

RESEARCH ARTICLE

## Inappropriate use of commercial Antinuclear Antibody Testing in a community-based US hospital: a retrospective study

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Healthcare providers use antinuclear antibodies (ANAs) to screen and diagnose patients with autoimmune diseases. In the recent years, commercial multiplex ANA kits have emerged as a convenient and fast diagnostic method. Diagnostic testing should follow sequenced algorithms: initial screen followed by specific antibody analysis. Second-level testing as an initial screen for autoimmune disease is inappropriate. We reviewed 68 patients with ANA comprehensive panels over a 6-month period from May 2015 to October 2015. We assessed appropriateness and estimated incurred losses from inappropriate testing. We found 92.6% (63 out of 68) of the ANA comprehensive panel results to be negative. Incurred losses from inappropriate ANA comprehensive panel testing were \$66,000. Physicians should become familiar with ANA-sequenced diagnostic algorithms to avoid unnecessary higher level testing.

Keywords: *antinuclear antibody; comprehensive antinuclear antibody panel; rheumatology; order sets*

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Healthcare providers use antinuclear antibodies (ANAs) to screen and diagnose patients with autoimmune diseases. ANAs have different subtypes that bind to different proteins within the cell nucleus. We can test for the presence of ANAs and subtypes. The initial test in the evaluation of rheumatologic diseases is ANAs. Testing for subtypes called extractable nuclear antigens (ENAs) should follow a positive ANA result. Kavanaugh and colleagues published guidelines supporting this in 2000 (1). Common tests for the detection of ANAs are indirect immune fluorescence test and enzyme linked immunosorbent assay (ELISA). These two methods determine the presence of antibodies directed toward the human cell nucleus.

In the recent years, commercial multiplex ANA kits at the ACL labs have emerged as a convenient and fast testing method with fewer false positives and completed with a single run. Also, it was noted that the unfamiliarity and confusing names of the order sets were contributing to the inappropriate ordering of ANA comprehensive panels.

### Methods

In this retrospective, single center study, we reviewed charts from 68 patients with ANA comprehensive panels. Inclusion criteria were patients  $\geq 18$  years old and had an ANA CP billing code between May 2015 and October 2015. Variables included age, sex, specialty of the ordering physician, test indication, and ANA result. The primary outcome was appropriateness of second-level comprehensive panel testing.

Our institutional review board determined this study was non-Human Subjects Research; institutional approval was not required. Categorical variables are summarized with frequencies and percentages. Continuous variables are summarized with means  $\pm$  standard deviations. Analysis was performed using SPSS 22<sup>®</sup> (Chicago, IL).

### Results

Demographic information for the total sample ( $n = 68$ ) is displayed in Table 1. The mean age of the sample was  $54.4 \pm 19.4$  years old, and 60.3% were female. Three subjects (4.4%) had a past history of rheumatological disease.

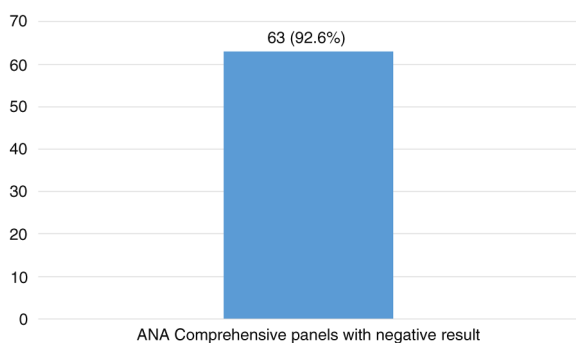
**Table 1.** Demographics and comprehensive antinuclear antibody order indications

	Total Sample (n = 68)
Age (mean $\pm$ SD)	54.4 $\pm$ 19.4
Female	41 (60.3%)
History of rheumatological disease	3 (4.4%)
Ordering specialty	
Internal medicine	57 (83.8%)
Family medicine	5 (7.4%)
Emergency medicine	2 (2.9%)
Psychiatry	2 (2.9%)
Other	2 (2.9%)
Indication for screen	
Hypercoagulable work up	6 (8.8%)
Transaminasemia	6 (8.8%)
Skin rash	6 (8.8%)
Other	50 (73.6%)

Table 1 includes physician specialty and the test indication. Internal Medicine ordered the majority of ANA CPs (83.8%) followed by Family Medicine (7.4%), Emergency Medicine (2.2%), and Psychiatry (2.2%). Hypercoagulable work up, transaminasemia and skin rash were the most frequent indications for ordering the ANA CP (8.8% for each indication). The remaining indications (73.6%) covered a broad spectrum and combined as an 'other' category. All the ANA CPs ordered were considered to be inappropriate including the three patients who had previous history of rheumatological disease and did not require re-testing. Sixty-three ANA comprehensive panels were negative for rheumatological disease (92.6%, Fig. 1).

