Abstract

The Global Action Plan to End Tuberculosis (TB) has set out a 90-90-90 strategy, aiming to reach at least 90% of people with active TB, place at least 90% of them on appropriate treatment and ensure that 90% of those on treatment complete their treatment course. A key intervention to achieve this goal is the validation of a non-sputum-based diagnostic TB biomarker that can detect all forms of active TB.

TB biomarker studies have been ongoing for decades. HIV is a major risk factor for TB, but previous TB biomarkers have not performed well in HIV-infected patients. Recently, the activity of indoleamine 2,3-dioxygenase 1 (IDO), has attracted attention as a TB biomarker. IDO converts tryptophan (T), an essential amino acid, to kynurenine (K). In TB, IDO directs the host immune response to *Mycobacterium Tuberculosis* (Mtb) towards an anti-inflammatory profile.

In the first section of this work, we reviewed the literature regarding IDO in TB disease as well as in HIV infection. Broad evidence available indicates that IDO-mediated tryptophan catabolism via the kynurenine-nicotinamide pathway represents a common axis upregulated in TB and HIV-infection. Evidence spanning over three decades suggests that elevated IDO activity (high K/T ratio) is a key factor marking progression from latent TB infection to active disease, particularly in HIV-infected individuals.

The gold standard method for detecting IDO activity is the measurement of the product (kynurenine)-to-substrate (tryptophan) (K/T) ratio using mass spectrometry, a time-consuming and costly procedure. In the second part of this study, we evaluated an enzyme-linked immunosorbent assay (ELISA) as an alternate method for plasma K/T ratio quantification. ELISA is a low-cost, high-throughput method, with the potential for automation, making it easy to use in peripheral laboratories. The assay requires small sample volumes and can be completed within a relatively short time. We found strong reproducibility and good agreement between plasma K/T ratio measured by ELISA and mass spectrometry. The mean bias between ELISA and mass spectrometry was 0.01 with 95% limits of agreement from -0.06 to 0.10.

We then used the ELISA method to determine plasma K/T ratio in two nested case-control studies, aiming to test whether plasma IDO activity measured by K/T ratio meets the proposed target product profile of a non-sputum-based TB biomarker.

In the first study, we used residual plasma samples from HIV-infected and uninfected individuals with and without active TB from Cape Town. We found that HIV-infected patients with active TB had higher plasma K/T ratio than those without TB (median 0.101 [interquartile ranges (IQR) 0.091-0.140] versus 0.061 [IQR 0.034-0.077], $p < 0.0001$). At a cut-off value of 0.080, plasma K/T ratio gave a sensitivity of 90%, specificity of 80%, a positive predictive value (PPV) of 82% and a negative predictive value (NPV) of 90%. In a receiver operating characteristic (ROC) analysis, plasma K/T ratio had an area under the curve (AUC) of 0.93.

In HIV-uninfected patients, we also observed a higher plasma K/T ratio in those with active TB compared to those with latent TB infection (median 0.064 [IQR 0.040-0.088] versus 0.022 [IQR 0.016-0.027], $p < 0.0001$). A cut-off of 0.040 gave a sensitivity of 85%, a specificity of 92%, a PPV of 91% and an NPV of 84% with an AUC of 0.93.

In the third section of this work, we validated our findings using the Tshepiso HIV-infected pregnant women cohort and explored whether pregnancy is a confounder to the use of plasma K/T ratio as a diagnostic biomarker for active TB. We found that plasma K/T ratio was significantly elevated during pregnancy compared with non-pregnant periods ($p < 0.0001$). At the time of TB diagnosis, plasma K/T ratio was markedly elevated in pregnant patients with active TB compared to individuals without TB ($p < 0{\cdot}0001$). Applying a previously optimised cut-off value of 0.080, plasma K/T ratio gave a diagnostic sensitivity of 96%, specificity of 86%, a PPV of 77% and an NPV of 99%. A ROC gave an area under the curve of 0.95. The optimal cut-off in HIV-infected pregnant women was however, calculated as 0.100 with a sensitivity of 94%, specificity of 90%, a PPV of 85% and an NPV of 98%. Thus, we assert that the higher cut-off is more appropriate in pregnancy. A novel finding from this work was that in our control group, pregnant women who received isoniazid preventative treatment (IPT) had lower plasma K/T ratio than those who did not receive IPT, suggesting that isoniazid alone decreases IDO activity.

Additionally, in both cohorts, we noted that plasma K/T ratio inversely correlated with body mass index (BMI) in TB patients, but not in controls. Relative loss in weight is a cardinal symptom of TB; thus, our findings suggest that elevated K/T ratio accompanies lower BMI as a factor associated with active TB.

Conclusion

In conclusion, plasma K/T ratio is a suitable blood-based biomarker of active TB in both HIV-infected and uninfected individuals. Plasma K/T ratio can be used as an initial blood-based test for active TB. Pregnancy was not a confounder to the use of plasma K/T ratio to diagnose active TB in HIV infected individuals, although a higher cut-off value of 0.100 rather than 0.080 was preferable. Plasma K/T ratio can be measured using a high-throughput, low-cost, widely available ELISA. Plasma K/T ratio should be evaluated prospectively in further studies to determine its impact on patient care.