EVALUATION OF AN ANTIBODIES-IN-LYMPHOCYTE-SUPERNATANT (ALS) ASSAY AS A DIAGNOSTIC MARKER OF INFECTION WITH SALMONELLA ENTERICA SEROVAR TYPHI IN PATIENTS WITH TYPHOID FEVER IN BANGLADESH.

Saruar Bhuiyan, M.S., Fahima Chowdhury, M.B.B.S., Amit Saha, MBBS, KMA Jamil, MBBS, PhD, M.B.B.S., M.P.H., Stephen B. Calderwood, M.D., Edward T. Ryan, M.D., Alejandro Cravioto M.D, PhD, Abdullah Brooks, M.D., Firdausi Qadri, Ph.D.,

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B, Dhaka, Bangladesh); Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts. USA

Background: A specific and sensitive diagnostic test for early detection of typhoid fever is needed. Both blood culture and serological assays such as the Widal test are able to specifically diagnose typhoid fever in only 30-70% of cases. Bone marrow aspiration and culturing is more sensitive, but of limited clinical utility. PCR-based diagnostic kits have had variable predictive value. For this reason, an improved early diagnostic marker for typhoid fever is needed. Evaluation of serum for Typhi-specific antibodies during typhoid fever has been either non-sensitive (during early disease) or non-specific (during early or late stage disease). The antibody-in-lymphocyte-supernatant assay is an assay based on collection of peripherally circulating white blood cells that are then cultured in vitro for 24-48 hours. Supernatants of these cultures can then be evaluated for the presence of antigen-specific antibodies, using either an ELISA or dot-blot format. We hypothesized that the ALS assay could thus be used to amplify low level specific anti-Typhi antibody responses, facilitating the diagnosis of individuals with typhoid fever, especially during early stage disease.

Aim: The aim of the study was to evaluate the ALS procedure for detection of serovar Typhi-specific antibodies in the blood of individuals with suspected enteric fever, and to compare sensitivity and specificity with blood culture and the Widal serological assay.

Methods: From May 2007 through June 2007, we enrolled patients at the ICDDR,B meeting a case definition of suspected typhoid fever (individuals 2-59 years of age with fever of at least 39.0°C, of at least 3 days duration, without an obvious other source), and collected blood samples at enrollment, then 5 and 20 days later. We evaluated ALS supernatants in both ELISA and dot-blot format against a range of typhi antigens, including TcfB (a pilus antigen of Typhi), Vi antigen (the capsule antigen of Typhi), PagC peptides (an immunogenic PhoP-induced Typhi antigen), Typhi LPS, a Typhi membrane preparation, and whole cell organism preparation. We also performed ALS analysis using blood of healthy controls and individuals with cholera. The study was approved by the IRB of the ICDDR,B.

Results: Of the 253 individuals with suspected typhoid fever enrolled in this study, 13% were positive by blood culture (serovar Typhi, n=26; serovar Paratyphi A, n=6). 43% were positive by the Widal test (titer ≥320 on at least one blood sample). Using a definition of a positive ALS result of > 10 mAB/min in the ELISA format, 132 (52%) of the study participants were positive for at least one Typhi antigen. Of the 32 patients from whom serovar Typhi or serovar Paratyphi A were recovered by microbiological culture of blood, all (100%) were positive by the ALS method (GM=40 mAB/min). Of study participants meeting the case definition for suspected typhoid fever but with negative blood cultures, 44% were positive by the ALS method (GM=40 mAB/min). ALS results were comparable whether PBMCs or buffy coats were used, and results did not appear to be age-specific (the magnitude of responses were similar in children and adults). Healthy controls or cholera patients tested negative in the ALS assay (GM=4 mAB/min).

Conclusions: Our results suggest that the ALS assay may have utility in diagnosing individuals with typhoid fever. Further evaluation of this technology is warranted.