Clinical pathology selected abstracts

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Use of whole blood real-time PCR testing to diagnose early Lyme disease

January 2023—*Borrelia burgdorferi* is the leading cause of Lyme disease in the United States, with approximately 35,000 new cases reported to the CDC each year. The agency recommends a two-tiered approach to testing. The first tier is a sensitive enzyme immunoassay (EIA) targeting *B. burgdorferi* antibodies and the second is an immunoblot specific for immunoglobulin M (IgM) or immunoglobulin G (IgG) antibodies. However, orthogonal EIA in a modified two-tiered testing (MTTT) algorithm can replace the second tier of testing. The two-tiered approach to Lyme disease diagnosis has a sensitivity of 66 to 78 percent and specificity of 95 to 99 percent. Direct molecular testing for *B. burgdorferi* DNA in whole blood also has high specificity for early Lyme disease diagnosis, but spirochetemia in early Lyme disease usually lasts only several days after infection. The authors conducted a study to assess the impact of concurrent molecular and serologic testing for early Lyme disease and determine the utility of whole blood real-time polymerase chain reaction (WB-RTPCR) in assisting with the diagnosis. They performed a retrospective analysis of 33,199 blood specimens that were concurrently evaluated with WB-RTPCR and an antibody capture enzyme immunoassay (ACEIA) method (group A). An additional 56 pairs of specimens from a separate data set were retrospectively identified and analyzed at both initial and follow-up testing time points to determine if seroconversion occurred (group B). Lastly, 2,526 specimens that were evaluated by molecular testing and the MTTT EIA serology methods were analyzed (group C). The authors found that 1,379 of the 33,199 results in group A were positive for early Lyme disease when tested concurrently with ACEIA and WB-RTPCR. Of this latter group, 1,179 were found to be positive via serology, 131 via molecular testing, and 69 via both testing methods. Of the 56 pairs of specimens in group B, 20 pairs initially were found to be positive via serology, 17 via molecular testing, and 10 via both methods, while nine were negative by both methods. The 47 pairs that were positive for Lyme disease were serology-positive on follow-up testing. In the 2,526 specimens evaluated in group C, 358 results were consistent with early Lyme disease. Of these, 308 were found to be positive via serology, 31 via molecular testing, and 19 via both methods. The data showed that WB-RTPCR in clinically suspected cases of early Lyme disease can identify an infection that serology alone would miss. Furthermore, newer molecular testing techniques may improve the limit of detection and, therefore, extend the diagnostic testing window, further enhancing the utility of molecular testing for early Lyme disease. The authors concluded that WB-RTPCR will identify additional cases of Lyme disease that would have been missed with serology alone. In addition, WB-RTPCR and serologic testing for Lyme disease could be most appropriately utilized in situations involving uncharacteristic erythema migrans rash or an absence of such rash in those with a recent history of tick bites.

Pratt GW, Platt M, Velez A, et al. Utility of whole blood real-time PCR testing for the diagnosis of early Lyme disease. *Am J Clin Pathol*. 2022;158:327–330.

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Assessment of neurofilament light chain levels in patients with ICANS

Neurofilament light chain, a biomarker of neuronal injury, is elevated in several neurodegenerative diseases, including Alzheimer disease and multiple sclerosis, and in acute concussion. Patients receiving an infusion of cellular products are at risk for neuronal injury and may develop immune effector cell-associated neurotoxicity syndrome (ICANS). Approximately 40 to 60 percent of patients will develop ICANS after chimeric antigen receptor (CAR) T-cell therapy. Symptoms of ICANS range from encephalopathy to aphasia to cerebral edema. Grade three or higher ICANS can cause significant morbidity and mortality. While fewer than 10,000 patients are treated annually with cellular therapy, the indications for such therapy are growing rapidly. Neurofilament light chain (NfL) may be elevated before or after infusion of cellular products and observed up to five days prior to peak ICANS. Determining

whether NfL elevations in ICANS occur before or after infusion is important for identifying high-risk patients and determining whether neuroaxonal injury is latent or a result of treatment. The authors conducted a study to quantify serial NfL levels in patients undergoing cellular therapy, and they examined the association between serial NfL levels, ICANS, and potential risk factors. They performed a retrospective two-center study that examined plasma NfL levels in 30 patients who had detailed medical and treatment histories that included risk factors. The authors excluded from the study patients with dementia and severe symptomatic central nervous system involvement. NfL levels were measured at seven time points: baseline (prelymphodepletion), during lymphodepletion, and at post-infusion days one, three, seven, 14, and 30. The prediction accuracy for developing ICANS was modeled using receiver operating characteristic (ROC) classification. Univariate and multivariate modeling were also performed to determine the association between NfL levels, ICANS, and potential risk factors related to demographics, oncologic history, neurologic history, and history of exposure to neurotoxic therapies. The study results showed that patients who developed any grade of ICANS had elevated NfL levels before lymphodepletion and CAR T-cell infusion (87.6 pg/mL) compared with those who did not develop ICANS (29.4 pg/mL). Baseline NfL levels predicted development of ICANS with a high degree of accuracy (area under the ROC curve, 0.96), sensitivity (0.91), and specificity (0.95). Of interest, NfL levels remained elevated across all time points up to 30 days post-infusion. Baseline NfL levels correlated with ICANS severity but not with the other potential risk factors. The authors demonstrated that the risk of developing ICANS is associated with preexisting neuronal injury that can be quantified with plasma NfL levels. Preinfusion NfL levels may help identify those patients most at risk for ICANS. Additional studies should address whether NfL can serve as a predictive biomarker for early preemptive or prophylactic intervention.

Butt OH, Zhou AY, Caimi PF, et al. Assessment of pretreatment and posttreatment evolution of neurofilament light chain levels in patients who develop immune effector cell-associated neurotoxicity syndrome. *JAMA Oncol*. doi:10.1001/jamaoncol.2022.3738

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