Prediction of Incident Active TB in PLHIV by Detection of Free-Circulating *M. tuberculosis* DNA in patient plasma: A Retrospective Longitudinal Study

(MSS 265R)

Executive summary

- Background

Tuberculosis (TB) remains the major cause of death in PLHIV/AIDS. Isoniazid Preventive Treatment (IPT) has been demonstrated to reduce TB incidence in patients receiving ART, yet universal treatment may expose large number of patients who have no known TB contact with unnecessary isoniazid hepatotoxicity. IPT would be more justifiable if it is targeted to those with significantly increased risk of TB. However, no existing single test can accurately predict incident active TB among PLHIV.

- Aim and Objectives

To develop a droplet digital PCR (ddPCR) assay for detection of free-circulating *M. tuberculosis* DNA in human plasma to predict subsequent active TB development in PLHIV.

- Project design

Longitudinal archived plasma collected at different CDC clinical stages of at least 200 PLHIV/AIDS will be analysed with the ddPCR assay. Using clinically or culture-confirmed TB as the reference standard, the predictive value of the ddPCR assay for incident active TB was calculated. The performance was compared with tuberculin skin test, the current standard method for detection of latent TB.

- Target population

PLHIV/AIDS who had been followed up in ITC under Special Preventive Programme for at least three years. By the time of study entry, all patients had no evident of active TB.

- Main achievements

A total of 829 longitudinal archived plasma from 280 non-duplicated PLHIV/AIDS were collected. According to the clinical record (updated on 21 June 2018) provided by Special Preventive Programme in Department of Health, a total of 38 patients were defined as confirmed active TB group, whereas the rest of 242 were defined as no evident of active TB. A ddPCR was designed to detect 68-bp fragment of insert element IS6110 specific to *M. tuberculosis* in plasma samples of HIV patients. All 829 archived plasma were tested with ddPCR assay in triplicate (totally 2,487 tests). Among the 38 active TB cases, 19 showed positive ddPCR result before active TB diagnosis was confirmed, resulting in a predictive value of 50.0%, which is higher than that of the existing method, tuberculin skin test (23.7%). On the other hand, the specificity of ddPCR was found to be 89.7%, which is also higher than tuberculin skin test (73.9%).

- Conclusions

This project demonstrated that cell-free *M. tuberculosis* DNA is present in plasma samples of PLHIV/AIDS before the diagnosis of active TB and is detectable using our ddPCR assay. The predictive value of using ddPCR for incident active TB is 50%, which is higher than that of the tuberculin skin test (23.7%). In this study, the archived plasma samples were collected from 1999 to 2013, and thus have been stored in freezer for at least 5 years. Considering the instability of cell-free circulating DNA, the clinical value of ddPCR for prediction of active TB is underestimated in this study. A prospective evaluation of the clinical utility of ddPCR for prediction of incident active TB in fresh patient plasma is warranted.

背景

結核病仍然是愛滋病毒攜帶者或愛滋病患者的主要死亡原因。異煙肼預防治療已經被證明可以降低接受抗愛滋病毒治療的患者的結核病發病率，但盲目的廣泛治療可能會使大量沒有感染結核病菌的患者不必要地接觸到異煙肼的肝毒性。如果可以針對性地對一些結核病風險顯著增加的人使用異煙肼，這治療法將更合理。但是，現在沒有測試能準確預測在愛滋病患者中活性結核病發病的風險。

- 目標

開發液滴數位聚合酶鏈反應(droplet digital PCR, ddPCR)測定法來檢測在血漿中自由循環的無細胞結核分枝桿菌 DNA，以預測在愛滋病患者中活性結核病發病的風險。

- 項目設計

使用 ddPCR 測定法分析在至少 200 個愛滋病患者在不同 CDC 臨床階段收集的縱向存檔血漿。使用臨床或培養證實的結核病個案作為參考標準，計算 ddPCR 測定法對活性結核病的預測值。結核菌素試驗是目前檢測潛伏性結核病的標準方法。在本項目中，ddPCR 的效能會與結核菌素皮膚試驗進行比較。

- 目標人口

在 ITC 特殊預防計劃內接受了至少三年診斷的愛滋病病毒感染者/愛滋病患者。在研究開始時，所有患者都沒有活性結核病的臨床特徵。

- 主要成就

共收集了來自 280 個愛滋病患者，共 829 個早前雪藏的縱向血漿。根據衛生署特別預防計劃提供的臨床記錄（2018 年 6 月 21 日更新），共有 38 名患者被確診為活性結核病，而其餘 242 名患者被定義為無活性結核病。我們設計的 ddPCR 針對地檢測在患者血漿樣品中結核分枝桿菌獨有的 *IS6110* 基因片段。所有 829 個的血漿用 ddPCR 一式三份進行測試（總共 2,487 次測試）。在 38 個活性結核病例中，19 個在確診活性結核病之前顯示陽性 ddPCR 結果，預測值為 50.0%，高於現有結核菌素皮膚試驗方法（23.7%）。另一方面，ddPCR 的特異性為 89.7%，也高於結核菌素皮膚試驗（73.9%）。

- 結論

該項目證明，在診斷活性結核病之前，無細胞結核分枝桿菌 DNA 已存在於患者的血漿樣品中，並且可以使用我們的 ddPCR 測定法檢測。ddPCR 對活性 TB 的預測價值為 50%，高於結核菌素皮膚試驗（23.7%）。在這項研究中，血漿樣本從 1999 年至 2013 年收集，因此已在冰箱中儲存至少 5 年。考慮到無細胞循環 DNA 的不穩定性，本研究低估了 ddPCR 預測活性結核病的臨床價值。有必要收集新鮮血漿樣品，再對 ddPCR 用於預測活性 TB 的臨床效用進行前瞻性評估。