Autoantibody Signatures Unravel Disease Complexity

Chronic diseases such as autoimmune, cancer and neurological diseases are complex, difficult to diagnose, and problematic to treat due to their inherent heterogeneity. Cancer and neurodegenerative diagnostics, for example, rely heavily on symptom reporting and diagnostic imaging, procedures conducted later in the disease pathogenesis.

Liquid biopsy derived markers of cancer, Alzheimer's and Parkinson's do exist (*Hampel et al., 2022; Li & Le, 2020; Tumor Markers in Common Use, 2021*); however, testing for these markers may be invasive (lumbar puncture), and is usually conducted following symptoms, only employing one or a few markers to confirm diagnosis. Misdiagnosis can result in great patient discomfort, uncertainty, unnecessary treatments and a high cost burden (*CDC, 2019; Hansson, 2021; Hunter et al., 2015*). Further, current markers have not been thoroughly evaluated for long term prognosis. Great effort is underway to discover better biomarkers that are easy to obtain & detect, highly predictive and observed before symptoms emerge.

Blood derived biomarkers are advantageous because sera contains a plethora of accessible, easy to measure analytes. Because blood tests are common practice in the medical community, they offer the potential to provide quicker results with less discomfort to patients compared with tissue biopsies or lumbar puncture (*Hampel et al., 2022; Hayes Balmadrid et al., 2017; Laranja et al., 2019*). Evaluating antibodies from patient sera is a minimally invasive approach with huge potential. Antibodies are manifestations of disease, direct consequences of illness that appear early and persist in blood circulation throughout the disease. In fact, antibodies have been shown to predate cancer and autoimmune disease diagnosis by years (*Anderson & Labaer, 2005; Arbuckle et al., 2003; Trivers et al., 1996*). Antibody production is not only stimulated by infectious organisms, chronic diseases can also initiate antibody production. Aberrantly expressed proteins that result from the disease process induce production of autoantibodies. Tumors, for example, can produce unique tumor specific proteins as well as ectopically express host proteins. In ALS, the transactivating enzyme TDP-43 becomes overexpressed, inducing anti-TDP43 autoantibody production (*Conti et al., 2021*). The humoral immune system recognizes these inconsistencies and produces autoantibodies as a result, sensing disease often before symptoms appear. This is why antibody screening and immunodiagnostics have existed for over a century. Antibodies offer the promise of more robust biomarkers for early disease detection with greater prognostic value compared with many current biomarkers in clinical use (*Bizzaro, 2007; Damoiseaux et al., 2015; Wang et al., 2020; Zaenker & Ziman, 2013*).

Until recently, it was difficult to capture all the information held by antibodies. Protein microarrays provide an excellent mix of throughput, sensitivity and cost. Thousands of antibodies from a single sample can be quickly evaluated for disease association on a single slide. However, antibodies recognize small discontinuous antigen epitopes (*Barlow et al., 1986; Muro et al., 1994*). Antibody-antigen binding is highly sensitive to antigen shape and not sequence. Protein microarray data benefits from the use of properly folded proteins on the microarray, for example the i-Ome Discovery array (*Immunoproteomic Services | Autoantibody Biomarker Profiling | Sengenics*). This technology maintains conformational epitopes and ensures optimal antibody-epitope binding for the rigors of antibody screening. Combined with a carefully laid out protein library, and modern machine learning, protein microarrays have advanced biomarker discovery by uncovering distinct disease associated antibody signatures, a panel of autoantibodies that match the real complexities of disease. Multiple markers are more likely to be indicative and prognostic of disease pathophysiology than single makers. Further, autoantibody signatures can help identify patient endotypes for better stratification of patients into therapeutically efficacious categories and clinical trial enrichment.

In a recent, comprehensive study by Patel et al. (*Patel et al., 2022*), 60 different autoantibodies of interest were uncovered from a screen with the Sengenics i-Ome microarray of more than 1600 antigens across a cohort of 157 patients with non-small cell lung cancer (NSCLC). Eighteen of the 60 autoantibodies correlated with survival rates. Evaluating various permutations of these 18 autoantibodies with machine learning revealed that 13 strongly correlated with poor 5-year patient survival. These 13 autoantibodies were also predictive in an independent validation cohort, demonstrating the reproducibility of the approach. Interestingly, a number of these autoantibodies were cancer testis antigens (CTA) (*Patel et al., 2022*) - fetal antigens that are silenced in all adult somatic tissues except the testes – potentially indicating that the patients with a poor prognosis (male and female) had a distinct, more cancer stem cell-like sub-type of NSCLC. These findings provide new insights and potential therapeutic targets for treating NSCLC. For example, the ectopic expression of CTAs may provide an

excellent, well-aimed therapeutic target – indeed, certain CTAs such as NY-ESO-1 and MAGEA3 have been proposed as vaccine targets in cancers such as melanoma. Future screens will undoubtedly unveil other prognostic panels (*Patel et al., 2022*).

Antibodies have been used to detect disease for over a century. With high specificity for disease related antigens, antibodies are ideal biomarkers. Modern technologies like the Sengenics KREX technology and new machine learning algorithms provide comprehensive immunoprofiles with antibodies that can advance patient care by detecting complex diseases and predicting patient outcomes including survival, adverse events, and therapeutic response.

