|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Name of the BBMs** | | **Underlying**  **Pathophysiology** | **Current Categorization of BBBMs (NIA-AA'2024)** | **Relevance in AD** | **Trend of the biomarker in Plasma** | **Assessment techniques** |
| **Amyloid β** | Aβ42 | Plasma proteinopathy- Amyloidogenic | Core 1 biomarker  **(A)** | Early detection of AD in people without symptoms  - progression from normal cognition to MCI or AD | Decreased in AD and MCI in comparison to controls | 1: ELISA  2: Luminex xMAP Technology  3: SIMOA  4: LCMS  5: Immunoprecipitation Mass Spectrometry |
| Aβ40 | Plasma proteinopathy- Amyloidogenic | Core 1 biomarker  **(A)** | Early detection of AD in people without symptoms  - progression from normal cognition to MCI or AD | Decreased in AD and MCI in comparison to controls |
| Aβ42/40 | Plasma proteinopathy- Amyloidogenic | Core 1 biomarker  **(A)** | Identify early stages of AD and predict cognitive decline in concordance with CSF and neuroimaging biomarkers. | Decreased Aβ42/Aβ40 Ratio in AD and MCI compared to control |
| **Tau** | p-tau217 | Plasma proteinopathy-Tauopathy phosphorylated and secreted AD tau | Core 1 biomarker  **(T1)** | Early detection of AD in people without symptoms  -Accurately predict the progression of individuals from subjective cognitive decline (SCD) and MCI to  dementia when combined with other risk factors. | Increased in AD and MCI in compared to controls | 1: ELISA  2: Luminex xMAP Technology  3: SIMOA  4: LCMS  4: Immunoprecipitation Mass Spectrometry |
| p-tau181 | Plasma proteinopathy-Tauopathy phosphorylated and secreted AD tau | Core 1 biomarker  **(T1)** | Early detection of AD in people without symptoms -Distinguishes  between Aβ-PET (+) and Aβ-PET (-) individuals, along with the disease progression to dementia and tau-burdened brain areas with AD-related atrophic changes. | Increased in AD and MCI compared to controls |
| p-tau 231 | Plasma proteinopathy-Tauopathy phosphorylated and secreted AD tau | Core 1 biomarker  **(T1)** | -Early detection of AD in people without symptom  -Discriminates  patients with and without AD pathology during post-mortem assessment | Increased in AD and MCI compared to controls |
| MTBR-tau243 | Plasma proteinopathy-Tauopathy  AD tau proteinopathy | Core 2 biomarker  **(T2)** | Elevated in later stages of AD  Staging of biological disease severity along with Core 1 biomarker  -strongly associated with tau-PET and disease progression. | Increased in AD and MCI compared to controls |
| Non-phosphorylated mid-region tau fragments | Plasma proteinopathy-Tauopathy  AD tau proteinopathy | Core 2 biomarker  **(T2)** | Elevated in later stages of AD  -staging of biological disease severity along with Core 1 biomarker | Increased in AD and MCI compared to controls |
| **α-synuclein (αSyn)** | αSyn/tau | Proteinopathy-related biomarkers of non-core AD pathology Synuclein pathology | Biomarkers of non-AD co-pathology  **(S)** | Total α-synuclein levels in the blood may not  differ significantly between patients with neurodegenerative diseases. The oligomeric or phosphorylated form of α-synuclein accelerates cognitive dysfunction. | Decreased in AD and MCI compared to controls | 1: Seed Amplification Assays:  -Protein Misfolding Cyclic Amplification (PMCA)  -Real-Time Quaking-Induced Conversion (RT-QuIC)  2: ELISA and Western blotting  3: Quantitative Mass Spectrometry  4: Luminex xMAP Technology  5: SPR- DLS  6: Immuno-PCR |
| αSyn /Aβ 42 | Biomarkers of non-AD co-pathology |  | Increased in AD and MCI compared to controls |
| **DKK-1 or Dickkopf-1** | | Proteinopathy-related biomarkers of non-core AD pathology. | Research Biomarker | Elevated levels correlate with disease severity, particularly  cognitive decline and synaptic loss and help differentiate AD from other neurodegenerative conditions | Increased in AD | 1**:** ELISA and Western blotting  2: Luminex xMAP Technology  3: Immuno-PCR  4: Mass Spectrometry |
| **VILIP-1** | | Proteinopathy-related biomarkers of non-core AD pathology. | Research Biomarker | Increased levels are seen in AD, but there is no significant difference in concentrations with AD-MCI patients  and other neurodegenerative groups. | Increased in AD | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3. Immuno-PCR  4. Mass Spectrometry |
