Assessment of the Antibody-in-Lymphocyte Supernatant Assay for Enteric Fever Diagnosis in Two Human Challenge Studies and Prospective Evaluation in an Endemic Area of Nepal

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Background: Antibody-in-lymphocyte supernatant (ALS) assay, which detects *ex vivo* antibody production by newly formed B cells, could offer improved sensitivity and specificity over blood culture for enteric fever diagnosis. Here, we characterise ALS assay in two human challenge studies in Oxford and evaluate assay performance in febrile adult patients in Kathmandu, Nepal.

Methods: Heparinised blood samples were taken from participants during challenge with *Salmonella* Typhi or *S.* Paratyphi A; and from patients in Kathmandu on presentation and at 1, 12 and 26 weeks. Peripheral blood mononuclear cells were separated, washed, and cultured *ex vivo*. *In vitro* IgA antibody production specific for membrane preparation (MP), lipopolysaccharide or flagellin antigens was measured by ELISA of ALS. Diagnostic performance of anti-MP IgA responses at presentation (primary outcome) were compared with blood culture (reference standard) by discordant pairs and area under the curve (AUC) receiver-operator characteristic (ROC) curve analyses.

Findings: In 23 participants challenged with typhoid, anti-MP responses were 91% (95%CI 72–99) sensitive for typhoid diagnosis (fever and/or bacteraemia). In 40 participants challenged with paratyphoid, anti-MP responses were 95% (74–100) sensitive and 57% (34–78) specific for paratyphoid diagnosis, with an AUC ROC of 85% (74–97). In both studies, higher ALS responses were associated with longer duration of bacteraemia ($p<0.05$). In 173 patients in Nepal, anti-MP responses were 86% (70–95) sensitive and 51% (43–60) specific for blood culture confirmed typhoid/paratyphoid diagnoses, with an AUC ROC of 79% (70–88). High ALS responders were more symptomatic ($p=0.024$), and had lower white cell counts than low responders ($p=0.001$).

Conclusions: The ALS assay is sensitive in identifying bacteraemic enteric fever patients, however better reference standards are needed to ascertain accurate test specificity. The ALS assay could be used to improve enteric fever burden assessments and the accuracy of vaccine efficacy studies.