The potential of whole blood interferon-gamma release assay to detect Leishmania exposure in people living with HIV: results from the Ethiopian PreLeisH cohort

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Background: Surveillance of asymptomatic Leishmania infections remains difficult. Current screen-and-treat strategies employ a Leishmania diagnostic battery (Leish-DB) relying on parasite-based (PCR, KAteX) or antibody-based (rK39-RDT, rK39-ELISA, DAT) tests, all showing suboptimal sensitivity and specificity in PLWH. We evaluated whether whole blood cytokine release assays (CRA) with soluble Leishmania antigen (SLA) stimulation, may improve detection of Leishmania exposure in Ethiopian PLWH.

Methods: A total of 181 Ethiopian PLWH from the PreLeisH cohort were followed over 21 months and retrospectively classified, according to the Leish-DB, as Leishmania unexposed PLWH (HIV; n=34), with asymptomatic Leishmania exposure (AL-HIV; n=40), experienced past VL episode (pastVL; n=50), VL developers (VL-HIV; n=7), and "potential asymptomatic superspreaders" (n=2). To define positivity thresholds, a non-endemic healthy volunteer cohort was included (NEHV, n=47). Leishmania-specific IFN-γ, IL-2 and IP-10 responses from the CRA were measured by cytometric bead assay. Cross-sectional, longitudinal and correlation analyses were performed.

Findings: CRA IFN-γ emerged as the most discriminatory marker for Leishmania exposure detection, whereas IL-2 lacked discriminatory value and IP-10 showed cross-reactivity. Combining Leish-DB with CRA IFN-γ achieved nearly complete case detection in the pastVL cohort (49/50; 98%) versus Leish-DB alone (46/50; 92%). For AL-HIV, 70.0% of cases were confirmed and ten additional Leishmania-exposed individuals were identified within the HIV group. Moreover, test positivity over consecutive visits improved when combining Leish-DB and CRA IFN-γ. CRA IFN-γ results were consistently negative in "potential asymptomatic superspreaders" and became negative three months before disease onset in VL developers.

Interpretation: Whole blood IFN-γ release assays capture Leishmania-specific cellular responses undetected by conventional diagnostics, enabling more robust surveillance of asymptomatic Leishmania exposure in Ethiopian immunocompromised individuals, with potential to predict VL development in PLWH.

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