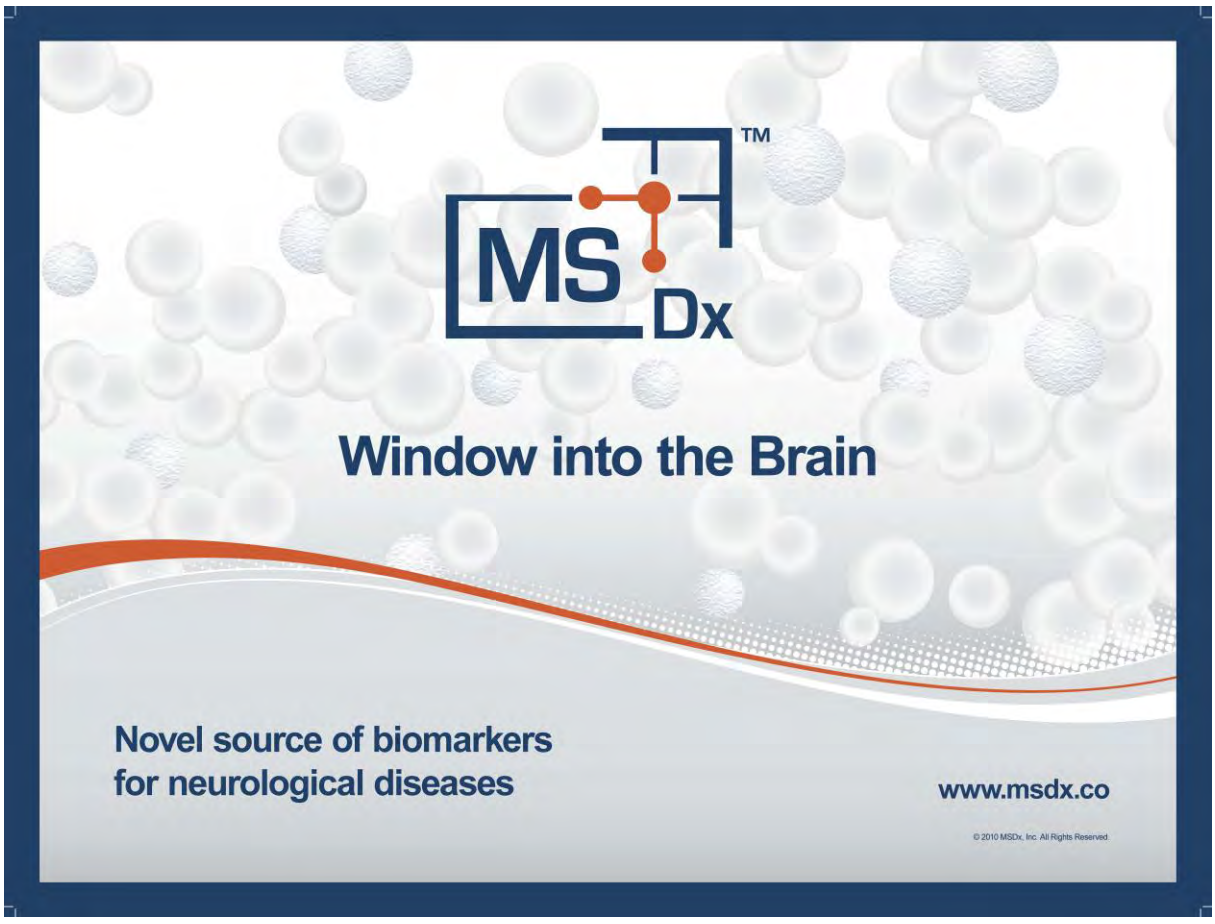
MEDICAL SOLUTIONS Dx

MSDx Biomarkers for CNS Disease

Ramesh C. Nayak, Ph.D.
Director of Research
MSDx, Inc.

