WHITE PAPER

VIBRANT AMERICA ANA+ENA PANEL FOR CONNECTIVE TISSUE DISORDER

Table of Contents

1. Diagnosis of Connective Tissue Disorder (CTD)

Connective tissue disorders (e.g., systemic lupus erythematosus (SLE), Sjögren's syndrome (SSj), systemic sclerosis (SS), rheumatoid arthritis (RA), etc) are normally tested for autoantibodies in order to reach consensus of diagnosis. Antinuclear antibodies (ANA) are a heterogeneous group of autoantibodies that plays an important role in diagnosis, prognosis assessment, and monitoring of the clinical evolution of patients with CTDs. [1] The gold standard technique for ANA detection is by indirect fluorescence assay (IFA) assay on human epithelial type 2 (HEp-2) cells. Followed by a positive ANA result, an extractable nuclear antigens (ENA) testing is usually performed to detect the presence of one or more autoantibodies in the blood that react with proteins in the cell nucleus for differential diagnosis, as shown in Figure 1. [2]

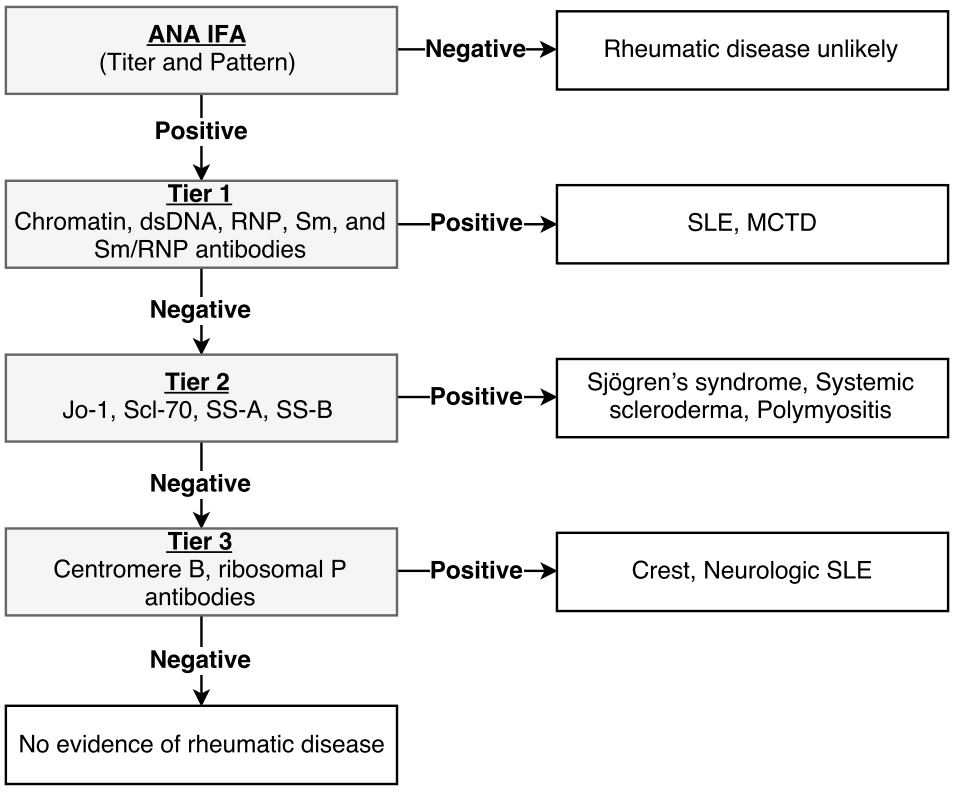

Figure 1. Traditional algorithm for CTD diagnosis.

2. Limitations of ANA IFA

While ANA IFA assay detects a large number of nuclear and cytoplasm antigens, its sensitivity and specificity for diagnosis has been inherently limited by several factors.[3] ANA IFA can be subjective due to its heavy dependence on humans' operation (large number of seral dilutions) and interpretation (visual determination of staining patterns). Another significant limitation is the existence of false positives due to ANA's presence in other autoimmune diseases, infections, tumors and in 25% healthy individuals.[4] In addition, false negatives may also present at the very beginning stage of the disease. There has been a pressing need to achieve early detection of autoantibodies for asymptomatic subjects with ensured consistency and reliability.

3. Predictive Detection by ENA

Vibrant America provides a microarray-based assay that enables simultaneous ANA IFA detection and ENA testing (panel shown in Table 1). A multiple-visit follow-up study was conducted aiming at clarifying the clinical significance and predictive value of Vibrant America's multiplexed panel. This comparative study showed that the Vibrant America's multiplex panel is not only more sensitive than performing ANA detection alone but also provides invaluable information at a very early stage for recognition of CTD. The combined detection of ANA and ENA can significantly reduce the number of false negatives.

