Blood Protein Signature for the Early Diagnosis of Alzheimer Disease

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Alzheimer disease (AD) has become one of the main health concerns for the elderly population in the United States. Current treatments target symptoms only, but several advanced clinical trials are testing new drugs that are potentially disease modifying. Because AD is still difficult to diagnose in its earliest stages and the disease process is estimated to start many years before current clinical diagnosis is made, accurate and simple diagnostic tools are urgently needed. We recently described a blood-based panel of secreted signaling proteins that distinguishes between blinded samples from patients with AD and control subjects with high accuracy. The same proteins also predicted progression to AD in preclinical patients with mild cognitive impairment several years before clinical diagnosis for AD was made. Herein, we describe these findings and discuss the potential for a more general application of our proteomic approach in understanding and diagnosing disease.

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disease as early as possible and to develop new treatments. While there is a long history for using molecular biomarkers in blood as surrogate markers for physiological and disease-related processes in various tissues, no such markers are available for neurological disorders to date. This is in part explained by the preconceived notion that the brain is relatively isolated from the blood by the blood-brain barrier. However, over the past decade, it has become clear that the brain maintains intricate relationships with the immune system, for example, and that secreted proteins from the brain can regulate physiological processes throughout the body.

In AD, the characteristic amyloid plaques and tangles in the brain are accompanied by prominent local stimulation of innate immune and inflammatory responses, and there is increasing evidence from AD mouse models that peripheral immune cells can be recruited from the blood to the brain and modulate the disease. Other studies have reported differences in the distribution or reactivity of blood cell subsets or normal levels of cytokines, chemokines, and growth factors in the brain parenchyma, CSF, or blood of patients with MCI or AD.

Based on this body of scientific evidence, we formulated the hypothesis that pathological processes associated with AD (or other central nervous system diseases) would produce disease-specific molecular changes in the blood. We focused specifically on secreted signaling proteins, including cytokines, chemokines, and growth factors, as these are the primary means of communication between cells in our body. Imbalances in the network of communication between cells in disease may not only be a diagnostic indicator but could potentially reveal mechanistic insight into pathophysiological processes. Herein, we review our recently published discovery of an AD-specific signature of signaling proteins in blood and discuss its potential relevance for clinical application and basic research.

**PROTEIN SIGNATURE IN BLOOD PREDICTS PROGRESSION TO AD IN PATIENTS WITH MCI YEARS BEFORE DIAGNOSIS OF AD**

To find a blood-based signature for AD, we measured with a proteomic multiplex method 120 cytokines, chemokines, growth factors, and related signaling proteins in plasma of roughly 40 patients with mild to moderate AD and 40 age-matched controls without dementia from 7 different AD research centers and clinics in the United States and Europe. Statistical comparison of the measurements led to the discovery of 18 proteins whose concentrations in plasma of patients with AD is characteristically changed (proteins are listed in the Figure 1 legend). To confirm that this panel is a potential AD signature, we tested it in a similarly sized, independent, blinded sample set containing plasma from patients with AD, patients with other types of dementia, and controls without dementia. The 18 proteins were able to distinguish between samples from patients with AD and various controls with almost 90% accuracy. The same proteins also discriminated patients with AD from patients with other neurological disorders or rheumatoid arthritis. Together, this indicated that we discovered a relatively robust AD-specific protein signature in blood.

Such an AD signature would be most helpful if it could identify those patients with MCI whose disease later converted to AD. We therefore analyzed plasma samples from 47 patients with MCI who gave blood at the point of diagnosis of MCI and who were followed up clinically. At follow-up diagnosis 2 to 6 years later, 22 patients’ disease had progressed to AD and the prediction by the AD signature was consistent with the clinical diagnosis for 20 of them. Moreover, the same proteins also entirely discriminated AD converters from 8 patients with MCI who developed other types of dementia. Interestingly, of the 17 samples from patients who continued to have MCI
were classified as being AD converters and it will be interesting to see if this prediction is correct. Our data indicate that a highly specific plasma biomarker signature can identify presymptomatic AD in patients with MCI years before a clinical diagnosis is made.

AD SIGNATURE SHOWS SPECIFIC PROTEIN EXPRESSION PATTERNS IN THE BLOOD

To explore and compare expression patterns of the 18 signaling proteins in plasma of patients with AD, other dementia, or MCI and healthy individuals, we used a so-called clustering algorithm that arranges samples in clusters based on similarity of expression patterns of the 18 proteins. Most of the samples from patients with AD and patients with MCI whose disease converted to AD were grouped into 1 main cluster, whereas most other samples were arranged into another cluster (Figure 1), illustrating that the 18 proteins can be used to discriminate patients with AD from patients with other dementias or healthy individuals.

