Chapter 12
Biomarkers: Potential Perspectives in Detection, Diagnosis, and Prognosis of Neurodegenerative Disorders

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Abstract The cornerstone for diagnosis and treatment of neurodegenerative diseases is the ability to diagnose the disease before the onset of irreversible cellular damage and intervene with suitable therapy to stop or slow down the disease progression. Neurodegenerative diseases are mostly polygenic in origin and present with heterogeneous symptoms in the clinic. Therefore, specific and sensitive biomarkers are required for the early diagnosis and management of these diseases. The four most common neurodegenerative diseases by prevalence are Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. For familial forms of these diseases, characterized by the early age of onset, the genetic biomarkers are highly reliable for risk assessment, diagnosis, and prognosis. For sporadic forms of the diseases, with typically a late age of onset, the symptoms develop gradually over decades with little or no manifest symptoms at the beginning of the disease. At present, no cure exists for any of the neurodegenerative diseases; therefore, prevention and management of disease through reliable biomarkers for each type and substage of the disease is necessary. The current biomarkers for sporadic form of the diseases—genetic biomarkers, biochemical biomarkers, and neuroimaging-based biomarkers—provide limited information on disease prognosis and progression. However, the field of biomarkers for neurodegenerative diseases is in a state of flux; the traditional approach for a unique biomarker for each disease condition is being replaced by a panel of biomarkers that report on the systemic health of the patient and not just the nervous system.

Keywords Biomarkers · Neurodegenerative diseases · Alzheimer's disease · Parkinson's disease · Amyotrophic lateral sclerosis · Huntington's disease

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Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
C9ORF72	Chromosome 9 open reading frame 72
CSF	Cerebrospinal fluid
CT	Computerized tomography
DPET	Dual photon emission tomography
DTI	Diffusion tensor imaging
ELISA	Enzyme-linked immunosorbent assay
FDG	^{18}F-6-fluoro-2-deoxyglucose
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FUS	Fused in sarcoma
HD	Huntington's disease
Htt	Huntingtin protein
MRI	Magnetic resonance imaging
PD	Parkinson's disease
PET	Positron emission tomography
PMCA	Protein-misfolding cyclic amplification
PNCSs	Phrenic nerve conduction studies
RT-QuIC	Real-time quaking induced conversion
SNIP	Sniff nasal inspiratory pressure
SOD1	Superoxide dismutase 1
SPECT	Single-photon emission computerized tomography
TDP-43	Tar DNA binding protein of 43 kDa
Tw Pdi	Twitch trans-diaphragmatic pressure
α-Syn	α-Synuclein

12.1 Introduction

Etiologically neurodegenerative diseases are of two types: the sporadic form and the familial form. The majority of the neurodegenerative diseases are sporadic in origin and are strongly associated with increased age. The typical age of onset, i.e., when the definitive symptoms manifest, is more than 60 years. Neurodegenerative diseases are caused by loss of neuronal function; therefore by the time symptoms manifest, a critical number of neurons have already died. The lost neurons cannot be regenerated with the present scientific know-how, making the cure impossible. Thus, the best strategy to alleviate the burden of neurodegenerative diseases on healthcare systems is to find preventive measures. Even for preventive measures, we should be able to diagnose presymptomatic individuals. This can only be achieved by discovering or developing biomarkers for early diagnosis for specific neurodegenerative diseases

(DeKosky and Marek 2003). The power of early diagnosis and appropriate preventive measures is highlighted by the observation that a 5-year delay in the onset of Alzheimer's disease can reduce the disease risk by 50%, while a delay by 10 years likely prevents it completely (Brookmeyer et al. 1998).

Based on prevalence, the common neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (Fig. 12.1). All these diseases share many common clinical and molecular features. Clinically, late age of onset, motor-neuron dysfunction, and dementia are common symptoms, while at molecular level, amyloid deposits of intrinsically disordered proteins and progressive spread coupled with the loss of function are the main features. Also, all neurodegenerative diseases show high patient-to-patient variability. The diagnostic and pathological hallmark of all these diseases is the presence of protein aggregates and the loss of specific neurons (Table 12.1). The exact molecular mechanisms in each neurodegenerative disease however are distinct, which necessitates the development of a specific set of biomarkers for each condition.

12.2 Biomarkers

Biological markers or biomarkers are defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working 2001).

The ideal biomarker should have high specificity and sensitivity for the disease and the substages of the disease; it should be noninvasive and affordable. Depending on the use of biomarkers, they can be diagnostic, predictive, prognostic, and pharmacodynamic. Diagnostic biomarkers help determine the presence of a disease and identify the stage of a pathological condition. Predictive biomarkers evaluate the effectiveness of a given therapy. Prognostic biomarkers provide an overall outcome for the patient, regardless of the therapy. Pharmacodynamic biomarkers indicate the effect of the drug on the patient and the efficacy of the treatment. All clinical biomarkers need to be validated in diagnosed pathological cases. For neurodegenerative diseases especially, the biomarkers should be able to distinguish between the four common diseases and specific types of dementia, which shows highly overlapping clinical symptoms.

Biomarkers for neurodegenerative diseases are broadly classified into three categories: genetic, biochemical, and imaging-based. Combinations of all these biomarkers are used to diagnose and stratify susceptible individuals. Stratification identifies and classifies susceptible individuals or patients in groups of relative risk of developing a disease (DeKosky and Marek 2003).