| **Plasma Neurofilaments (NfL)** | | Injury, dysfunction, or degeneration of neuropil | Biomarkers of non-specific processes involved in AD pathophysiology  **(N)** | Increased levels in Aβ-positive patients with AD and MCI are associated with the degree of cognitive impairment as well as used as monitoring biomarkers to indicate the severity of neurodegeneration. | Increased in AD and MCI vs controls | 1. ELISA (Enzyme-Linked Immunosorbent Assay)  2. Luminex xMAP Technology  3. ECLIA  4. Mass Spectrometry  5. SIMOA |
| **SNAP-25** | | Neuronal and synaptic injury-pre synaptic dysfunction | Biomarkers of non-specific processes involved in AD pathophysiology  **(N)** | CSF concentrations can distinguish between various neurodegenerative diseases like AD, PD, and ALS | Decreased in AD compared to controls | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **Neuronal pentraxin 2 (NPTX-2)** | | Neuronal and synaptic injury-pre synaptic dysfunction | Biomarkers of non-specific processes involved in AD pathophysiology  **(N)** | Potential as a probable biomarker for early detection of AD. | Decreased in AD compared to control | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **Growth-associated protein (GAP-43)** | | Neuronal and synaptic injury-pre synaptic dysfunction | Biomarkers of non-specific processes involved in AD pathophysiology  **(N)** | Potential as a probable biomarker for early detection of AD. | Increased in AD compared to control | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **Neurogranin (NG)** | | Neuronal and synaptic injury- post-synaptic protein dysfunction | Biomarkers of non-specific processes involved in AD pathophysiology  **(N)** | Potential as a probable biomarker for early detection of AD. | Decreased in AD compared to control | 1. ELISA  2. Luminex xMAP Technology  3. ECLIA  4. Mass Spectrometry  5. SIMOA |
| **Fms-like tyrosine kinase-1 (Flt-1)** | | Vascular Damages related to AD | Research Biomarker  **(V)** | Assess total vascular involvement and early detection of vascular changes  associated with AD | Increased in AD compared to control | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **Endothelin 1 (ET-1)** | | Vascular Damages related to AD | Research Biomarker  **(V)** | Indicates vascular impairment in AD | Increased in AD compared to AD | 1. ELISA (  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **Atrial natriuretic peptide (ANP)** | | Vascular Damage related to AD | Research Biomarker  **(V)** | Causes reduced cerebral blood flowand impairment of Neurovascular health  . | Increased in AD compared to control | 1. ELISA  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **MIG/CXCL-9** | | Vascular Damage related to AD | Research Biomarker  **(V)** | Indicate ongoing chronic neuroinflammatory processes | Increased in AD compared to control | 1. ELISA  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **heart-type fatty acid binding protein (H-FABP)** | | Vascular Damage related to AD | Research Biomarker  **(V)** | Potential as a probable biomarker for early detection of AD as it was found to be elevated in the pre-clinical phase of AD dementia | Increased in AD compared to control | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **Vascular adhesion molecular** | sVCAM-1 | Vascular Damage related to AD | Research Biomarker  **(V)** | Indicate the burden of atherosclerosis in AD with elevated sVCAM, indicating a significant correlation between age and the severity of  cognitive decline | Increased in AD compared to control | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| sICAM-1 | Vascular Damage related to AD | Research Biomarker  **(V)** | Indicate the burden of atherosclerosis in AD with elevated sICAM | Increased in AD compared to control | 1. ELISA and Western blotting  2. Luminex xMAP Technology  3.Immuno-PCR  4. Mass Spectrometry |
| **Metabolic products secondary to lipid peroxidation (LPO)** | malondialdehyde (MDA) | Oxidative Stress | Research Biomarker | Increased levels in familial AD that carry APP and presenilin-1 gene mutations | Increased in AD compared to control | 1. HPLC  2. LC-MS  3. ELISA  4. GC-MS |
