi Sachan collected the information. Alika Sarkar and Neeraj Rawat wrote the initial draft of manuscript. Nidhi Sachan and Alika Sarkar contributed in making the figure. Mahendra Pratap Singh comprehensively revised the manuscript. All authors have seen and finalized the manuscript and concur with its content.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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