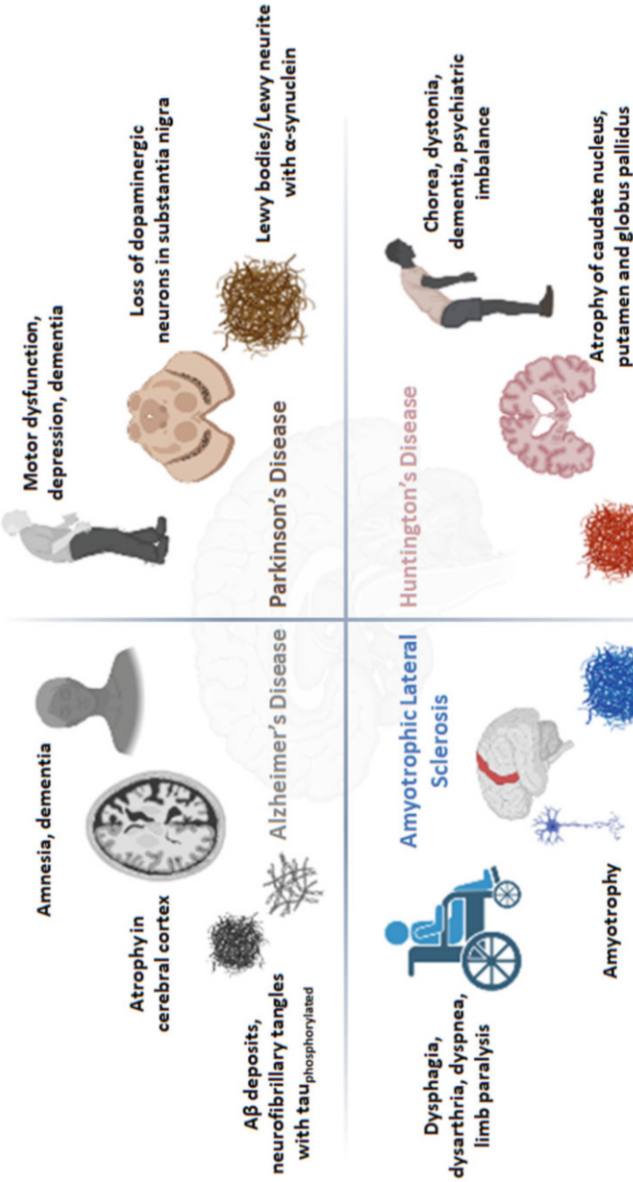

Fig. 12.1 Common neurodegenerative diseases with clinical symptoms at the individual level, changes at the tissue level, and amyloid deposits at the molecular level

Table 12.1 List of common neurodegenerative diseases with associated proteins and neurons

Disease	Key proteins	Affected neurons
AD	Amyloid β, p-tau, presenilin	Cerebral cortex and hippocampus
PD	α-Synuclein, LRRK2, parkin	Substantia nigra pars compacta
ALS	FUS, TDP-43, C9ORF72	Upper and lower motor neurons
HD	Huntingtin	Caudate nucleus, putamen, and globus pallidus

Table 12.2 Genes commonly mutated in familial forms of neurodegenerative diseases

Disease	Genes
AD	APP, Presenilin-1, Presenilin-2, ApoE (polymorphism)
PD	SNCA, LRRK2, GBA, PINK1, UCHL-1
ALS	TDP-43, FUS, C9ORF72, SOD1, ALS
HD	HTT

12.2.1 Genetic Biomarkers

Nucleic acid-based biomarkers—DNA and RNA—form the genetic biomarkers. Mutations and polymorphisms in genes that lead to familial forms of neurodegeneration are the primary biomarkers for the respective diseases for risk assessment, diagnosis, and prognosis. Examples of genes mutated in the familial form of neurodegenerative disease are listed in Table 12.2. The updated list of mutations—missense/nonsense, splicing, regulatory, small deletions, small insertions, small indels, gross deletions, gross insertions/duplications, complex rearrangements, and repeat variations—in these genes can be accessed through The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). Any of the validated mutations can be developed into a genetic assay to be used as a biomarker. DNA-based biomarkers allow early risk assessment of the susceptible individual, can predict the early vs. late onset of the condition, and can predict the course of the disease (prognosis). For familial forms of neurodegeneration, DNA biomarkers can be tested anytime from childhood to early adulthood in individuals with a family history of the disease, as the mutations or polymorphisms are present in the genomic DNA.

In recent years, RNA-based biomarkers like mRNAs, miRNAs, long noncoding RNAs, etc. are under intense investigation for each neurodegenerative disease. RNA biomarkers provide information about the dynamics of gene expression and regulation. Since a majority of the neurodegenerative diseases are sporadic in nature, the loss of gene function arises at an extragenetic level. Sporadic DNA mutations can also lead to loss of gene function, but their effect is restricted to the neurons in which they arise, as neurons do not divide. Hence, sporadic DNA mutations are seldom a cause of sporadic neurodegenerative diseases. An exception to this hypothesis is if the neurodegenerative diseases spread in a prion-like manner, which implies that misfolded proteins from one neuron can nucleate misfolding of other proteins in the neighboring cells. Prion-like spread of neurodegenerative diseases involves the

transport of misfolded proteins from one cell and uptake into another neuron (Goedert et al. 2010).

