To the Editor:

We appreciate the feedback from Fritzler, *et al*¹, on our article “A Negative Antinuclear Antibody Does Not Indicate Autoantibody Negativity in Myositis: Role of Anticytoplasmic Antibody as a Screening Test for Antisynthetase Syndrome,” published in *The Journal*². Our primary goal was to simply call attention to the many clinical laboratories in the United States that will report a negative antinuclear antibody (ANA) and not report the presence of cytoplasmic staining, which could indicate the presence of an antisynthetase autoantibody. This indeed may be in the setting of other commercial autoantibodies (i.e., rheumatoid factor, antineutrophil cytoplasmic antibodies, etc.) also being reported as negative. The clinical correlate of this is that the patient with the antisynthetase syndrome (particularly those with non-Jo1 autoantibodies) may not fully manifest the entire clinical spectrum of the antisynthetase syndrome but could certainly present with lung dominant disease. Thus, when autoantibody testing is ordered on this subset of patients, there is the possibility that the report yields a “negative” ANA. This form of autoimmune interstitial lung disease (ILD) may then be considered idiopathic pulmonary fibrosis or some other nonautoimmune pulmonary problem such as infection when indeed, the process is immunologically driven. The management of this misdiagnosed patient in this setting would then likely not include immunosuppression if the managing clinician believes that the lack of any positive autoantibody testing result mitigates the potential diagnosis of an immune-driven process. Our point is that this clinical decision is inaccurate and the patient would not receive potential lifesaving immunosuppression to treat autoimmune ILD. That being said, we certainly agree in principle with Mahler, *et al*, who note that ANA reporting should represent a spectrum of autoantibodies that leads to cytoplasmic indirect immunofluorescence (IIF) patterns³. It has been our experience over the last many years that this is not what is currently being reported in many clinical laboratories, as highlighted by our article. We appreciate the efforts of the International Consensus on ANA Pattern (ICAP) group to bring about standardization in ANA reporting and nomenclature⁴. However, changing the terminology of ANA to *anticytoplasmic antibody* may not be practical, given that ANA terminology is clinically entrenched in the minds of clinicians, as reported in the literature. Certainly the optimal solution is to eventually educate clinicians and laboratory personnel to standardize ANA reporting as “positive” for antigenic targets in the nucleus as well as mitochondria, cytoskeleton, endosomes, Golgi complex, endoplasmic reticulum, or the cytosol, etc. We also agree that immunology laboratories should report IIF patterns based on an international consensus (i.e., cytoplasmic fine speckled as in the case of antisynthetase syndrome; www.anapatterns.org/trees.php); however, currently this is not being done consistently. We certainly recognize the limitation of our study given that we simplified reporting to positive or negative and restricted the recognition of an anticytoplasm pattern on IIF to “speckled” or “diffuse.” However, our study included reporting dating back to the 1980s and 1990s, when ICAP standardization of IIF patterns was unavailable^{4,5}.

Another issue raised by Fritzler, *et al* is that our 1:40 cutoff was too sensitive and not consistent with the current recommendations of a 1:160 cutoff using the conventional Hep-2 substrate⁶. Again, our data date back many years to when a 1:40 serum dilution titer for ANA was a standard practice, particularly in a research laboratory. Further, a 1:160 dilution titer to define a positive ANA would have improved specificity, but at a further loss of sensitivity. We appreciate the authors’ clarifying that our study used HEp-2000 substrate (ImmunoConcepts), which can detect SSA/Ro60 autoantibodies as well⁷. Therefore, diagnostic laboratories using HEp-2 cell substrates from different suppliers may not have similar sensitivity and specificity, as in our study.

We would agree with the authors that if the ANA at a serum dilution of 1/40 is performed on HEp-2000 cells as a screen for anticytoplasmic antibodies (anti-CytAb), then testing for specific antisynthetase autoantibodies on a multianalyte array platform such as addressable laser bead assays

and line immunoassays is likely to be requested. However, in certain situations, checking ANA/anti-CytAb may represent an “inexpensive and quick” approach. The reason is that practically, ANA and anti-CytAb, which can be quickly and inexpensively checked in any immunology laboratory, can help in the early identification of the antisynthetase syndrome, and guide the appropriate therapy for autoimmune ILD. This is particularly important when clinicians are managing patients with worsening ILD who are very ill, perhaps even being mechanically ventilated, where time is of the essence. In these instances, the multianalyte array platform for specific antisynthetase autoantibody testing is not available in most community hospitals in the United States, leading to a significant delay before results are available.

The bottom line is that there is a clear need for better standardization of ANA/anti-CytAb as well as myositis autoantibodies. We need to educate rheumatologists and pulmonologists about the clinical spectrum of the myositis autoantibodies, especially those associated with ILD without clinical myositis. A comparative study of anti-CytAb detected on HEp-2 substrates versus more specific myositis autoantibody commercial assays would be a useful addition to the literature. However, clinical laboratory reporting must be convenient and timely from a clinical perspective.

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