A Liquid Biopsy for Neurodegenerative Diseases

Neurodegenerative diseases are complex, chronic debilitating diseases often with no known cause and uncertain pathology. Diagnosis is difficult, relying on a combination of behavioral symptoms, cognitive testing, functional imaging, and biomarker screening, most often collecting samples via lumbar puncture. Consequently, most diagnoses happen after symptoms occur.

Detecting disease before symptoms can have a profound scientific impact by enabling early intervention, providing greater detail on disease pathophysiology, and contributing to the development of new treatment strategies to abrogate disease progression. A great deal of research is underway to identify new, reliable, blood-based, or liquid biopsy biomarkers for neurodegenerative diseases. This has been challenging because of the blood-brain barrier and privileged nature of the central nervous system. Until recently, scientists believed the best source of fluid biomarkers was cerebrospinal fluid (CSF), the fluid surrounding the brain and spine, that contains measurable levels of the protein aggregates identified in many neurological diseases. However, the concentration and number of markers in CSF is limited and access requires lumbar puncture, a delicate, uncomfortable procedure.

Serum antibodies are ideal biomarkers because they are linked directly to disease, have been shown in cancer and autoimmune diseases to predate symptoms by years, are easily accessible and highly reliable. In neurodegenerative diseases, antibodies have been underexplored, partly because antibodies are too large to cross the blood brain barrier and the lack of clarity regarding B cell access to intracellular proteins known to contribute to neurodegenerative diseases. Evidence is accumulating, though, that antibodies and autoantibodies are prominent in many neurodegenerative diseases.

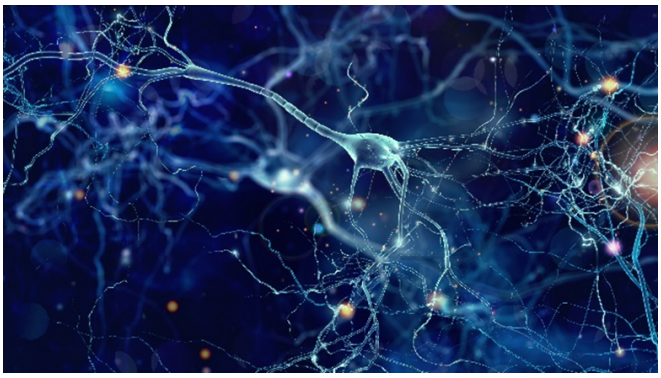
Over the last 15 years, scientists have discovered numerous cases of overlap between the humoral immune system and neurological disease, most notably with the discovery of an anti-NMDAR autoantibody in autoimmune encephalitis (*Pruss, 2021*). This has prompted many neuroscientists to examine the diagnostic, prognostic, and therapeutic potential of sera antibodies in neurodegenerative diseases. In Alzheimer's, for example, anti-nuclear autoantibodies are found in Alzheimer's patients with cognitive impairment, suggesting that blood circulating antibodies could be useful in detecting Alzheimer's (*Lopez et al., 1992*). In amyotrophic lateral sclerosis (ALS), the human endogenous retrovirus HML-2, a normally silent component of the human genome, activates near the onset of symptoms, and once activated, initiates production of antiviral autoantibodies. The autoantibody titers diminish as the disease progresses, implying a protective role for the anit-HML2 antibody (*Garcia-Montojo et al., 2022*). In this case, the HML-2 antibody has potential to both predict and provide guidance for the treatment of ALS.

Similar findings have been noted with the 43 kd transactive response DNA binding protein, TDP-43, known to form aggregates throughout the ALS brain. TDP-43 overexpression and protein assemblies result in antibody production found in Alzheimer's and ALS patient sera, encouraging researchers to study this protein as a biomarker for these and other neurodegenerative diseases. In sera from ALS patients, TDP-43 antibodies have been observed that may not only indicate the disease but may also be prognostic and protective. The research indicates that TDP-43 antibodies may hold greater diagnostic value than the TDP-43 protein levels. Antibodies were easier to collect, not requiring a spinal tap, and more consistently linked to diagnosis across patients (*Conti et al., 2021*).

In Parkinson's, diagnosis still relies heavily on motor symptoms, primarily bradykinesia, resting tremor and rigidity (*Li & Le, 2020*), however, research is ongoing to find fluid-based biomarkers that appear early and are clearly associated with Parkinson's. In fact, high-affinity anti-α-synuclein natural autoantibodies have been identified, and disappear as Parkinson's worsens (*Brudek et al., 2017*). Altogether, the common neurodegenerative disease associated protein aggregates stimulate antibody production.

Until recently, most examination of sera autoantibodies in neurodegenerative diseases focused on targeting autoimmune disease related autoantibodies or natural autoantibodies such as anti-TDP-43 antibodies. Knowledge that these proteins stimulate antibody titers measurable in sera samples has motivated researchers to engage high throughput technologies to search for additional novel markers. Protein micro array technology with machine learning is now being applied to find antibodies and antibody panels associated with disease for early detection and diagnosis. Antibody panels are more likely to detect disease and predict prognosis than single markers

while also uncovering potential disease related biochemical pathways. In one small pilot study of mild dementia, using the Sengenics i-Ome protein microarray composed of 1600+ antigens, six autoantibodies were identified in clinically diagnosed Alzheimer's patients compared with non-Alzheimer's dementia patients. The panel distinguished between the patient groups, and provided new pathways to investigate with potential to illuminate new facets of pathology and new therapeutic targets. For example, pantothenic acid kinase 3 (PANK3) antibodies in Alzheimer's patients. PANK3 is an enzyme involved in the production of Coenzyme A utilizing vitamin B5 as a precursor. This finding supports previous research connecting metabolic dysfunction to Alzheimer's and suggests diet could play a role in the disease pathology (*Wang et al., 2020*).



A major advantage of detection of serum autoantibodies over those found in CSF is the greater number and concentration of markers available in the specimen. In a larger screen of early stage Alzheimer's patients with mild cognitive impairment and low A 42 levels, 193 autoantibodies were found unique to the patients compared with healthy control subjects, the top 50 correlating with Alzheimer's disease at 99.6% accuracy. The panel discriminated among disease severities as well (*Demarshall et al., 2016*). Autoantibody profiling may also be more powerful for identifying and predicting disease than current markers. In a recent longitudinal study of Parkinson's patients, researchers followed the progress of at-risk patients with hyposmia with and without dopamine transporter deficit (DAT) who showed no other symptoms of Parkinson's. Overall, 116 autoantibodies were unique to hyposmia subjects. A subpanel of 22 autoantibodies in the hyposmia with DAT group correlated significantly with phenotype conversion (pheno-conversion) to Parkinson's over the 4-year observation period. The panel performed with 90.5% sensitivity and 70.6% specificity. This meant autoantibodies were able to discriminate among at risk patients and predict prognosis. In addition, pathway analysis revealed 56 new biomarkers not previously associated with Parkinson's related genes (*Anuar et al., 2022*).

The humoral immune system is an accessible component of human physiology that can be exploited for early detection of disease, making it the perfect liquid biopsy for neurodegenerative diseases. Immunoprofiling with antibodies can offer the benefit of early disease detection, stratification of patients by disease subtypes and new biochemical pathways to explore.

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