12.2.2 Biochemical Biomarkers

Cerebrospinal fluid (CSF) circulates through the brain ventricles and drains into venous blood via the arachnoid villi of venous sinuses. CSF is an ultrafiltrate of plasma with a minimal concentration of blood metabolites and proteins. Therefore, biomolecules and metabolites of CSF are representative of neural homeostasis. Metabolites from intra-neural and extra-neural biochemical processes are removed by the circulating CSF. Regeneration of bulk of CSF takes place during deep sleep. Lack of sound sleep results in the accumulation of metabolites in the cerebral interstitial fluid that drains into CSF. Emerging evidence shows that elderly people with chronic sleep disturbances are more likely to develop neurodegenerative diseases (Lucey et al. 2018; Shokri-Kojori et al. 2018; Ju et al. 2017; Kang et al. 2009). Therefore, CSF, blood, and urine can yield potential biochemical biomarkers to map the presence and progression of neurodegenerative diseases. The accessibility and noninvasiveness of the body fluid follow the sequence: urine > blood > CSF. However, the concentration of the metabolites, in respective body fluids, is in the reverse order. Aggregated α-synuclein, Aβ peptides, tau, etc. are detectable in the CSF from presymptomatic individuals, but due to the highly invasive nature of CSF collection, it cannot be performed routinely for mass screening and follow-ups, whereas low concentration of the metabolites in blood and urine poses technical challenges for detection. The sensitivity of CSF as a source of biomarkers is due to its direct contact with the brain tissue, while the blood is separated by the blood–brain barrier and the urine is separated by a dual barrier of blood–brain and glomerular filtration. Each degree of separation from the brain tissue reduces the types and quantity of potential biomarkers (Table 12.3).

Absolute measurement of a single biomarker is prone to biological variations due to unknown factors like other health conditions and medications. Therefore, a ratio of two or more biomarkers normalizes the measurements and improves the accuracy. A pair of biomarkers that represent the total vs. altered biomarker status is an efficient method for diagnosis and staging, e.g., measurement of $A\beta_{42}/A\beta_{4o}$ and $tau_{total}/tau_{phosphorylated}$. CSF and blood biomarkers are measured by standard techniques like ELISA (enzyme-linked immunosorbent assay), electrochemiluminescence, xMAP® technology, etc. (Parnetti et al. 2019). The aggregated forms of proteins in CSF/blood sample are detected by using the prion-like behavior of the aggregated forms. They act as seeds for aggregation in assays like protein-misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC) (Paciotti et al. 2018).

Table 12.3 Selected biochemical biomarkers used for neurodegenerative diseases

Disease	Biomarker	Information	Type
AD	CSF, plasma: phosphorylated-tau	AD pathology	Diagnostic, predictive
	CSF, plasma: $A\beta_{1-42}$	AD pathology	Diagnostic
	Glycosylated acetylcholinesterase	Acetylcholine signaling	Diagnostic
	Glycosylated butyrylcholinesterase	Acetylcholine signaling	Diagnostic
	Aggregated β-amyloid peptides	AD pathology	Diagnostic
	ε-ApoE	Lipid metabolism	Prognostic
PD	Lewy bodies	PD pathology	Diagnostic
	Oligomeric α-Syn	PD pathology	Diagnostic, predictive
	Altered dopamine transporter	Dopamine signaling	Diagnostic, prognostic
	β-Glucocerebrosidase	Lysosomal function	Predictive
	β-Hexosaminidase	Lysosomal function	Predictive
	Aggregated α-synuclein	PD pathology	Diagnostic, prognostic
	Lewy neurites	PD pathology	Diagnostic
ALS	Aggregated FUS	ALS pathology	Diagnostic, prognostic
	Aggregated TDP-43	ALS pathology	Diagnostic, prognostic
	8-Hydroxy-2′-deoxyguanosine	Oxidative stress	Predictive
	3-Nitrotyrosine	Oxidative stress	Predictive
	4-Hydroxy-2,3-nonenal	Oxidative stress	Predictive
	8-Oxodeoxyguanosine	Oxidative stress	Predictive
	^{15}-F(2 t)-isoprostane	Oxidative stress	Predictive
HD	CSF: Htt	Neuron damage	Prognostic
	Aggregated Huntingtin protein	HD pathology	Diagnostic

12.2.3 Imaging-Based Biomarkers

The least invasive and the most direct way of learning about and assessing brain function is through neuroimaging techniques available in the clinic and laboratories. The imaging techniques used in biomarker discovery and to study neurodegeneration include the following:

- Single-photon emission computerized tomography (SPECT).
- Positron emission tomography (PET)/dual photon emission tomography (DPET).
- Magnetic resonance imaging (MRI)/computerized tomography (CT).

