

A multi-marker approach to diagnosing autoimmune diseases

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Autoimmune diseases are complex by nature, making their diagnosis challenging to even the most experienced of rheumatologists. Diseases like rheumatoid arthritis (RA), systemic sclerosis (SSc), and systemic lupus erythematosus (SLE) frequently have overlapping symptoms, with patients moving through periods of relapse and remission. In order to reach an accurate and timely diagnosis, patients must rely upon their doctor's expertise and the results from small-scale multi-marker panels, typically looking at just three to five biomarkers. Unfortunately, the power of such marker panels remains limited, meaning many patients receive an incomplete or even an incorrect diagnosis.

If we are to accurately screen for and discriminate among different autoimmune diseases, we need to have more sophisticated and accurate *in vitro* diagnostics (IVDs). These need to be able to account for the heterogeneity of autoimmune diseases, limit the generation of false positives, and overcome the shortcomings of existing individual biomarkers, which by themselves are relatively weak diagnostic classifiers due to their sometimes-low prevalence.

A lack of sensitivity and specificity

The measurement of specific autoantibodies remains the most commonly employed IVD for autoimmune disease. Their presence and levels can be indicative of not only a disease itself, but also a patient's risk of progressing to clinical onset of an autoimmune disease. However, these autoantibodies present inconsistently among patients with particular autoimmune diseases.

Rheumatoid factor (RF) autoantibodies, for example, are present in 75 percent of RA patients and three percent to five percent of healthy individuals; 98 percent of SLE patients are positive for antinuclear antibodies (ANAs), but so are up to 15 percent of the general population, especially in higher ages.¹ For IVDs like these to be successful, they need to combine high sensitivity and specificity.

Despite the drawbacks of some autoimmune biomarkers, we have seen an almost complete lack of novel markers developed in over a decade. This is particularly detrimental for the diagnosis of early autoimmune diseases, for which existing markers lack sensitivity. One solution has been to combine existing markers into a multi-marker panel. However, individual existing markers within these panels carry their own diagnostic thresholds, restricting the possibility of using classifiers or algorithms to amalgamate them into a single cohesive assay.

Creating strength from weakness

Although existing markers may be weak classifiers individually, it should be possible to combine them in order to generate a strong classifier marker panel. The algorithm behind such a multi-marker panel should be able to account for heterogeneity inherent to autoimmune diseases. By increasing the size and complexity of multi-marker analysis to include novel and existing markers capable of working together—independent of their individual thresholds—it

would be possible to create a powerful IVD tool.

This is precisely what is happening in the field of SLE. Recent research has produced a multi-marker test based on the analysis of more than 1,500 patients with SLE, RA, SSc, and other autoimmune diseases. This panel looks at 87 autoantigens—including 40 novel ones—and measures autoantibody markers linked to SLE pathologies and different clinical manifestations. The inclusion of classic markers such as ANAs, anti-double-stranded (ds)-DNA, and anti-cyclic citrullinated peptide (CCP) brings the power to first identify an appropriate SLE population.

From here, patients with high disease activity and/or an SLE-specific signature are probed through the analysis of autoantibodies against interferon (IFN)-inducible genes such as SSA/Ro, SSB, SP100 and Histones. Autoantibodies associated with secondary syndromes or organ-specific damage are also included in this multi-marker panel; high titers of anti-dsDNA antibodies are associated with lupus nephritis, for example, while anti-U1-RNP autoantibodies are associated with Raynaud's phenomenon and a reduced probability of nephritis.

The technology also analyzes linked sets of autoantibody reactivities to generate data for relevant patient stratification. This results in the identification of four distinct patient subgroups/clusters with clearly defined characteristics. This system should allow clinicians and diagnosticians to define homogenous groups of SLE to tailor optimized therapies.

A useful companion

The power of multiplex approaches doesn't end in the diagnosis of autoimmune diseases: we can use the insight these panels provide into a patient's disease for the development of treatment response prediction and ultimately companion diagnostics. Attempts to use multiplexing in this manner have initially relied on a handful of cytokines and antibodies as biomarkers. For example, 24 cytokines and autoantibodies were used in a screen for RA due to their ability to predict outcomes in response to a tumor necrosis factor (TNF) blocker, etanercept.² This achieved predictive values of 58 percent to 72 percent and negative predictive values of 63 percent to 78 percent.

Companion diagnostic tools provide vital information about the nature of patient subgroups with respect to their individual disease profile and personalized responses to a particular drug or treatment. Predictive IVDs in this context would contribute to the improvement of clinical trial design, as trial endpoints could be defined based on detailed phenotypes (e.g., disease severity or progression), making them more likely to be achieved. This in turn increases the probability that a new treatment will progress through clinical trials to the point of achieving regulatory approval.

The future of diagnostics

This is just one example of how multi-marker panel approaches to diagnostics can be used in an attempt to deal with the com-

plexity and heterogeneity that is so characteristic of autoimmune diseases. Next-generation multiplexing tools based on strong classifiers facilitate earlier diagnoses and a powerful disease stratification approach that could be beneficial to future clinical programs. While such tests represent a significant investment, they have the potential to provide the basis for treatment-specific companion diagnostics. In fact, initial investment in predictive biomarker panels can lessen the financial impact of trials designed to test new treatments, as they allow clinicians to select those patients most likely to respond from the outset. Identification of these responder groups can improve the quality of data from placebo vs. treatment groups, minimize potential serious adverse effects, and reduce trial time and costs.

The development and analysis of multiplex panels that examine complete autoantibody profiles has far-reaching benefits: they will improve both the diagnosis and prognosis of patients with autoimmune diseases; lead to the generation of clinically relevant patient subgroups; and possibly even improve response prediction and contribute to more robust clinical trials. 🔄

REFERENCES

1. Mariz HA, Sato EI, Barbosa SH, Rodrigues SH, Dellavance A, Andrade LE. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. *Arthritis Rheum.* 2011;63(1):191–200.
2. Hueber W, Tomooka BH, Battliwalla F, et al. Blood autoantibody and cytokine profiles predict response to anti-tumor necrosis factor therapy in rheumatoid arthritis. *Arthritis Res Ther.* 2009;11(3):R76.



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