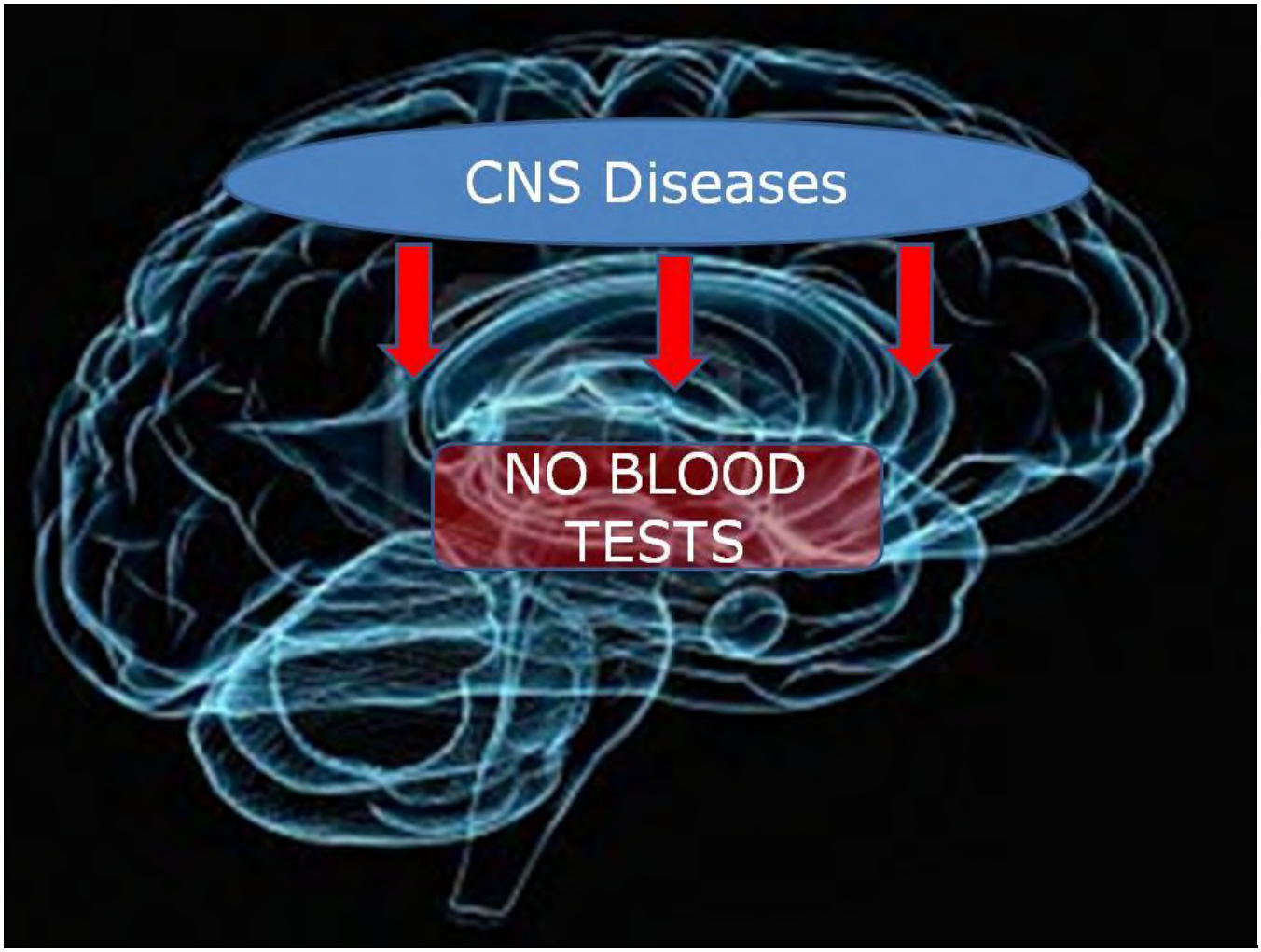


Obtaining Critical Data by Measuring Disease Processes

MEDICAL SOLUTIONS Dx

Blood Based Biochemical Monitoring of Major Diseases of the CNS

In the vast majority of diseases of the CNS there are no blood tests to aid in the diagnosis and long term management of patients. Consequently, there is a pressing clinical need for inexpensive blood tests that allow the physician to closely monitor the physiological status of patients with CNS disease/injury and their response to disease modifying therapies. To produce tests that provide critical and timely information requires biomarkers that are involved in the disease mechanism. That requires an understanding of how the disease process evolves over time so that key processes can be monitored. Diseases such as Alzheimer's disease, Parkinson's disease Multiple Sclerosis and the sequelae of mild (closed cranium) Traumatic Brain Injury (Sports concussion, military over pressure injury (bomb blast)) are the principle areas of need!