Neuroimaging is a powerful method as it provides quantitative and qualitative measurements in real-time and can be used to follow the disease progression (Table 12.4). PET and SPECT imaging is based on the detection of photons emitted from the target tissue labeled with specific radiotracers. Radiotracers are radioactive

Table 12.4 Some of the neuroimaging biomarkers used for neurodegenerative diseases

Disease	Biomarker (Technique)	Target	Use
AD	Amyloid plaques (MRI)	Cerebral cortex	To detect the presence of amyloids
	Brain volume (MRI)	Whole brain or specific regions	To detect brain atrophy and mild cognitive impairment
	^{18}F-fluorodeoxyglucose (PET)	Glucose metabolism	To assess neuron health
	^{11}C-PiB (PET)	Amyloid-β	To detect and follow aggregation of amyloid-β
	^{11}C6-OH-BTA-1 (PET)	Amyloid-β	To detect and follow aggregation of amyloid-β
	^{18}F MK-6240 (PET)	Tau protein	To detect and follow aggregation of tau
PD	^{18}F-Dopa (PET)	Mass of dopamine neuron	To diagnose hemi-, pre-, and symptomatic PD
	^{11}C- DTBZ (PET)	Endocytic vesicles with monoamines	To measure the number of vesicles with dopamine
	^{123}I-β-CIT (SPECT)	Dopamine transporter ligands	To measure dopamine levels in the striatum
ALS	Diffusion tensor imaging (MRI)	White matter	To diagnose early ALS
HD	Brain volume (MRI)	Whole brain or specific regions	To detect brain atrophy
	^{11}C-β-CIT (PET)	Presynaptic dopaminergic neuron	To map disease progression
	^{11}C-DTBZ (PET)	Presynaptic dopaminergic neuron	To map disease progression
	^{11}C-SCH22390 (PET)	Postsynaptic dopaminergic neuron	To map disease progression
	^{11}C-raclopride (PET)	Postsynaptic dopaminergic neuron	To map disease progression
	^{11}C-FLB457 (PET)	Postsynaptic dopaminergic neuron	To map disease progression
	^{18}F-FDG (PET)	Glucose metabolism	To assess neuron health
	^{15}O-H_2O (PET)	Blood flow	To assess brain function

molecules that bind specific receptors or enzymes. Both the techniques have nanomolar sensitivity, with positron-emitting radioactive isotopes (^{15}O, ^{11}C, ^{18}F, ^{76}Br) in PET and γ-emitting isotopes (^{123}I, ^{99m}Tc) in SPECT imaging (Brooks 2005). The ability to replace natural isotopes with positron-emitting isotopes—^{11}C, ^{15}O, ^{13}N, and ^{18}F for hydrogen—allows to develop specific labels for virtually all the biomolecules in the body (Phelps and Mazziotta 1985).

Radiotracers against specific receptors or enzymes measure activity in all the neurons that use them. For example, radiotracers like ^{18}F-6-fluoro-2-deoxyglucose (FDG) labels all the neurons undergoing glucose metabolism. Healthy neurons with normal metabolism show higher FDG signals and appear as bright spots on images,

while dead or dying cells show reduced signals and appear as less bright spots on the image. The ability to visualize live cells and neuron function—through custom radiotracers—allows to study, diagnose, and determine the disease progression.

Magnetic resonance imaging (MRI) is based on the stimulation and relaxation of the proton spin in the water molecules. Typically, the subject is placed in an external magnetic field that aligns all the proton spins in the direction of the field. Next, a series of radiofrequency pulses are passed through the tissue under examination, and the protons absorb the radio energy that changes their spin direction. At the end of the pulse, the proton spin realigns with the external magnetic field while releasing energy. MRI detectors detect the released energy and build a composite image from multiple stimulation–relaxation cycles. MRI allows label-free imaging of the soft tissues of the body (higher water content provides more protons, hence a higher signal-to-noise ratio). Brain MRIs are sensitive to distinguish gray and white matter tissue, in addition to the various other parts like cerebellum, amygdala, hypothalamus, thalamus, striatum, etc. (Rosas et al. 2003). MRI scans are very efficient in determining the volume of the whole brain as well as of specific regions. Thus, MRI is routinely used as a diagnostic biomarker for various brain conditions including neurodegenerative diseases. Computerized tomography (CT) scan is based on X-ray images and complements the information from an MRI scan. All the neuroimaging techniques require specialized infrastructure and trained manpower; thus, they are not universally affordable.

12.3 Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of neurodegenerative disease. Loss of memory—amnesia—and consistent decline in cognitive functions—dementia—over a period of up to 20 years are the primary clinical symptoms for AD. The progressive degeneration of the cerebral cortex and hippocampus region leads to loss of cholinergic neurons resulting in the manifestation of AD. Postmortem brain autopsy that shows the presence of neurofibrillary tangles and/or amyloid plaques positive for phosphorylated tau protein and amyloid β (Aβ), respectively, confirms the diagnosis. AD pathology is broadly categorized as amyloid-β positive or negative ($A\beta^{+}/A\beta^{-}$) depending on the presence or absence of the Aβ deposits (Masters et al. 1985; Grundke-Iqbal et al. 1986; Braak et al. 2006). The clinical diagnosis, with 70–80% accuracy, based solely on the clinical symptoms—behavior and neuropathology—is poor and necessitates for better diagnosis to improve disease management and cure. An optimal diagnosis regimen should cover various stages of disease development and progression.

Molecular diagnosis is based on the molecular mechanisms of the disease. Since the main component of the AD amyloid plaques is the $A\beta_{1-42}$ form of amyloid-β along with tau protein (Miller et al. 1993; Masters et al. 1985; Grundke-Iqbal et al. 1986), the first ELISA-based diagnostic assays for AD measured the CSF level of total-tau, phosphorylated-tau, and $A\beta_{1-42}$. The diagnostic profile in these assays

included an increased level of tau proteins (total + phosphorylated) and decreased levels of $A\beta_{1-42.}$ (Andreasen et al. 1999; Vandermeeren et al. 1993; Vanmechelen et al. 2000). Reduced levels of CSF $A\beta_{1-42}$ correlate with increased $A\beta_{1-42}$ amyloid deposit in the brain, as corroborated by PET imaging (Fagan et al. 2006). A decrease in CSF $A\beta_{1-42}$ levels can be measured before amyloid aggregates can be imaged by PET, showing that the accumulation of $A\beta_{1-42}$ in the brain is a precursor for aggregation and subsequent clinical symptoms of dementia. The CSF ratio of $A\beta_{42}/A\beta_{4o}$, rather than $A\beta_{1-42}$ levels alone, normalizes the background variations and improves the specificity of the diagnosis as $A\beta_{40}$ levels do not change in AD (Palmqvist et al. 2016).

