Patient and External Quality Assessment Samples Demonstrate Similar Bias Between Manufacturers in Titer of Antibodies to Nuclear Antigens: Implications for Commutability

To the Editor.—The presence of antibodies to nuclear antigens (ANAs) above a threshold titer is a defining criterion for classification of systemic lupus erythematosus (SLE) and participation in clinical trials, and the probability of diagnosis of many autoimmune rheumatic diseases varies with the ANA titer.1,2 Consistent ANA titer results are therefore necessary for clinical and research purposes. Indirect fluorescent antibody (IFA) assays using HEp-2 cell substrates are widely considered the reference method for ANA detection. However, there is significant variability in titer results between laboratories performing IFA, challenging the concept of a universal threshold titer.3

Variability in ANA titer could result from reagents, HEp-2 substrates, microscopy conditions, and subjective visual interpretation. We recently analyzed results reported for 11 years from the College of American Pathologists (CAP) ANA proficiency testing survey and found that reported ANA titer was strongly influenced by reagent manufacturer.3 We found remarkable consistency in the relative rank order of ANA titer for each kit over time among all participating laboratories, including specimens producing a variety of different ANA patterns. Although this finding supports the potential for harmonization of ANA titer between laboratories using different kits, it remains unclear whether the consistent difference between manufacturers is also present for clinical specimens. Thus, we sought to determine whether the manufacturer bias that we observed in proficiency testing specimens is consistent in clinical specimens.

We used data reported by Copple et al4 who evaluated commercially available ANA IFA assays with serum samples from patients with autoimmune rheumatic diseases. This study evaluated 8 serum samples from patients with SLE and 11 serum samples from patients with scleroderma, each on HEp-2 substrates from 5 manufacturers. Consistent with our observations using proficiency testing specimens, some assays typically produced higher titers and others produced lower titers for each patient sample. We used the reported titer from each assay for each specimen to determine the manufacturer rank order. We then compared this manufacturer rank order for clinical samples versus the rank order for proficiency testing samples that we previously reported.3 The kit rankings for proficiency testing samples were nearly identical to the kit rankings for patient specimens (Figure). Linear regression demonstrated strong correlation of kit rankings for proficiency testing specimens and samples from patients with both SLE ($r = 0.90$, $P < .001$) and scleroderma ($r = 1.0$, $P < .001$).

These results demonstrate that differences in ANA titer between kits are consistent between CAP proficiency testing materials and specimens from patients with autoimmune rheumatic diseases, supporting commutability of proficiency testing material for relative ANA titer, where commutability is defined as a property of materials having the same interassay relationships as clinical samples.5 Together with our previous study, these data support the potential to harmonize IFA-based ANA quantitation. Harmonization could be achieved by collaboration between the clinical laboratory community, manufacturers, and organizations including proficiency test providers. Standardization using reference materials to align titer results between manufacturers could improve consistency between clinical laboratories, thus promoting diagnostic accuracy for patients with autoimmune diseases.

The authors gratefully acknowledge Christine Bashleben, MT(ASCP), from the College of American Pathologists Laboratory Improvement Programs.

Susan L. Fink, MD, PhD1; Michael A. Linden, MD, PhD2; Mark H. Wener, MD1,3

1Department of Laboratory Medicine and Pathology, University of Washington, Seattle; 2Department of Laboratory Medicine and Pathology, University of Minnesota Medical Center, Minneapolis; 3Division of Rheumatology in the Department of Medicine, University of Washington, Seattle


Finding of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Within Placental Tissue 11 Weeks After Maternal Infection

To the Editor.—We read with particular attention the article of Schwartz et al1 recently published in your journal. We want to discuss a case of long persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in placental specimens, associated with histiocytic intervillositis, marked increase in perivillous fibrin deposition, and chronic high-grade villitis. The mother was previously hospitalized for a symptomatic SARS-CoV-2 infection at gestational week (GW) 26, and spontaneous delivery occurred at GW 37. The reverse transcription polymerase chain reaction result of the nasopharyngeal swab taken at delivery was negative in both mother and child. Placental weight was less than the third centile for gestational age, no intrauterine growth restriction was recorded during pregnancy, and the neonatal outcome was unremarkable.

Here we show a pattern of histiocytic intervillositis associated with the immunohistochemical permanence of SARS-CoV-2 in Hofbauer cells and in the walls of intravillous fetal vessels, not in trophoblastic cells. The clone used to identify the virus (anti-nucleocapsid GTX135361, GeneTex) was previously tested for this purpose.2 In addition, we found other hallmarks of chronic persisting damage, such as a marked increase in perivillous fibrin deposition, chronic high-grade villitis, and chronic deciduitis (Figure, A through D).

Viruses can be vertically transmitted from mother to infant through intrauterine (hematogenous or ascending paths), intrapartum, and postpartum routes. Even though a large majority of infants born to pregnant women with coronavirus disease 2019 (COVID-19)2019 have been uninfected, new evidence shows that vertical transmission can occur. A recent analysis revealed that nearly 70% of SARS-CoV-2–positive neonates acquired the infection through postpartum transmission, with the remaining 30% through intrapartum or intrapartum mechanisms. Among all (122), 5.7% were stated to have confirmed congenital infection.3 It has been reported that, once the transmission has taken place, its effect on the fetus and the newborn can be relevant, leading to preterm delivery, admission to neonatal intensive care, or even stillbirth. A review of literature has shown only a case of persistence of the virus associated with placental lesions suggestive of inflammation after a previous SARS-CoV-2 infection. However, in this case, the infection occurred at GW 8, leading to a spontaneous abortion at GW 13.4 Although the placental lesions previously described in the work of Schwartz et al3 were related to an acute, symptomatic infection, here we demonstrate a histiocytic intervillositis with specific viral persistence, even after 11 weeks from the previous maternal symptomatic infection, in an otherwise healthy mother and newborn.

All these findings may be the consequence of an immune-mediated persistent injury to the maternal-fetal...