PESAB06

Cryptococcus qPCR assays: the future for routine mycology labs and clinical trials dealing with HIV-associated cryptococcosis

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Background: Routine laboratory testing for cryptococcal meningitis currently consists of Cryptococcal antigen (CrAg) testing in blood and cerebrospinal fluid (CSF), CSF India ink, and CSF fungal culture. Quantitative cryptococcal culture (QCC) is labor intensive and not feasible in most settings. We evaluated quantitative (qPCR) and reverse transcriptase qPCR (RT-qPCR) assays to quantify cryptococcal load in CSF, plasma, and blood. We investigated the dynamics of fungal DNA and RNA detection during antifungal treatment.

Methods: We developed a qPCR assay that can differentiate serotypes A, D and B/C of *Cryptococcus neoformans* and *Cryptococcus gattii* based on the amplification of a unique nuclear Quorum sensing protein 1 (QSP1) and a multicopy 28S rRNA gene and evaluated the assays on 205 patients samples from the AMBITION-cm trial in Botswana and Malawi (2018–2021). CSF, plasma and whole blood samples were stored per patient and were sampled at day 0 (baseline), day 7 and 14 for CSF and at day 1, 3 and 7 for plasma and whole blood post antifungal treatment initiation. A Roche LightCycler480 and Graph pad prism were used for data analysis.

Results: Using the *QSP1* qPCR, 138 (81.7%) were serotype A, 28 (16.6%) were serotype B/C and 3 (1.8%) were a mixed infection of serotype A and B/C. There was no amplification with 36 (17.6%) samples. QCC showed a good correlation with *QSP1* qPCR (slope = 0.797, R^2 = 0.73) and with 28S rRNA qPCR (Slope = 0.771, R^2 = 0.778) assays. The fungal load at D0 was significantly higher in patients who died at week 10 (w10) as compared to patients who survived post week 10 ($p < 0.01$). Detection of *Cryptococcus* DNA (28S rRNA qPCR) in plasma or whole blood within the first 24 hours of treatment was significantly associated with early mortality at w10 ($p < 0.01$). *QSP1* RT-qPCR showed that detection of DNA was due to viable fungal cells as the quantification of *QSP1* whole nucleic acids was systematically higher (2 to 5-fold) than that of DNA.

Conclusions: Quantification of *C. neoformans* and *C. gattii* load in CSF and plasma at D0 is useful in identifying patients at risk of death and may be a promising tool for monitoring treatment response in the future.