CSF levels of total and phosphorylated tau proteins also directly correlate with the progression of AD. The ratio of $tau_{total}/tau_{phosphorylated}$ is a reliable indicator of the severity of AD. Tau can be phosphorylated at multiple sites—81, 199, 231, 235, 396, 404—each of which can be used as a biomarker (Blennow and Zetterberg 2018). Many other proteins like neurogranin, neuron-specific enolase, visinin-like protein, and neurofilament light chain protein also show elevated levels in AD patients but their association with specific molecular pathology is poorly understood (Keshavan et al. 2017). Higher CSF levels of neurofilament light chain protein are detected in early clinical stages that show beginning of the brain atrophy in multiple regions and mild cognitive impairment (Zetterberg et al. 2016). Tau_{total} and neurogranin levels reflect advanced $A\beta^{+}$ clinical stage (Mattsson et al. 2016).

Altered $A\beta_{1-42}$ and neurofilament light chain protein levels are also associated with AD when measured in plasma samples. Long-term studies show that neurofilament light chain levels begin to increase up to 10 years before the onset of dementia symptoms; tau levels in plasma are however ambiguous. One of the reasons for the poor detection of tau proteins in the plasma is the likely degradation of the protein in the bloodstream (de Wolf et al. 2020). Changes in protein levels and biochemistry in blood complicate standardization of blood biomarkers, resulting in a paucity of blood biomarkers for AD.

In terms of invasiveness, urine biomarkers are the most promising ones due to safety and ease of sample collection. Unlike the CSF that is derived from the brain tissue, urine mostly contains secondary metabolites dominated by the biochemistry of visceral organs. Thus, to qualify as an AD biomarker, AD pathology must be corelated with the metabolic function at a systemic level. Sucrose levels and catabolic products of spermidine (N-acetylisoputreanine-γ-lactam) are higher in patients with AD, suggesting changes in glucose and polyamine metabolism (Kurbatova et al. 2020). Recent high-throughput genomic, proteomic, and metabolomic studies report dysregulation of multiple proteins and RNA. However, direct evidence of these changes and their role in AD is lacking.

Neuroimaging of suspect AD patients is a noninvasive, highly informative screening technique. It allows to study the structural features of the brain like gray and white matter volume, ventricular volume, etc. by MRI. PET and SPECT imaging enables probing biochemical functions by using specific radiotracers. MRI scans are commonly used to detect changes in the brain structures of AD patients. AD brains typically show gray matter atrophy in regions of median temporal lobe, insula, and

temporoparietal cortices along with the increase of ventricular volume and reduction in total brain tissue volume (Busatto et al. 2008). The atrophy begins in the median temporal lobe and extends to the temporal neocortex and then to the parietal and frontal lobes. Atrophy in the median temporal lobe coincides with mild cognitive impairment as a clinical symptom, which often develops into typical AD in the following years. Thus, an MRI scan is a robust imaging biomarker for presymptomatic and early-stage AD patients (Whitwell et al. 2007).

Functional brain imaging with PET scans for glucose metabolism (^{18}F-fluorodeoxyglucose) and SPECT scans for blood flow imaging in AD patients shows hypometabolic profile and reduced blood flow in the temporoparietal cortex. Impaired temporoparietal function in clinical cases with mild cognitive impairment develops into typical AD in time (Schroeter et al. 2009). Although functional brain imaging for one or more brain functions is predictive of the high risk to develop AD, it should be complemented with approaches like MRI/CT scans that assess the whole brain structure.

Amyloid deposits in the brain tissue of AD patients can be imaged by Aβ-specific ligands like ^{11}C-PiB, ^{11}C6-OH-BTA-1 (derived from amyloid binding dye thioflavin-T), and tau-specific ligands like ^{18}F-MK-6240, using amyloid PET (Mathis et al. 2003; Marquez and Yassa 2019). The presence of Aβ amyloids however does not correlate with a decline in cognition or AD pathology. Various amyloid imaging experiments show that amyloid deposits appear and increase with age; they may or may not be associated with dementia or AD (Marquez and Yassa 2019).