| 4-hydroxynonenal (4-HNE) | Oxidative Stress | Research Biomarker | Increased levels in familial AD that carry APP and presenilin-1 gene mutations | Increased in AD compared to control |
| increased F2-isoprostanes (F2-IsoPs) | Oxidative Stress | Research Biomarker | potential marker of oxidative stress during the MCI phase of AD, and its quantity correlates with the disease continuum from SCD to MCI to AD | Increased in AD compared to control |
| **Free radicals** | ROS | Oxidative Damage | Research Biomarker | ROS modifies neuronal macromolecules and induces τ protein hyperphosphorylation during prodromal AD phases. | Increased in AD | 1. DCFDA  2. Electron Spin Resonance (ESR) Spectroscopy  3.Nitroblue Tetrazolium (NBT) Assay  4. Flow Cytometry with ROS-sensitive dyes |
| RNS | Oxidative damage | Research Biomarker | Nitrosylation of critical proteins in neurons impairs their function, promoting neurodegenerative processes. | Increased in AD | 1. Nitrotyrosine ELISA 2. Electron Spin Resonance (ESR) Spectroscopy 3. Western Blot for 3-Nitrotyrosine-modified Proteins |
| **Nucleoside 8-hydroxyguanosine (8-OHG)** | | Oxidative Damage | Research Biomarker | It is notable for determining the gradient of DNA oxidative damage in AD patients. It allows us to determine oxidative damage to plasma DNA early in AD. | Increased in lymphocytes of AD patients compared to control | 1. ELISA  2. HPLC and Electrochemical detection  3. LC-MS  4. Western Blot using specific anti-5.8-OHG antibodies  6. Immunoprecipitation  7. GC-MS |
| **Mitochondrial respiratory complex I-V genes (OxPHOS genes)** | | Bioenergetic abnormality | Research Biomarker | An imbalance in nuclear and mitochondrial genome-encoded OXPHOS transcripts may cause a negative feedback loop that lowers mitochondrial translation and compromises OXPHOS efficiency. This would likely result in the generation of harmful reactive oxygen species. | Reduced expression in early AD patients | 1. qPCR 2. Western Blot 3. IHC 4. Blue Native Gel Electrophoresis |
| **SNO-Drp1** | | Bioenergetic abnormality | Research Biomarker | SNO-Drp1 can result in increased mitochondrial fission, loss of synapses, and neuronal damage in mice models and primary neuronal culture, as well as in post-mortem tissue. | Increased in peripheral blood lymphocytes in AD patients. There are also contradictory findings that SNO-Drp1 does not differ significantly in AD compared to controls. | 1. Biotin Switch Assay 2. Mass Spectrometry 3. Nitroso-Proteome Profiling 4. Immunoprecipitation and Western blot |
| **mitochondrial DNA (mt-DNA)** | | Bioenergetic abnormality | Research Biomarker | mtDNA copy number acts as an indirect indicator for several functioning mitochondria and thus provides info regarding bioenergetics as a factor for AD progression. | Decreased in patients with AD | 1. qPCR 2. Digital droplet PCR 3. Southern Blotting |
| **8oxoG sSNVs** | | Bioenergetic abnormality | Research Biomarker | due to their inflammatory endophenotype, the circulating cf-mtDNA (ccf-mtDNA) 8oxoG variant can be used as an improved biomarker. | Increased in AD patients | 1. 8-oxoG DNA Glycosylase (OGG1) Assay 2. Comet Assay with Fpg (Formamidopyrimidine-DNA Glycosylase) 3. ELISA 4. HPLC with electrochemical detection |
| **circulating cf-mtDNA (ccf-mtDNA)** | | Bioenergetic abnormality | Research Biomarker | cellular mt-DNA copy number can be used as a potential biomarker of mitochondrial biogenesis and cellular energetics to reflect upon mitochondrial health in AD | Increased in AD patients | 1. qPCR 2. Digital droplet PCR 3. Southern Blotting |
| **The intermediate filament glial fibrillary acidic protein (GFAP)** | | Neuroinflammation and Immune Dysregulation | Research Biomarker  **(I)** | The marker of astrogliosis can be seen in chronic inflammatory processes like progressing AD. | Increases in AD patients | 1. ELISA 2. ECLIA 3. mesoscale discovery immunoassay V-PLEX |
| **CX3CL1 (Fractaline)** | | Neuroinflammation and Immune Dysregulation | Research Biomarker  **(I)** | significantly elevated in the plasma of MCI and AD compared to other neuroinflammatory disease processes. | Increases in AD and MCI | 1. ELISA 2. Western blot 3. IHC 4. Flow Cytometry 5. Luminex |
| **CCL23** | | Neuroinflammation and Immune Dysregulation | Research Biomarker  **(I)** | Their plasma concentration has also been found to have a predictive value toward MCI-to-AD progression | Increases in AD | 1. ELISA 2. Western blot 3. IHC 4. Flow Cytometry 5. Luminex |