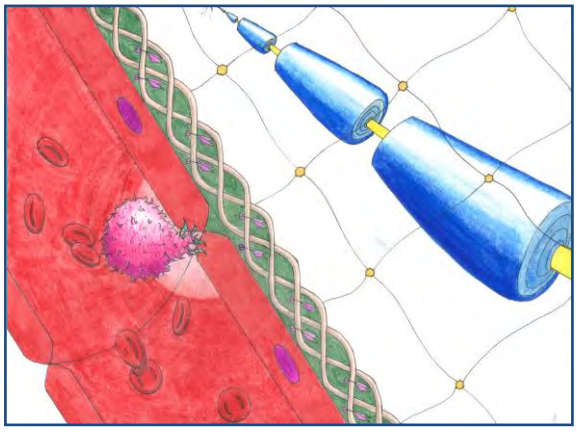
MEDICAL SOLUTIONS Dx

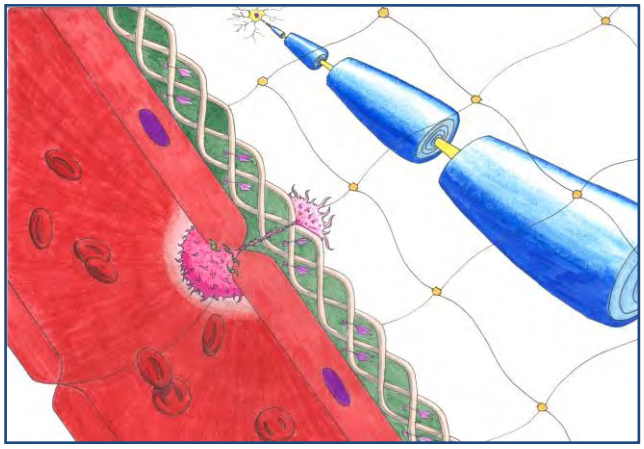
Exploiting Immunophysiology to retrieve information

When an organ or tissue is injured an inflammatory response is initiated to begin wound healing regardless of the cause of the injury. White blood cells are recruited to the site of injury to aid in repair and in the case of infection to kill and remove infectious agents, in cancer to kill and remove cancerous cells. In chronic diseases such as autoimmune diseases the initiating insult may have been eradicated but for reasons that are still not known the inflammatory response is not turned off and even escalates. In other chronic diseases such as atherosclerosis the insult is persistent over time (ie persistently elevated blood lipids). A common feature of inflammation in all of these scenarios is that phagocytic cells (monocytes/macrophages) are recruited to the site of damage and they engulf and clear the debris of damaged and dead cells. We discovered that some of these debris laden phagocytes re-enter the blood circulation. Consequently they can be removed by a simple blood draw and be examined for the type of debris present in the phagocytes.

The process by which white blood cells are recruited into an area of damage is complex. The following description is a simplification that allows an understanding of how the biomarkers we measure are generated and their relevance to monitoring disease activity.

Molecular signals (eg chemokines) generated at the site of injury attract white blood cells to the site of injury. To get to the site of injury, white blood cells travel in the blood and travel towards these signals (illustration 1). To get to the site of injury the white blood cells must cross the blood vessel wall. First they must pass through or between the endothelial cells of the blood vessel wall (diapedesis).