12.4 Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. Like AD, the definitive diagnosis of PD is also made by brain autopsy of the suspected patients. The clinical symptoms of PD include both motor and nonmotor disorders. The motor disorders affect the normal range and strength of muscle movements like bradykinesia, rigidity, tremors, and unstable posture. These symptoms are caused by lack of dopamine due to loss of dopaminergic neurons in substantia nigra pars compacta. Many of the motor symptoms can be ameliorated by levodopa supplements. The nonmotor disorders include impaired brain function like sleep problems, depression, dementia, and fatigue. These symptoms are caused by the loss of a heterogeneous group of neurotransmitters and do not respond to levodopa; this form of PD is called atypical Parkinsonian syndrome (Kalia and Lang 2015). The presence and magnitude of all these symptoms are highly variable in suspected patients, leading to ambiguity in clinical diagnosis based solely on the symptoms. Moreover, symptoms of PD do not manifest until the loss of 70–80% substantia nigra neurons (Schapira 1999). Tissue analysis, postmortem, in PD patients show the presence of Lewy bodies and Lewy neurites accompanied by loss of substantia nigra neurons (that produce dopamine). The main component of

Lewy bodies and neurites is α-synuclein protein in the amyloid form. Familial forms of PD are caused by mutations in a number of genes: α-synuclein, PARKIN, LRRK2, DJ-1, etc. Screening for mutations in these genes, using PCR-based techniques, in individuals with familial history of PD provides risk assessment and prognostic evaluation.

α-Synuclein (α-Syn) aggregates are central to various forms of dementia—Parkinson's disease, multiple system atrophy, dementia with Lewy bodies, Lewy body variant of AD, and neurodegeneration with brain iron accumulation—which are characterized by the presence of α-Syn aggregates; these conditions are collectively called α-synucleopathies. Detection of α-Syn in CSF, blood, and other body fluids, as a biomarker, is thus the most obvious choice for synuclein-associated pathologies. However, a biomarker should be able to distinguish PD from other synucleopathies, as the etiology, prognosis, and therapy for each condition are typical. In general, total α-Syn levels in PD patients' CSF are lower than in control samples. However, the range of total α-Syn concentration varies considerably; this limits its use as a diagnostic biomarker. Two main sources of variation include contamination from blood (erythrocytes have a high level of α-Syn) and heterogeneity of stage, age, and genetic background of patients enrolled in trials (Mollenhauer et al. 2016; Barbour et al. 2008). Contamination from erythrocyte α-Syn is also a problem when measuring blood α-Syn levels for diagnostic purposes. Some forms of neurodegeneration like progressive supranuclear palsy, caused by extensive neuron damage in the brain stem, show increased levels of CSF α-Syn. In cases with an overlap of synucleopathies and progressive supranuclear palsy (a form of tauopathy), the total CSF α-Syn levels can be normal due to a decrease caused by synucleopathies and an increase caused by progressive supranuclear palsy. Overall, the heterogeneity between synucleopathies, other forms of neurodegeneration and contamination from erythrocytes, renders total CSF α-Syn levels a nonselective biomarker for PD (Parnetti et al. 2019).

Lewy body-associated α-Syn pathology proceeds through the aggregation of monomeric α-Syn to oligomers and fibrils. The oligomeric form of α-Syn is elevated in CSF and blood of PD patients; however, the diagnostic accuracy of the assays used and heterogeneity observed is not fit for clinical use as yet (Eusebi et al. 2017). Modified forms of α-Syn monomers/oligomers—phosphorylated, ubiquitinated, nitrated, oxidized, and truncated—are under investigation for use as a specific and selective biomarker for PD (Schmid et al. 2013). Aggregation of α-Syn depends on the α-Syn concentration; thus, enzymes in cellular pathways that regulate the degradation of α-Syn—autophagy-mediated lysosomal degradation—are also potential biomarkers for PD. A combined reduction in β-glucocerebrosidase and β-hexosaminidase activity in CSF shows a good correlation with PD but lacks accuracy for clinical use (Balducci et al. 2007).

Structural MRI allows detection of dopaminergic lesions and brain atrophy in PD patients that corroborate the clinical symptoms for PD. Functional MRI based on PD-specific patterns of low-frequency fluctuations in the striatum, supplementary motor neurons, frontal gyrus, and occipital cortex can distinguish PD patients from healthy subjects with high accuracy (Wu et al. 2015). A combination approach of

brain MRI, ^{123}I-mIBG SPECT, and ^{18}F-FDG PET enables differential diagnosis of PD and atypical PD. PET scan alone can identify several PD subtypes (Pagano et al. 2016). Although highly specific and informative, none of the neuroimaging modalities are used in the clinic as a routine diagnostic method as yet.

12.5 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease caused by progressive degeneration of spinal cord neurons that results in atrophy of the lower and upper motor neuron function. About 10% of ALS cases are inherited and are called familial ALS, while about 90% are sporadic in etiology. Clinical symptoms of ALS include progressive muscle weakness, spasticity, and twitching that progresses to dysphagia, dysarthria, dyspnea, and limb paralysis due to the death of motor neurons (amyotrophy). The average life expectancy after the onset of symptoms is 2–5 years, and death occurs mainly due to respiratory failure. ALS is a heterogeneous disorder with symptoms overlapping with other neurodegenerative diseases like frontotemporal dementia (FTD) and frontotemporal lobar degeneration (FTLD). Neurodegeneration and neuroinflammation are the molecular hallmarks of ALS. The most common genes (familial form) and proteins (sporadic form) associated with ALS are TDP-43 (Tar DNA binding protein of 43 kDa), FUS (fused in sarcoma), C9ORF72 (chromosome 9 open reading frame 72), and SOD1 (superoxide dismutase 1). In ALS and FTLD, loss of neuronal function is accompanied by the presence of cytoplasmic aggregates of FUS, SOD1, dipeptide repeats, and TDP-43 protein (Ustyantseva et al. 2020).