| **C-C chemokine ligands or RANTES** | | Neuroinflammation and Immune Dysregulation | Research Biomarker  **(I)** | Elevated in AD and correlate with the neuroinflammatory burden | Increases in AD | 1. ELISA 2. Western blot 3. IHC 4. Flow Cytometry   5. Luminex |
| **YKL-40** | | Neuroinflammation and Immune Dysregulation | Research Biomarker  **(I)** | Increasing expression of YKL-40 in astrocytes during neuroinflammatory changes has been observed. Plasma YKL-40 levels show a positive correlation with the results of the Sensitive Free and Cued Selective Reminding Test | Increases in AD | 1.ELISA  2. Western blot  3. IHC  4. Flow Cytometry  5. Luminex |
| **Progranulin** | | Neuroinflammation and Immune Dysregulation | Research Biomarker  **(I)** | Studies have revealed that the increased progranulin-expressing gene GRN is in the blood of MCI and AD patients. | Increases in AD | 1. ELISA  2. Western blot  3. IHC  4. Flow Cytometry  5. Luminex |
| **Triggering receptor expressed on myeloid cells 2 (TREM2)** | | Neuroinflammation and Immune Dysregulation | Research Biomarker  **(I)** | mRNA levels in peripheral mononuclear cells have been found to have the distinguishing ability between aMCI, AD, and healthy control individuals and to be dependent on the APOE genotype | Increases in AD | 1. ELISA  2. Western blot  3. IHC  4. Flow Cytometry  5. Luminex |
| **NDE** | P-S396-tau | Tauopathy | Research Biomarker | Can predict the development of AD up to 10 years before the clinical onset of sporadic AD | Increased in AD | Proteomic Analysis of the EV’s like  ELISA |
| p-tau 181 | Tauopathy | Research Biomarker | Can predict the development of AD up to 10 years before the clinical onset of sporadic AD | Increased in AD and MCI vs healthy controls | 1 ELISA  2. Ultra-sensitive inhouse SIMOA |
| Synaptotagmin | Synaptopathy | Research Biomarker | Its impairment leads to decreased neurotransmission, neuroplasticity, and long-term potentiation, thus hampering memory formation. | Reduced in AD | 1. ELISA 2. LC-MS 3. SIMOA |
| synaptophysin | Synaptic loss and dysfunction | Research Biomarker | Loss of proper functioning synapse leads to impaired signal transmission and, thus, cognitive impairment. | Reduced in AD | 1. ELISA 2. LC-MS 3. SIMOA |
| P-S312-IRS-1 | Neuroinflammation and Insulin Resistance | Research Biomarker | Its increment promotes insulin resistance, leading to progressive neurodegeneration. | Increased in AD vs. controls | 1. ELISA 2. LC-MS 3. SIMOA |
| P-panY-IRS-1 | Insulin resistance and Synaptic dysfunction | Research Biomarker | Its reduction promotes insulin resistance, leading to progressive neurodegeneration. | Downregulated in AD | 1. ELISA 2. LC-MS 3. SIMOA |
| N-(1-carboxymethyl)-L-lysine (CML) | ROS mediated damage | Research Biomarker | can differentiate early to moderate AD. | Downregulated in AD | 1. ELISA 2. LC-MS 3. SIMOA |
| **MDE** | | Tauopathy | Research Biomarker | When neurons absorb MDEs containing tau, it triggers further abnormal tau aggregation. | Increases in AD | 1. ELISA 2. LC-MS |
| **ADE** | | Neuroinflammation | Research Biomarker | Levels of various complement components (such as C1q, C3b, factor D, etc.) in plasma may serve as predictive biomarkers for the progression of mild cognitive impairment to AD. | Increases in AD | 1. ELISA 2. LC-MS |

**Abbreviations:** BBBM, Blood-Based Biomarkers; AD, Alzheimer’s Disease; ELISA, Enzyme-Linked Immunosorbent Assay; SIMOA, Single Molecular Array; LC-MS, Liquid Chromatography-Mass Spectroscopy; ECLIA, Electrochemiluminescence Immunoassay; IP-MS, Immunoprecipitation-Mass Spectroscopy; SPR, Surface Plasmon Resonance; DLS, Dynamic Light Scattering; ECLIA, Electrochemiluminescence Immunoassay; HPLC, High Performance Liquid Chromatography; GC-MS, Gas Chromatography-Mass Spectrometry; DCFDA, Dichloro-fluorescein Diacetate Assay; IHC, Immunohistochemistry; Immuno-PCR, Immuno-polymerised Chain Reaction; q-PCR, Quantitative polymerised Chain Reaction; V-PLEX, Validated Plasma exchange; NDE, Neuron Derived Exosome; ADE, Astrocyte Derived Exosome; MDE, Microglia Derived Exosome; HSPA1A, Heat Shocked Protein family A (70kDA)