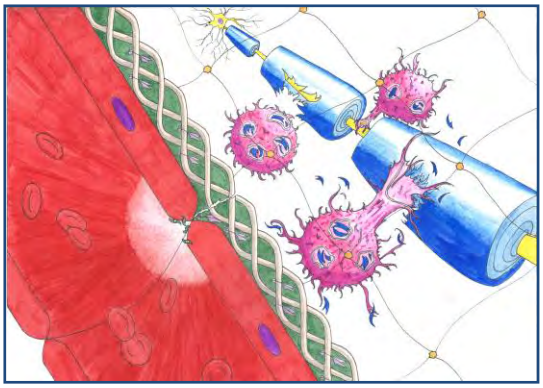




Once through the endothelium, they encounter the endothelial basement membrane. This may be considered to be like a chain link fence. It is a physical barrier that must be breached. The endothelial basement membrane is made of several proteins and is breached by the white blood cells by secretion of enzymes that cut these proteins (illustration 2).

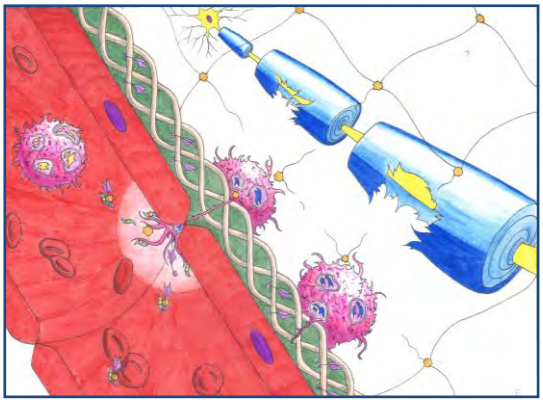
MEDICAL SOLUTIONS Dx

In breaching the basement membrane these proteins (fibronectin) are released and enter the blood circulation. Now the white blood cell has to move through the protein matrix that supports the cells of the brain, the extracellular matrix (ECM). Similarly, to move through the ECM enzymes that cut proteins are employed which release Fibulin-1from the matrix surrounding the nerves. Some Fibulin-1 is also released into the blood supply. We discovered that fibronectin and fibulin-1 bind to each other and to fibrinogen B in the blood forming extremely large protein complexes that we can measure. We call this complex MSDx complex-1 and have found that it is elevated in multiple sclerosis and Parkinson's disease and other neuroinflammatory conditions.

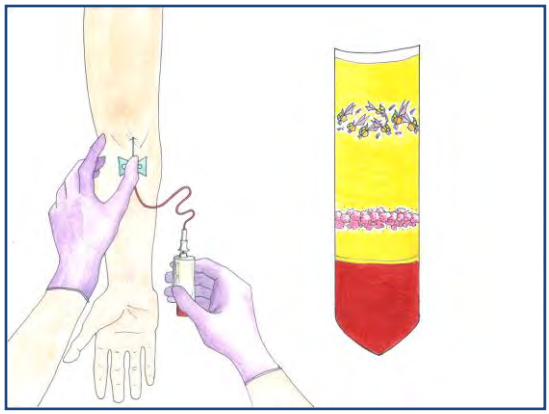


Having penetrated the ECM and reached the dead and dying nerves, the phagocytes proceed to engulf the debris and clear the site for repair (illustration 3).

These debris laden cells may stay in the brain or exit into the lymphatic system or the blood supply (illustration 4).



Because the job of the phagocytes is to degrade and recycle the debris, it quickly disappears. Also phagocytes in the blood are short lived. Consequently detection of brain specific debris in circulating phagocytes is an indicator of recent damage and may be usable as a marker of active neurodegeneration. It can be viewed as a kind of brain biopsy as we get information about what is happening in the brain. We call this approach ***Window into the Brain™***.



By doing a normal blood draw we can obtain both the white blood cells and the liquid part of the blood (plasma). The plasma contains MSDx complex-1 and the white blood cells contain the debris laden phagocytes. These can be measured in a lab. Measuring substances that would normally only be found in nerve cells or myelin cells in these blood phagocytes is a novel strategy for monitoring disease. The results may be useful for neurologists as an aid to monitor patients.

MEDICAL SOLUTIONS Dx

MSDx's initial panel of biomarkers to measure disease processes.