The earliest symptoms of ALS include weight loss, respiratory insufficiency, and muscle stiffness. Thus, the earliest biomarkers of ALS include monitoring weight loss that is accompanied by a decline in respiratory function. Sniff nasal inspiratory pressure (SNIP) is a volitional noninvasive test that measures inspiratory muscle strength. SNIP performance declines with motor neuron degeneration (Heritier et al. 1994). SNIP is recommended as a predictive biomarker in ALS patients that may need noninvasive ventilation within 3 months (Tilanus et al. 2017). Respiration is controlled by phrenic nerve stimulation; tests for phrenic nerve stimulation, though invasive, also acts as a biomarker for respiratory function. Phrenic nerve conduction studies (PNCSs) measure the diaphragm action potential that allows measuring the nerve function. In ALS patients, PNCSs show increased latency in the nerve stimulation that correlates with weak muscles and poor respiratory function (Evangelista et al. 1995). Twitch trans-diaphragmatic pressure (Tw Pdi) measures inspiratory muscle strength in a nonvolitional manner. Tw Pdi shows a linear decline with the progression of ALS and thus has predictive potential for mortality stratification (Polkey et al. 2017). Both PNCSs and Tw Pdi are performed by electrophysiologists in a clinical setup. Therefore, phrenic nerve tests are slightly invasive and need trained personnel and dedicated infrastructure.

Specific and reliable CSF-blood biomarkers for ALS are not known. Some biomarkers like levels of neurofilament heavy/light chain proteins and tau proteins are elevated in CSF from ALS patients, but these are indicative of extensive axon damage and neuron degeneration, and not ALS specifically. Moreover, their levels plateau out after significant neuron damage. TDP-43 levels in CSF and blood are reported to change, but more evidence is required to classify it as an ALS biomarker (Verber et al. 2019). SOD1, one of the key proteins associated with familial and sporadic ALS, maintains the redox homeostasis in neurons. Loss of function of SOD1 in ALS increases the concentration of biomarkers for oxidative stress in various body fluids, e.g., 8-hydroxy-2′-deoxyguanosine and 3-nitrotyrosine in CSF, 4-hydroxy-2,3-nonenal in serum and CSF, and 8-oxodeoxyguanosine and ^{15}F(2 t)-isoprostane in urine (Verber et al. 2019). However, oxidative stress biomarkers need further investigation and protocol optimization for clinical use.

One of the proposed mechanisms for the spread of ALS is through the extracellular vesicles (microvesicles and exosomes) that carry misfolded proteins to neighboring neurons and induce a prion-like propagation of the disease. ALS-associated proteins, TDP-43, FUS, and SOD1, are found in extracellular vesicles isolated from blood as well as CSF; however, there is no consensus, as yet, about their optimization as a biomarker either for diagnosis or disease progression (Sproviero et al. 2018; Feneberg et al. 2014; Gagliardi et al. 2021).

Key ALS-associated proteins, FUS, TDP-43, and C9ORF72, are RNA binding proteins. FUS and TDP-43 are involved in miRNA biogenesis; thus, loss of function in these proteins is expected to reflect in RNA metabolism. Consequently, RNA levels in the extracellular vesicles in CSF as well as in the blood of ALS patients also change (Kawahara and Mieda-Sato 2012; Morlando et al. 2012). Panels of upregulated and downregulated miRNAs are promising biomarkers, but more research is needed to find optimal candidates.

MRI imaging in ALS patients shows brain atrophy that is not limited to the motor cortex. Reduction in brain volume in ALS is not a reliable prognostic or diagnostic biomarker for ALS; it is mainly used in the clinic to exclude conditions that mimic ALS symptoms (Filippi et al. 2010). Diffusion tensor imaging (DTI), a type of MRI for visualization of white matter, shows loss of white matter in the early stages of ALS. DTI is recommended as a diagnostic biomarker for relatives of familial ALS patients to detect early damage in upper motor neurons (Filippi et al. 2010). PET imaging using ligands like ^{11}C-flumazenil, ^{11}C-WAY 100635, ^{11}C(R)-PK11195, and ^{11}C(l)-deprenyl correlate changes in neural biochemistry with symptoms broadly observed in motor-neuron diseases, but their specificity to ALS is not proven. Thus, more specific ligands for ALS need to be developed for use as ALS biomarkers (Filippi et al. 2010).

12.6 Huntington's Disease

Huntington's disease (HD) is a progressive neurodegenerative disease that is mainly inherited in an autosomal dominant manner. The typical age of onset in adults is 30–40 years. The clinical symptoms of HD include poor motor control, characterized by involuntary movements (chorea), dystonia, decline in cognition, and psychiatric imbalance. At the cellular level, the symptoms originate from the progressive cell death in the striatum, i.e., atrophy of caudate nucleus, putamen, and globus pallidus. The cause for cell death is not precisely understood but is suggested to stem from dysregulated cellular proteostasis that includes synaptic dysfunction, mitochondrial toxicity, and hampered axonal transport. At the molecular level, HD is caused by an expansion in the trinucleotide repeat—CAG—of the huntingtin protein (Htt). An increase in the number of CAG repeats from 36–39 (nonpathological) to >40 leads to pathogenesis. The mutant Htt, with expanded repeats, aggregates into amyloids resulting in cytoplasmic and nuclear Htt-positive inclusion bodies, which are the pathological hallmark of HD (McColgan and Tabrizi 2018; Arrasate and Finkbeiner 2012).