## Discussion

Multiplex immunoassays have led to a paradigm shift in the methodological testing of autoimmune diseases. High throughput multiplex immunoassays have supplanted the use of traditional methods like indirect immunofluorescence (IIF) and ELISA. IIF testing is subject to poor specificity, has a high false positive rate, lack of standardization in substrate and dilution protocols, and

**Fig. 1.** ANA comprehensive panels that are negative.

interobserver variability in pattern interpretation (2). Enzyme immunoassays (EIA) testing removes the subjective variations of IIF testing (3); however, there exist interlaboratory method variations and heterophile antibody interferences causing false-positive results. The correlation between ELISA and multiplex assays is high, with a 90% concordance (4). ANA testing with multiplexed microsphere fluorescence allows for rapid quantification and efficient profiling of multiple clinically significant antibodies in a single run of assay (5). The multiplex ANA screen is a composite screen which tests for 11 specific autoantibodies that are known to be associated with autoimmune diseases. If none of the specific antibodies are present, the ANA screen is reported as negative. Positive screens are reflexed, and the reflexed antibodies are resulted semi-quantitatively as numeric antibody indices (AI) (5).

The authors identified that the major reason behind inappropriate 'ANA comprehensive panel' ordering could be due to physician unawareness regarding test components performed under the order panel. The anti-ENA multiplex order is termed 'ANA comprehensive panel (ANA CP)' in our ordering system. ANA testing by multiplex assays should be performed in a bi-leveled sequence with an initial screen followed by comprehensive antibody testing only if ANA screen returns positive. The 'ANA comprehensive panel' is not a reflex laboratory test and examines directly for specific antibodies without going through an initial screen. Comprehensive antibody testing without an initial screen leads to unnecessary cost burden on the patient and a waste of laboratory resources. In our study, net calculated value of incurred losses resulting from 'Comprehensive Lab Order' (ANA CP) testing amounted to \$66,000 over a 6-month study period. This could have been avoided with bi-level ANA multiplex testing. The 'ANA comprehensive panel' does not provide any further utility over the 'ANA screen with reflex'. Hence, the ideal test of choice should be 'ANA screen with reflex'. Also, the term ANA comprehensive panel gives the physicians a sense of it being a more complete test over the ANA screen, thus leading to inappropriate use of the second-level testing without going through the initial screen. Over the last few years, ANA comprehensive panels have emerged as a very convenient test with fewer false positives and the convenience of being completed in a single run, resulting in the ordering physician choosing it over the appropriate ANA screen.

It is important to recognize that autoantibody testing is fraught with low specificity and false-positive results, leading to potential over-diagnosis and mistreatment. Providers should not order ANA testing with low clinical suspicion for rheumatological disease. In fact, false positives occur in a significant proportion of healthy population (6). Non-specific clinical symptoms do not warrant routine ANA testing (7). ANA testing in the wrong clinical

setting, especially in the absence of specific signs and symptoms suggestive of rheumatic diseases can potentially lead to misdiagnosis and unnecessary rheumatology consultations. Also, repeat ANA testing is not warranted in patients with established rheumatological disease (as noted in 3 of 68 in our study patients) except to monitor disease activity, in which case, repeating specific antibody titers may be considered and not the 'ANA comprehensive panel'. A retrospective study on patients who had ANA testing suggested that a large proportion (30%) of them had non-specific and unrelated signs and symptoms (7). Incorporation of ANA testing into initial lab order bundles in an effort to expedite diagnoses; overestimation of the pretest probabilities; failure to consider non-rheumatological factors expected to cause false-positive results contribute to ANA testing among physicians.

One of the limitations of our study was that it was a single institutional experience. We initially conducted this as a pilot study and based on the results in our center, we intend to conduct the study across all Advocate health-care hospitals.

### Conclusion

ANA testing with multiplex immunoassay systems allows for rapid profiling of multiple analytes in a single run of assay and have few false-positive results compared to conventional lab methods. However, it is important to educate physicians on following a sequenced diagnostic algorithm based on the guidelines laid down by American College of Rheumatology to avoid unnecessary higher level testing and increase cost burden on their patients. Also, inappropriate ANA testing without adequate

pretest probability estimation after careful history taking and clinical examination can lead to over-diagnosis with unnecessary consultations and should be avoided.

### Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study

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