AD SIGNATURE INDICATES IMBALANCE IN THE NETWORK OF SECRETED INTERCELLULAR SIGNALING PROTEINS

Cytokines, chemokines, growth factors, and related secreted signaling proteins are part of a soluble network of proteins in the extracellular space that cells use to communicate with each other. We call the universe of these intercellular communication factors the communicome, in reference to the other systemwide groups or -omes of molecules (eg, transcriptome, proteome). The 120 proteins that we measured are only a subgroup of the communicome and were selected with special emphasis on the fact that immune and inflammatory mechanisms in the brain and the periphery are increasingly implicated in AD. The relationship between factors of the communicome can directly be assessed by correlation networks. Focusing only on the 18 proteins of the AD signature, it becomes already quite obvious that the relationships between these factors changes considerably in patients with AD compared with controls without dementia (Figure 2). For example, while granulocyte colony-stimulating factor is strongly embedded in a network and correlated with 7 of the other 17 proteins in healthy individuals (Figure 2A), these relationships are all lost in patients with AD; instead, a weak correlation with IL-3 is observed (Figure 2B). In general, there seem to be more positively coregulated factors within these 18 proteins in patients with AD than in controls without dementia. If one considers the correlation network in controls without dementia as the baseline or homeostatic network, then the network for patients with AD could be considered off-baseline or imbalanced. Because of the coregulation of these 18 factors with other factors, this imbalance may “spread” into other parts of the communicome, disrupting existing communication or signaling networks. Computer-based analysis of biological pathways for these 18 factors points to dysregulation of hematopoiesis, inflammation, and apoptosis.

Interestingly, an independent microarray study comparing expression of hippocampal genes between AD and nondiseased control brains found similar pathways to be impaired.
RELEVANCE FOR CLINICAL APPLICATION AND BASIC RESEARCH

The National Institutes of Health Biomarkers Definitions Working Group standardized the definition of biomarker 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.24(p91)

An AD signature in blood such as the one we describe would fall under this definition. Clinically, a molecular blood biomarker for AD might have advantages over more complex and sometimes more invasive tests, such as CSF protein measurements, magnetic resonance, or positron emission tomography imaging, particularly as an early screening tool. Still, these existing tests could be used to follow up on a positive test result with a blood biomarker to establish a final or more differentiated diagnosis, much like prostate-specific antigen measurements can trigger biopsies or other testing for prostate cancer. Before our current blood biomarker can be used in the clinic, however, it needs to be validated in larger independent groups of samples obtained from other clinical centers.

One of the most intriguing scientific questions arising from our work is what might cause the observed changes in the communicome and the apparent disruption of relationships between communication factors in the blood in patients with AD. Understanding these causes may provide novel information about disease mechanisms and help identify new targets for therapeutic intervention. Interestingly, several of the 18 proteins that are part of the AD signature promote production and lineage commitment of myeloid cells toward macrophages, stimulate egression of these cells from the bone marrow, and control recruitment to target tissues. Because these factors were found to be reduced in AD plasma in our study, macrophages and related cells may be impaired in AD and not be recruited as efficiently to sites of injury in the brain. Indeed, studies in mouse models for AD support the concept that peripheral macrophages are recruited to the brain where they appear to limit disease progression.18,25 In related studies, treatment of “AD mice” with granulocyte colony-stimulating factor led to increased infiltration of the brain by hematopoietic stem cell–derived cells and improved memory function in comparison with mock-treated mice.26 Granulocyte colony-stimulating factor, which we observed to be reduced in AD plasma and “disconnected” in the AD relationship network (Figure 2), is currently undergoing clinical trials for stroke.27,28 Although it is still unclear how the studies in mice with AD-like pathological features relate to the human disease, our findings in plasma of patients with AD offer a framework to study the potential role of macrophages and related cell types in AD.

CONCLUSIONS

By focusing on secreted signaling proteins or intercellular communication factors rather than the entire plasma proteome, we were able to identify an AD blood signature that can potentially be used as a biomarker for the simple and early diagnosis of AD.20 While it does not predict the risk of a healthy person to develop AD, our current biomarker seems to be an early indicator of the disease process and conversion from MCI to AD. No doubt more work is necessary to validate this novel AD biomarker but our study suggests that changes in the cellular communicome in the blood can in principle be used to characterize a central nervous system disease. It is conceivable that other combinations of secreted signaling proteins can be used as surrogate markers of disease progression or even indicators of disease risk for AD as well as other diseases. Proteins that are part of such signatures may provide mechanistic insight into disease-associated biological pathways and serve as new targets for therapeutic strategies.

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