References

Anderson, K. S., & Labaer, J. (2005). The Sentinel Within: Exploiting the Immune System for Cancer Biomarkers. Journal of Proteome Research, 4(4), 1123-1133. https://doi.org/10.1021/pr0500814

Arbuckle, M. R., McClain, M. T., Rubertone, M. V., Scofield, R. H., Dennis, G. J., James, J. A., & Harley, J. B. (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med, 349(16), 1526-1533. https://doi.org/10.1056/NEJMoa021933

Barlow, D. J., Edwards, M. S., & Thornton, J. M. (1986). Continuous and discontinuous protein antigenic determinants. Nature, 322(6081), 747-748. https://doi.org/10.1038/322747a0

Bizzaro, N. (2007). Autoantibodies as predictors of disease: the clinical and experimental evidence. Autoimmun Rev, 6(6), 325-333. https://doi.org/10.1016/j.autrev.2007.01.006

CDC. (2019). Health and Economic Costs of Chronic Disease. CDC. Retrieved 03/08/2023 from https://www.cdc.gov/chronicdisease/about/costs/index.htm

Conti, E., Sala, G., Diamanti, S., Casati, M., Lunetta, C., Gerardi, F., Tarlarini, C., Mosca, L., Riva, N., Falzone, Y., Filippi, M., Appollonio, I., Ferrarese, C., & Tremolizzo, L. (2021). Serum naturally occurring anti-TDP-43 auto-antibodies are increased in amyotrophic lateral sclerosis. Sci Rep, 11(1), 1978. https://doi.org/10.1038/s41598-021-81599-5

Damoiseaux, J., Andrade, L. E., Fritzler, M. J., & Shoenfeld, Y. (2015). Autoantibodies 2015: From diagnostic biomarkers toward prediction, prognosis and prevention. Autoimmun Rev, 14(6), 555-563. https://doi.org/10.1016/j.autrev.2015.01.017

Hampel, H., Shaw, L. M., Aisen, P., Chen, C., Lleo, A., Iwatsubo, T., Iwata, A., Yamada, M., Ikeuchi, T., Jia, J., Wang, H., Teunissen, C. E., Peskind, E., Blennow, K., Cummings, J., & Vergallo, A. (2022). State-of-the-art of lumbar puncture and its place in the journey of patients with Alzheimer's disease. Alzheimers Dement, 18(1), 159-177. https://doi.org/10.1002/alz.12372

Hansson, O. (2021). Biomarkers for neurodegenerative diseases. Nat Med, 27(6), 954-963. https://doi.org/10.1038/s41591-021-01382-x

Hayes Balmadrid, M. A., Shelby, R. A., Wren, A. A., Miller, L. S., Yoon, S. C., Baker, J. A., Wildermann, L. A., & Soo, M. S. (2017). Anxiety prior to breast biopsy: Relationships with length of time from breast biopsy recommendation to biopsy procedure and psychosocial factors. J Health Psychol, 22(5), 561-571. https://doi.org/10.1177/1359105315607828

Hunter, C. A., Kirson, N. Y., Desai, U., Cummings, A. K., Faries, D. E., & Birnbaum, H. G. (2015). Medical costs of Alzheimer's disease misdiagnosis among US Medicare beneficiaries. Alzheimers Dement, 11(8), 887-895. https://doi.org/10.1016/j.jalz.2015.06.1889

Laranja, W. W., Sanches, B. C. F., Voris, B. R. I., Alonso, J. C. C., Simoes, F. A., Rejowski, R. F., & Reis, L. O. (2019). The Biopsychosocial Burden of Prostate Biopsy at the Time of Its Indication, Procedure, and Pathological Report. Prostate Cancer, 2019, 2653708. https://doi.org/10.1155/2019/2653708

Li, T., & Le, W. (2020). Biomarkers for Parkinson's Disease: How Good Are They? Neurosci Bull, 36(2), 183-194. https://doi.org/10.1007/s12264-019-00433-1

Muro, Y., Tsai, W. M., Houghten, R., & Tan, E. M. (1994). Synthetic compound peptide simulating antigenicity of conformation-dependent autoepitope. J Biol Chem, 269(28), 18529-18534. https://www.ncbi.nlm.nih.gov/pubmed/7518436

Patel, A. J., Tan, T. M., Richter, A. G., Naidu, B., Blackburn, J. M., & Middleton, G. W. (2022). A highly predictive autoantibody-based biomarker panel for prognosis in early-stage NSCLC with potential therapeutic implications. Br J Cancer, 126(2), 238-246. https://doi.org/10.1038/s41416-021-01572-x

Trivers, G. E., De Benedetti, V. M., Cawley, H. L., Caron, G., Harrington, A. M., Bennett, W. P., Jett, J. R., Colby, T. V., Tazelaar, H., Pairolero, P., Miller, R. D., & Harris, C. C. (1996). Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease can predate a diagnosis of cancer. Clin Cancer Res, 2(10), 1767-1775. https://www.ncbi.nlm.nih.gov/pubmed/9816128

Tumor Markers in Common Use. (2021). National Cancer Institute. Retrieved 10/31/2022 from https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/tumor-markers-list

Wang, B. Z., Zailan, F. Z., Wong, B. Y. X., Ng, K. P., & Kandiah, N. (2020). Identification of novel candidate autoantibodies in Alzheimer's disease. Eur J Neurol, 27(11), 2292-2296. https://doi.org/10.1111/ene.14290

Zaenker, P., & Ziman, M. R. (2013). Serologic autoantibodies as diagnostic cancer biomarkers--a review. Cancer Epidemiol Biomarkers Prev, 22(12), 2161-2181. https://doi.org/10.1158/1055-9965.EPI-13-0621