Leukocyte trafficking	**MSDx Complex-1:** Movement of white cells into and out of the brain	Inflammation/repair
Recirculating phagocyte	**PBMC - Myelin Basic Protein**	Active demyelination
Recirculating phagocyte	**PBMC – Tau**	Active neurodegeneration
Recirculating phagocyte	**PBMC - Hippocalcin 1 like 1**	Active neurodegeneration
Recirculating phagocyte	**PBMC – Neuromelanin**	Early Diagnosis of PD

Multiple Sclerosis:

Multiple Sclerosis is an autoimmune disease. In autoimmune diseases the immune system (for unknown reasons) attacks one or more organs of the body, the ensuing damage causes organ dysfunction and disease. In the case of multiple sclerosis the target is the central nervous system (brain and spinal cord). We selected multiple sclerosis (MS) as our first disease of interest for development of biomarkers as several disease modifying therapies are available for treating patients unlike the other major CNS diseases.

There is a genetic predisposition to developing MS which, not surprisingly, principally seems to reside with genes that control immune responsiveness namely the HLA genes. Genome wide association studies have also identified more than 100 genes with minor contributory effects. These are the same genes that have to be matched in organ transplantation to prevent immunological attack (rejection). This genetic predisposition in combination with environmental factors leads to an event which triggers the immune system and causes disease activity that leads to Multiple Sclerosis (multiple sclerosis literally means many scars).

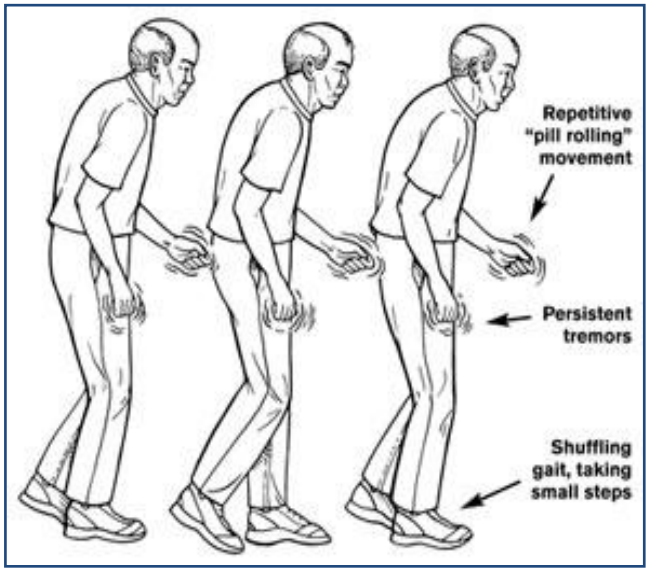
The processes involved in disease activity include autoimmune attack and inflammation (autoinflammation) which is regulated by specialized parts of the immune system (immunoregulation). When immunoregulation fails or is inadequate the disease activity is not held in check and the CNS is damaged leading to clinical signs of disease. Damage to the CNS includes damage to the myelin sheath that surrounds the nerve (demyelination) and damage to the nerve itself (neurodegeneration).

The first line disease modifying drugs for treatment of MS show some degree of efficacy in about 60% of patients treated. Consequently 40% of treated patients will accumulate neurological injury without any indication that the therapy is failing or failed until they experience a relapse or disease progression. Furthermore, these drugs do not completely suppress the disease activity in the patients that do respond. Some of the patients that respond initially experience secondary failure of disease modifying therapy seen as

breakthrough disease (relapse or progression while on therapy). Neurologists, however, have no biological tools to monitor patients on a regular basis to assess disease activity. Simple, inexpensive biological assays that monitor disease activity in the absence of symptoms holds the promise of allowing neurologists to intervene before enough damage has accumulated to show new lesions on MRI or cause neurological symptoms. Consequently, biomarkers that are involved in these processes will provide critical information to the neurologist for management of the disease. MSDx's biomarkers will enable monitoring treatment effects and disease activity with a simple blood test.

Parkinson's Disease:

Parkinson's disease (PD) is a neurodegenerative disorder that affects the substantia nigra and consequently causes motor impairments/symptoms. PD is diagnosed by clinical symptoms, most commonly motor symptoms (i.e. tremor). It is treated with dopamine replacement as the nerves that make dopamine are destroyed in PD. There is no interventional drug available for PD yet. There is great interest and activity in production of a therapeutic drug for PD. But as a large proportion of the nerves of the substantia nigra are affected by the time of diagnosis, drug therapy is likely to fail unless it can be started very early in the disease process. This defines the need for a blood test for early detection of disease activity in PD to facilitate early interventional therapy.