ANA IFA									
Speckled			Nucleolar		RIM	Cytoplasmic		Centromere	Homogeneous
ENA Panel									
Jo-1	Sm	RNP	SSA (Ro)	SSB (La)	Scl-70	Chromatin	Centromere	Histone	RNA POL III

Table 1. Vibrant America ANA+ENA panel for CTD

Methods

A multiple-visit follow-up study was performed for 110 subjects with a negative ANA result but a positive ENA result at their 1st visit. Vibrant America ANA+ENA complex panel was used to monitor their disease development for 2 years.

Results

(A) ENA+/ANA- to ENA+/ANA+

At 2 years of follow-up for the 110 subjects, 23 (20.9%) subjects showed ENA positive for an average of 385 (±144) days ahead of their ANA results becoming positive. Among them, 4 (3.6%) turned positive within the first half year, 7 (6.4%) turn positive within the second half year, and 12 (10.9%) turned positive in the second year after their 1st visit at Vibrant America. The investigation for this cohort is ongoing. We hypothesize more ANA- subjects would eventually develop into CTD or related autoimmune diseases after a longer period of time.

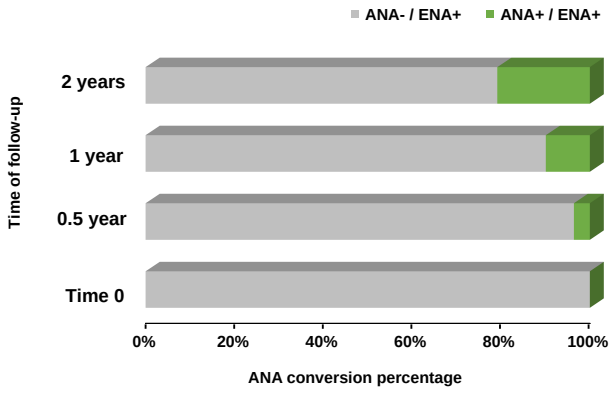

Figure 2. Out of 110 subjects who were ANA negative but had anti-ENA antibodies (ANA-/ENA+), 23 subjects (20.9%) sero-converted to ANA positive and continued to have anti-ENA antibodies (ANA+/ENA+) in two years.

Results

(B) Autoantibodies detected by the complex panel in ANA negative subjects

Histone (45.5%) and Chromatin (22.7%) antibodies were the most frequently found in the cohort with ENA+/ANA- result at their first visit. Antibodies against Jo-1 (5.5%) and Scl-70 (3.6%) were the least prevalent. After 2 years of follow-up, no obvious trend was observed between the conversion rate of ANA- to ANA+ and the prevalence of ENA autoantibodies. Therefore, a comprehensive ENA testing consisted of 10 autoantibodies is still considered necessary in order to reduce false negatives that could have be caused by performing ANA tests alone.

ENA	Prevalence at the 1st visit		Frequency of ANA coversion	
Histone	50	45.5%	5	10.0%
Chromatin	25	22.7%	3	12.0%
SSA (Ro)	22	20.0%	10	45.5%
Sm	15	13.6%	3	20.0%
RNA POL III	11	10.0%	1	9.1%
RNP	8	7.3%	5	62.5%
SSB (La)	8	7.3%	2	25.0%
Centromere	8	7.3%	1	12.5%
Jo-1	6	5.5%	2	33.3%
Scl-70	4	3.6%	0	0.0%

Table 2. The frequencies of the 10 autoantibodies detected by the ENA testing and the conversion rate of ANA+ in a cohort of 110 subjects with ENA+/ANA+ at the 1st visit.

4. Conclusion

Vibrant America's ANA+ENA panel has shown greater sensitivity than a single test of ANA IFA. Early detection of these antibodies by Vibrant America can be powerful in predicting possible development of CTD. Consequently, appropriate treatment or management can be implemented to reduce the severity impact of possible diseases.

5. Reference

1. Kumar, Yashwant et al. "Antinuclear Antibodies and Their Detection Methods in Diagnosis of Connective Tissue Diseases: A Journey Revisited." Diagnostic Pathology 4 (2009): 1.
2. Damoiseaux, J.G.M.C. et al. "From ANA to ENA: How to proceed?" Autoimmunity Reviews 5(1) (2006): 10-17.
3. Meroni, Pier Luigi et al. "Automated Tests of ANA Immunofluorescence as Throughput Autoantibody Detection Technology: Strengths and Limitations." BMC Medicine 12 (2014): 38.
4. Satoh, Minoru et al. "Prevalence and Sociodemographic Correlates of Antinuclear Antibodies In the United States." Arthritis and Rheumatism 64.7 (2012): 2319–2327.