Autosomal dominant nature of HD and monogenic etiology makes genetic screening for CAG expansion in the HTT gene the most robust prognostic biomarker in individuals with a family history of HD. Polymerase chain reaction-based techniques are standard methods to identify and characterize the CAG repeat length and to predict the severity and age of onset in suspected individuals. Mutant Htt protein is detectable in CSF and can be measured in femtomolar concentrations (Wild et al. 2015). The increase in mutant Htt coincides with an increase in CSF tau and neurofilament proteins, suggesting that the source of mutant Htt is damaged neurons. Therefore, mutant Htt can be a diagnostic marker for HD but is not as yet able to delineate the HD progression and prognosis. Mutant Htt levels for HD specific pathology and neurofilament level for global neuron damage are in clinical trials for validation and endpoint use in the clinic (Wild et al. 2015).

Volumetric MRI scans of the striatum are used as biomarkers in the clinic to map the progress of HD. Screening in susceptible individuals shows atrophy in various striatal structures 15–20 years before the onset of clinical symptoms. Changes in gray and white matter in specific areas or at the whole-brain level provide individual-specific changes in the brain structure as HD progresses. Diffusion tensor imaging reports the neuron damage prior to cell death that leads to volume reduction. Thus, MRI is routinely used to diagnose and follow HD progression, but it has little prognostic value (Rodrigues et al. 2018).

PET scans with specific radiotracer ligands are used to measure changes in the dopaminergic neurons (control muscle movements) by ^{11}C-β-CIT and ^{11}C-DTBZ ligands for the presynaptic dopaminergic system and ^{11}C-SCH22390, ^{11}C-raclopride, and ^{11}C-FLB457 ligands for the postsynaptic dopaminergic system. The pre- and postsynaptic dopaminergic systems include the D1 type and D2 type dopamine receptors in the substantia nigra pars compacta and globus pallidus. HD progression correlates with a decrease in D1 and D2 receptor activity (Wilson et al.

2017). Glucose metabolism and blood flow in the brain regions of HD patients are measured using ^{18}F-FDG and ^{15}O-H_2O labeled tracers, respectively. Reduced glucose metabolism in the striatum and cortex is associated with a decline in motor and cognitive functions (Kuwert et al. 1990). Neuroinflammation in HD brains via microglia activation in early HD stages can be measured using ^{11}C-PK11195 as a ligand, which binds to the activated microglial surface protein called translocator protein. Pro-survival cell-signaling via cyclic monophosphate signaling can be measured by ligands like ^{18}F-JNJ42258152 and ^{18}F-MNI-659 against phosphodiesterase 10A enzyme. However, these biomarkers for neuroinflammation and pro-survival signaling need more optimization to be used in clinics (Wilson et al. 2017).

12.7 Future of Biomarkers

Numerous studies show increased levels of tau, neurofibrillary proteins, and inflammatory molecules in the CSF of patients with AD, PD, ALS, and HD. This common CSF profile is indicative of the neuron damage that occurs with the progression of neurodegenerative diseases. A critical mass of neurons has already died before these biomarkers can be detected in the CSF and blood above the baseline, suggesting that they do not reflect early molecular and cellular changes at the onset of the disease. Moreover, with the present biomarkers, the sub-types of the four main neurodegenerative diseases cannot be reliably distinguished. MRI and PET-based neuroimaging provide information on both specific and global changes in the brain for each type of neurodegenerative disease, but they are poor in disease prognosis and have little predictive value.

With high-throughput techniques like the whole-genome sequencing, the whole exome sequencing, RNA sequencing, liquid and gas chromatography coupled to mass spectrometry, etc. becoming more affordable, the emphasis for biomarker discovery is shifting from a single biomarker to a panel of biomarkers that reflect changes not only in the brain but also in various other organs—from a reductionist to a systems biology approach. Biomarker discovery using genomics, proteomics, metabolomics, and lipidomics to find markers for genetic polymorphism, oxidative stress, neuroinflammation, DNA methylation, and neurofilament proteins will allow one to screen, predict the risk, and stratify the population; diagnose the disease onset in the early stages; and follow the progression of the disease and response to therapy at a systemic level. Regulatory miRNAs are promising biomolecules to probe cellular homeostasis. Numerous reports suggest upregulation and downregulation of specific groups of miRNAs in given neurodegenerative disease. However, the lab protocols to identify and measure these biomarkers are not standardized, as yet, so the results are not always reproducible. Moreover, the patient cohort in various studies represents different age groups, stages of the disease, and ethnic background that together makes direct comparison of results challenging.

12.8 Conclusion

Neurodegenerative diseases are a major concern in an aging world, primarily due to the absence of treatment options. Diagnosis, prognosis, and pharmacological intervention in neurodegenerative diseases are hampered due to the lack of optimal biomarkers. The biggest challenges in finding specific and sensitive biomarkers for neurodegenerative diseases are the heterogeneity of the clinical symptoms and the substantial overlap of symptoms between various conditions. Since adult-onset neurodegenerative diseases develop over decades with irreversible cellular damage, predictive and diagnostic biomarker are needed to reduce their burden in the coming years. For neurodegenerative diseases, especially, longitudinal studies with big sample size, standard protocols, age, and stage mapping are critical to characterize molecular and cellular hallmarks for disease risk, onset, progression, and response to therapy, as each of these stages may last for years and can be modified by lifestyle choices.

Conflict of Interest The authors declare no conflict of interest.

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