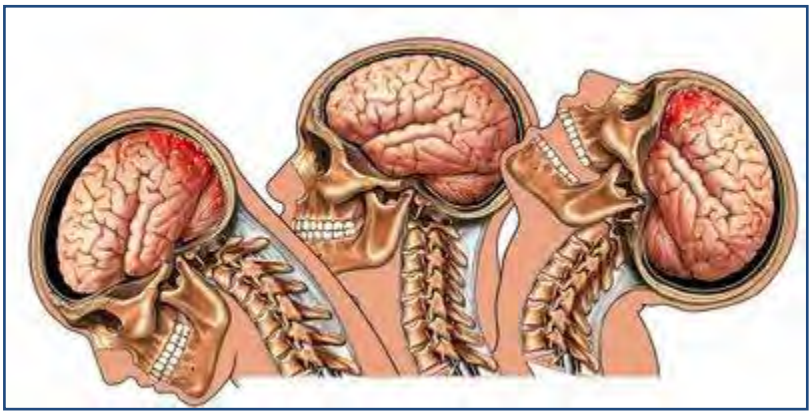


We can apply our "window into the brain" concept to solving the problem of early diagnosis of PD. The nerves of the substantia nigra contain a black pigment known as neuromelanin. When these nerves degenerate, neuromelanin is part of the debris and may be engulfed by phagocytes that will appear in the blood. In a pilot study of newly diagnosed PD subjects we showed that neuromelanin levels were substantially higher in blood phagocytes of PD subjects in comparison with age and sex matched controls. This proof of principle study is very encouraging. The application of this technology to early (pre-motor) diagnosis of PD holds the promise of revolutionizing PD therapy.

MEDICAL SOLUTIONS Dx

Mild Traumatic Brain Injury/ Concussion:

Multiple concussions can lead to a brain disease that has been named Chronic Traumatic Encephalopathy (CTE), which is a progressive neurodegenerative disease. The symptoms of the disease most often begin after a long period of latency ranging from several years to as much as several decades. The initial symptoms are insidious, and include impulsivity, irritability, depression, aggression, short-term memory loss and a heightened tendency for suicide.



The description of the clinical symptoms associated with the earlier stages of CTE were concerning as they were synonymous with the symptoms of post-concussion syndrome that are regularly recognized in individuals with mild traumatic brain injury (mTBI).

There is no reason to believe that every one of these individuals have CTE as we know that many of them can experience ongoing improvement over time. The Department of Defense recognizes greater than 300,000 individuals that have experienced a traumatic brain injury since 2000. The problem is that we do not have the ability to differentiate those who will go on to have CTE from those who will recover normally. There is a need for further understanding of this process both to a) identify individuals who will have ongoing and potentially worsening effects of their current injury and b) to allow for interventions that may impact the development of those long term effects.

Currently, CTE is definitively diagnosed by post mortem examination of the brain. Current evaluations in the living consist of imaging techniques that are still limited in their ability to even show brain injury and have no capacity to show degenerative changes until late in the process. CSF studies require the invasiveness of a lumbar puncture that can be associated with some morbidity such as bleeding and introduction of infection. Another even more invasive option to visualize degenerative change would be through brain biopsy. There is a need for a way to follow biomarkers that is both able to show the degenerative changes of the brain but that is less invasive leaving the patient wholly intact. The application of MSDx's biomarker strategy may meet this need.

MSDx Seeks Collaborators

MSDx is currently working to commercialize the biomarkers described in this document. While developing these assays, the company would like to gather feedback to better understand the needs of the clinician, researcher, and pharmaceutical/diagnostics companies in the neurological markets. In addition, MSDx is looking for opportunities to collaborate with researchers to become a part of "follow-on" patient studies. Dr. Ramesh Nayak can be reached at rnayak@msdx.co.